Biochemistry, 2017, 56 (28), pp 3632–3646Arch Biochem Biophys. 2018 Sep 1;653:97-106.4**DXHa**
ZN;dimer4DXH;4DWV;5VJ5,1SBY;3DJJ,1LQU,1YNQ,1HEJ,1KMS,1N92,1N8K,1C1D,1EK6,2J6L
1AXE;1A71,1HET,2JHG,1US0,3BCJ,3OQ6,1PWM,2J8T,1ZK4;3BTO;1HEU;1P1R;1U3U,1U3V,1U3W;1ZJZ,1SBY,1T2D,3JYO,2O23, 1ZJY

Horse Liver Alcohol Dehydrogenase: Zinc Coordination and Catalysis

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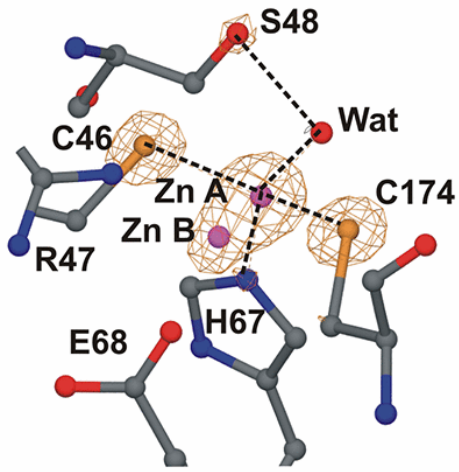
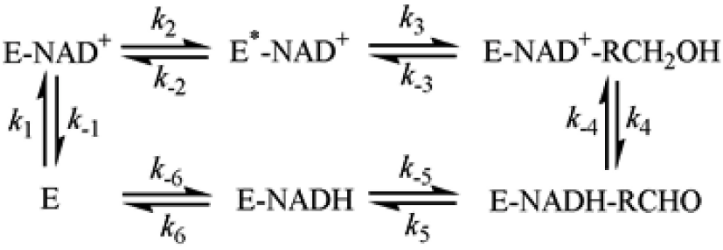
Highlights: The buried Glu-267 was substituted with His and Asn residues.

- The E267 H/N enzymes have decreased affinity for coenzymes.
- The conformational change of the enzyme-coenzyme complexes is hindered.
- Catalytic efficiency is significantly decreased.
- The E267H/N enzymes bind 3'-dephosphocoenzyme A.

Abstract During catalysis by liver alcohol dehydrogenase (ADH), a water bound to the catalytic zinc is replaced by the oxygen of the substrates. The mechanism might involve a pentacoordinated zinc or a double-displacement reaction with participation by a nearby glutamate residue, as suggested by studies of human ADH3, yeast ADH1, and some other tetrameric ADHs. Zinc coordination and participation of water in the enzyme mechanism were investigated by X-ray crystallography. The apoenzyme and its complex with adenosine 5′-diphosphoribose have an open protein conformation with the catalytic zinc in one position, tetracoordinated by Cys46, His67, Cys174, and a water molecule. The bidentate chelators 2,2′-bipyridine and 1,10-phenanthroline displace the water and form a pentacoordinated zinc. The enzyme–NADH complex has a closed conformation similar to that of ternary complexes with coenzyme and substrate analogues; the coordination of the catalytic zinc is similar to that found in the apoenzyme, except that a minor, alternative position for the catalytic zinc is ∼1.3 Å from the major position and closer to Glu68, which could form the alternative coordination to the catalytic zinc. Complexes with NADH and *N*-1-methylhexylformamide or *N*-benzylformamide (or with NAD^+ and fluoro alcohols) have the classical tetracoordinated zinc, and no water is bound to the zinc or the nicotinamide rings. The major forms of the enzyme in the mechanism have a tetracoordinated zinc, where the carboxylate group of Glu68 could participate in the exchange of water and substrates on the zinc. Hydride transfer in the Michaelis complexes does not involve a nearby water. Horse liver alcohol dehydrogenase ADH catalyzes the reversible oxidation of alcohols to aldehydes by an ordered mechanism Scheme 1, with the coenzyme NAD^+ binding first, isomerization to the E*–NAD^+ complex coupled to release of a proton and a conformational change that closes up the active site, binding of the alcohol, hydride transfer, release of aldehyde, and dissociation of NADH. Rate constants for each of these steps have been estimated for the EE isoenzyme ADH1E, and transient kinetics and solvent isotope effects indicate that hydride is transferred from a zinc alkoxide.1-3 The rate constants for the isomerization of the enzyme–NADH complex are too fast to determine with stopped-flow methods. The mechanism for the exchange of substrates for the water bound to the zinc also has not been established. Structures have been determined for the apoenzyme E and analogues of the ternary complexes, but structures for the binary enzyme–NAD^+ or enzyme–NADH complexes are needed to understand more details of the mechanism.





Scheme 1. Alcohol dehydrogenase kinetic mechanism.

Several studies have shown that substitutions of amino acid residues in the active site decrease affinity for coenzymes and alter the conformational change [8]. Inspection of the ADH structure led us to examine the role of Glu267, which is buried in a loop of the coenzyme binding domain connecting the end of a β-strand to an α-helix and includes Val268 and Ile269, whose carbonyl groups provide hydrogen bonds to the coenzyme [9]. The intrinsic p*K* is 4.5 of the γ-carboxyl group [10], and the hydrogen bonds (Fig. 1) would decrease the pK<4.5 and stabilize a negatively-charged carboxylate at pH 7.36 [11].

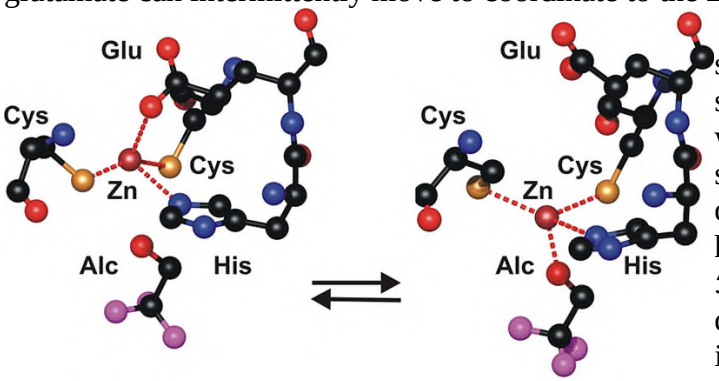
X-ray crystallography of horse ADH1E has determined the conformational states and the coordination of the catalytic zinc for the apoenzyme and for various complexes.(4-7) The catalytic zinc of the apoenzyme (no bound coenzyme) of liver ADH binds a water in a tetrahedral coordination with two cysteine sulfurs and one histidine imidazole.(4) In the holoenzyme complexes with NAD^+ and fluoro alcohols, the oxygen of the alcohol binds to the zinc and replaces the water.(8) The zinc acts as a Lewis acid and stabilizes the intermediate alkoxide, which

forms a low-barrier hydrogen bond with the Ser48 hydroxyl group and is connected by a proton relay system to His51 that acts as a base.(1, 9) In analogues of the ternary product complex with NADH and an aldehyde, the oxygen of a sulfoxide or a formamide also binds to the tetracoordinated zinc.(3, 5, 10-13)

The major issue addressed in this work is the coordination for the catalytic zinc in intermediate enzyme species and the mechanism for the exchange of the zinc-bound water (or hydroxide) with the substrate. A pentacoordinated zinc with adjacent oxygens from a water and the substrate was proposed for proton transfer in the enzyme–coenzyme–substrate complex to account for results available at that time.(14) The horse apoenzyme forms complexes with 2,2′-bipyridine and 1,10-phenanthroline, which appear to form a pentacoordinated zinc. 15-20 However, the proposed mechanism was abandoned when a structure of a ternary enzyme–NAD–substrate complex showed no such water and no space to accommodate a water.21 Nevertheless, later structural studies of enzyme–NADH complexes appear to show alternative positions for a water bound to zinc, with one water positioned close to C6N of NADH, and a mechanism in which a water bound to a pentacoordinated zinc activated the NADH was proposed.22, 23 As discussed below, nuclear magnetic resonance NMR and electron paramagnetic resonance EPR studies of the enzyme with Co(II) substituted for the catalytic zinc, NMR and perturbed angular correlation spectroscopy of the enzyme with ^{113}Cd(II) or ^{111}Cd(II), X-ray absorption spectroscopy, computations, and X-ray studies of other ADHs have led to various proposals about changes in metal coordination during the enzymatic reaction.24-32

X-ray crystallography has shown that the catalytic zinc has two different coordination environments in some ADHs. Complexes of human ADH3 with adenosine 5′-diphosphoribose or NAD(H) have an inverted tetrahedral configuration with coordination to the side chain carboxylate of Glu67, two cysteines, and a histidine, whereas the apoenzyme and some ternary complexes have the classical coordination with the oxygen of a water or substrate analogue, two cysteines, and a histidine.(33-36) Some subunits of the apoenzyme of *Escherichia coli* ADH have the inverted coordination of the zinc with Glu-59, two cysteines, and a histidine, whereas complexes with NAD have a water displacing the glutamate.(37, 38) The zinc in the yeast apoenzyme (in an open conformation) has an inverted tetrahedral configuration with coordination to the carboxylate of Glu67, two cysteines, and a histidine, whereas the ternary complex with NAD^+ and 2,2,2-trifluoroethanol (in the closed conformation) has the oxygen of the alcohol displacing the glutamate.(39, 40) The *Sulfolobus solfataricus* ADH also has these two different coordination geometries in apoenzyme and holoenzyme complexes.(41, 42)

Thus, an alternative mechanism for the exchange of zinc ligands could involve a double displacement in which the side chain carboxylate of the nearby glutamate residue displaces the water, inverting the configuration of the tetrahedral zinc, and then the substrate oxygen displaces the carboxyl group.(34) Such a mechanism can be illustrated with two structures observed for yeast ADH Scheme 2, from Protein Data Bank PDB entry **5ENV**.40 It is notable that the catalytic efficiency for alcohol oxidation is decreased when Glu67 is substituted with neutral amino acids in yeast ADH1 and human ADH3.(36, 43) Computational studies of horse ADH also suggest that the glutamate can intermittently move to coordinate to the zinc.(44)



Scheme 2. This study extends our knowledge of structures that are relevant to the mechanism. Partial data sets to 4.5 Å for binary complexes with NAD^+ or NADH were used previously to help define the alcohol binding site,(45) but higher-resolution structures are needed to describe the details. Some low-resolution structures for the horse ADH1E apoenzyme and complexes with adenosine 5′-diphosporibose and 1,10-phenanthroline have been determined; however, structure factors were not deposited in the PDB, and details of zinc coordination are lacking.(18, 46) As noted above, some atomic-resolution structures of horse ADH complexed with NADH apparently show some partial adducts of water with the reduced nicotinamide ring,(22, 23) but structures of ternary complexes of horse or human ADHs with NADH and formamides show no evidence of such a water.(11, 47) Atomic-resolution studies of ternary complexes of horse ADH with NAD^+ and fluoro alcohols have no water near the zinc or the nicotinamide ring.(8) A new atomic-resolution structure of the enzyme–NADH complex shows that the catalytic zinc is mobile and has alternative positions, which suggest that Glu68 participates in the exchange of water and substrates on the zinc. Although a pentacoordinated zinc may form transiently during the exchange of zinc ligands, the reactive ternary complexes are tetracoordinated. The conflicting results from the literature are analyzed in the discussion.

Experimental Procedures Crystallization Wild-type (natural) crystalline horse liver alcohol dehydrogenase (EC 1.1.1.1, UniProt entry P00327, GenBank entry M64864) and LiNAD$^+$ were purchased from Roche Molecular Biochemicals. 1,10-Phenanthroline, 2,2′-bipyridine, and adenosine 5′-diphosphoribose were obtained from Sigma. 2-Methyl-2,4-pentanediol (MPD) was obtained from Kodak and treated with activated charcoal before being used.

The crystals of the apoenzyme were prepared by a modification of the published procedure in which 1 mL of 10 mg/mL enzyme was dialyzed against 10 mL of 50 mM Tris-HCl (pH 8.4) at 5 °C as the concentration of MPD was gradually increased to a final concentration of 25% over several days.(48) The complex with adenosine 5′-diphosphoribose (ADPR) was prepared by soaking some apoenzyme crystals in the final outer dialysate for 1 h at 5 °C with 2 mM ADPR, relative to a K_i of 41 μM.(49) Crystals were also soaked for 3 h at 5 °C with 30 mM 2,2′-bipyridine, relative to a K_d of 0.31 mM,(19) or for 2 h at 5 °C with 5 mM 1,10-phenanthroline, relative to a K_d of 9–31 μM.(15, 19, 50)

Crystals of the binary enzyme–NADH complex were prepared as described by the general procedure used previously.(8) The enzyme (10 mg/mL) was dialyzed against 50 mM ammonium *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonate buffer and 0.25 mM EDTA (pH 7.0) (measured at 5 °C, pH 6.7 at 25 °C) at 5 °C with 1 mM NAD$^+$ as the concentration of 2-methyl-2,4-pentanediol was increased over several days to 12% when crystals formed. The concentration of the diol was finally increased to 30%. As determined from the X-ray data, the NAD$^+$ was reduced to NADH in the final complex. Crystals of a ternary complex were prepared with 1 mM NADH and 10 mM racemic *N*-1-methylhexylformamide (MHF; K_i = 5.4 μM(51)) under the same conditions described above. Three months after the crystals were produced, an ultraviolet (UV) spectrum of the outer dialysate showed that almost all of the NADH had been converted to NAD$^+$, so the crystals were harvested from the dialysis bag and placed in a fresh solution of the crystallization medium with 25% MPD. Crystals were prepared similarly with 1 mM NADH and 10 mM *N*-benzylformamide (BNF; K_i = 0.74 μM(51)).

X-ray Crystallography

Crystals were mounted on fiber loops (Hampton Research) and flash-vitrified by being plunged into liquid N_2. The data for the apoenzyme at 100 K were collected on IMCA-CAT beamline 17-ID with the ADSC Quantum detector at the Advanced Photon Source (APS) with a wavelength of 1.000 Å. The data for the apoenzyme complexed with ADPR at 100 K were collected on SBC-CAT beamline 19-ID at APS with the ADSC CCD detector with a wavelength of 1.0093 Å and high- and low-resolution passes. The data for the enzyme complexed with 2,2′-bipyridine or 1,10-*o*-phenanthroline were collected at 100 K on the Rigaku R-Axis IV^{++} instrument with the image plate and rotating anode at The University of Iowa X-ray Crystallography Facility with a wavelength of 1.5418 Å. The data were processed with d*TREK.(52)

The data for the ADH–NADH complex were collected at 100 K on the SBC 19-ID beamline with the ADSC detector at APS with an X-ray wavelength of 0.9537 Å. High-resolution data were collected at a 120 mm crystal to detector distance, with 0.3° oscillations and 10 s exposures with 2θ at 20° over a total of 216°; medium-resolution data were collected at 120 mm, with 0.5° oscillations over 360° and 1 s exposures, and low-resolution data were collected at 300 mm, with 0.5° oscillations over a total of 360° and 0.5 s exposures. Data were processed with XDS.(53) The data for the ADH–NADH–*N*-1-methylhexylformamide complex at 100 K were collected at the ESRF synchrotron on beamline 14-ID with a wavelength of 0.936 Å and high- and low-resolution passes. Data were processed with XDS.(53) Data were collected on two crystals of the complex with NADH and *N*-benzylformamide at 100 K at IMCA beamline 17-ID at APS with a wavelength of 1.000 Å with high- and low-resolution passes. Data were processed with d*TREK. Some data sets were not as complete as desired, because of the geometry of the beamline station, crystal orientation, lack of beam time, and radiation decay; however, the structures are of high quality, and the electron density maps support the reported structural details.

The structures for the apoenzyme and the complexes with ADPR and 2,2′-bipyridine were determined by molecular replacement using as a model of the structure in PDB entry **8ADH**, which has the same space group. The structure for the apoenzyme complexed with 1,10-phenanthroline was determined by molecular replacement using a model of the dimeric enzyme derived from PDB entry **1QLH**, which has the same space group. Regions of the structures that lacked readily interpreted electron density were examined with omit maps. The structure of the enzyme–NADH complex was determined by molecular replacement with the coordinates for the wild-type ADH–NAD$^+$–2,3,4,5,6-pentafluorobenzyl alcohol complex PDB entry **4DWV** after removal of the pentafluorobenzyl alcohol.(8) The structures for the enzyme complexed with NADH and *N*-1-methylhexylformamide or NADH and *N*-benzylformamide were determined by molecular replacement with the structure in PDB entry **1P1R**.(11)

The structures, with riding hydrogens, were refined by cycles of restrained refinement with REFMAC5.(54) The monomer dictionary used by REFMAC for NAD$^+$ was modified to remove the restraints on planarity and relax the restraints on bond distances (from ±0.02 to ±0.10 Å) for the nicotinamide ring so that puckered or

reduced rings could be properly refined. We named this modified NAD as “NAJ” in several structures in the RCSB data bank (e.g., **4DWV**, **4DXH**, **1N92**, and **1N8K**) to distinguish it from the NAD (with tight restraints) used in other structures. After the bound coenzyme was identified as NADH in the structures presented here, the REFMAC dictionary for NADH (named “NAI” in the RCSB data bank), modified with relaxed restraints on bond distances and angles for the reduced nicotinamide ring, was used for the final stage of refinement. Occupancies for alternative positions were adjusted to make the temperature factors similar. Model building used the program O.(55) SHELXL-2013(56) was finally used for analysis of the structure of the enzyme–NADH complex, with no restraints on the coenzyme for 10 cycles of CGLS refinement and one full-matrix cycle of least-squares refinement (BLOC 1, MERG 4) to determine puckering angles and bond distances and their errors for the nicotinamide ring. The structures were checked with MolProbity.(57) EXCEL was used for statistical analyses. Figures were prepared with the Molray web server in Uppsala, Sweden.(58)

Table 1. X-ray Data and Refinement Statistics for Horse Alcohol Dehydrogenases

Enzyme complex, zinc ligand	apoenzyme, H_2O	ADPR, H_2O	2,2′-bipyridine	1,10-phenanthroline	NADH, H_2O	NADH, MHF	NADH, BNF
PDB entry	**1YE3**	**5VKR**	**5VJG**	**5VJ5**	**4XD2**	**5VN1**	**5VL0**
space group, no. of subunits per asymmetric unit[a]	$C222_1$, 1	$C222_1$, 1	$C222_1$, 1	$P2_12_12_1$, 2	P_1, 2	$P2_1$, 4	$P2_1$, 4
cell dimensions Å	55.8, 74.3, 181.3	55.4, 74.3, 181.0	55.5, 73.7, 181.1	55.3, 73.4, 180.8	44.2, 50.9, 92.7	50.2, 180.8, 86.8	50.2, 180.3, 86.9
cell angles (deg)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	92.0, 103.0, 109.6	90, 106.1, 90	90, 106.2, 90
mosaicity	0.92	0.74	1.2	1.1	1.1		0.94
resolution range Å	20.1–1.59 (1.63)	19.93–1.80 (1.86)	19.9–1.90 (1.97)	19.84–1.90 (1.97)	19.8–1.1 (1.13)	20.0–1.25 (1.30)	20.0–1.2 (1.24)
no. of reflections (total, unique)[b]	205694, 36732	245973, 30816	198230, 27148	300828, 55508	687793, 257968	611039, 325043	2260931, 361271
redundancy (shell)	5.61 (5.43)	7.64 (5.68)	6.99 (5.04)	5.30 (3.38)	2.8 (1.89)	1.79 (1.59)	6.19 (4.34)
completeness (%) (outer shell)	71.9 (54.0)	91.7 (85.6)	95.3 (87.4)	96.1 (93.3)	86.8 (71.1)	80.0 (72.7)	79.4 (57.4)
R_{pim} (%) (outer shell)[c]	3.8 (9.9)	4.6 (23.2)	4.8 (17.0)	3.8 (24.9)	2.9 (39.6)	8.9 (25.5)	3.8 (27.7)
mean $\langle I\rangle/\sigma\langle I\rangle$ (outer shell)	12.9 (4.9)	9.0 (2.1)	9.8 (3.5)	8.8 (2.9)	9.1 (2.3)	6.6 (2.2)	8.3 (1.7)
R_{value},R_{free}% test %,no[d]	20.1, 25.6 (3.1, 1143)	19.2, 24.8 (4.1, 1328)	20.9, 28.3 (4.1, 1174)	24.8, 29.4 (2.1, 1169)	17.7, 19.8 (0.5, 1292)	15.2, 18.7 (0.5, 1650)	15.9, 19.9 (1.0, 3775)
RMSD for bond distances (Å)[e]	0.014	0.019	0.016	0.016	0.014	0.017	0.019
RMSD for bond angles deg[e]	1.53	2.07	1.80	1..81	1.75	1.89	2.06
estimated error in coordinates (Å)	0.069	0.125	0.153	0.195	0.032	0.028	0.036
mean *B* value (Wilson, Refmac) (Å^2)	25.4, 35.4	43.6, 50.6	29.2, 41.4	22.5, 35.7	8.8, 19.1	19.0, 25.0	13.0, 20.3
total no. of non-H atoms (mean *B* value)	2995	2868	2895	5828	6719	12774	12940
protein	2748 (32.0)	2785 (51.5)	2799 (41.0)	5568 (36.8)	5764 (17.7)	11195 (24.8)	11343 (19.8)
heteroatoms, zinc, ligands	10 (54.0)	38 (59.5)	14 (46.5)	32 (27.7)	126 (21.2)	250 (26.0)	240 (19.6)
waters	200 (39.6)	45 (51.4)	96 (40.5)	228 (38.4)	829 (36.8)	1329 (37.0)	1357 (32.5)
Ramachandran % (favored, outlier)	97.6, 0	96.0, 0	95.2, 0.27	96.0, 0.54	97.2, 0	97.2, 0	97.1, 0
MolProbity (clash, score, rank %)	3.71 (97th), 1.33 (95th)	2.99 (99th), 1.72 (83rd)	1.77 (100th), 1.49 (96th)	1.94 (100th), 1.75 (85th)	1.5 (97th), 1.03 (98th)	1.34 (98th), 1.05 (99th)	1.36 (98th), 1.01 (99th)

a The biological molecule is a dimer of identical protein subunits.
b Data cutoff, $\sigma F > 0$.
c $R_{pim} = R_{merge}/(n-1)^{1/2}$, where n is data redundancy.
d $R_{value} = (\sum||F_o| - k|F_c||)/\sum|F_o|$, where k is a scale factor. R_{free} was calculated with the indicated percentage of reflections not used in the refinement.(104)
e Deviations from ideal geometry.

Results Tertiary Structures Structures for three forms of horse liver alcohol dehydrogenase that have been deposited in the Protein Data Bank were re-determined at 100 K to extend the resolution of the data, secure the structure factors, and establish detailed structural features. Four structures of new complexes were also determined. Table 1 summarizes the X-ray data and refinements.

The apoenzyme structure determined here at 1.6 Å resolution PDB entry **1YE3** is in the open conformation, with essentially the same structure (RMSD of 0.33 Å for α-carbon atoms) as in PDB entry 8ADH, which was

determined at 278 K and 2.4 Å resolution.(4) Note the tetrahedral coordination of the zinc and the water molecule that is hydrogen bonded between Ser-48 and His-51 (Figure 1); this water was not identified in the first publication but was added when the structure was further refined and deposited in the PDB. This water is part of the proton relay system, which is replaced by the nicotinamide ribose O2D in ternary complexes.(21)

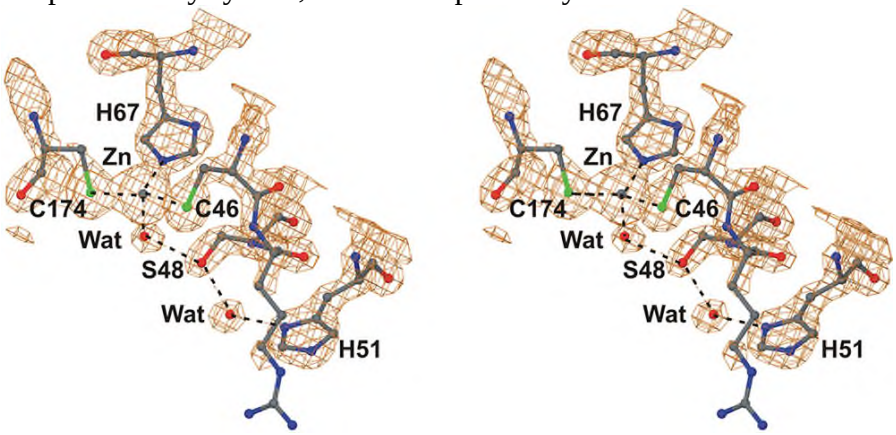


Figure 1. Zinc coordination in the apoenzyme. The stereoview is derived from PDB entry **1YE3**. The $2|F_o| - |F_c|$ map is contoured at ~0.3 $e^-/Å^3$.

The structure of the enzyme–adenosine 5′-diphosphoribose ADPR complex is very similar to that of the apoenzyme (PDB entry **8ADH**) and to that of the complex with ADPR determined at 2.9 Å at 278 K previously (PDB entry **5ADH**(46)) except for the conformation of the terminal ribose, which is disordered and has relatively high *B* values (Figure 2).The position of the adenosine 5′-diphosphate moiety is similar to that found in PDB entry **5ADH**, as well as in ternary complexes with NAD^+ or NADH, but because the protein conformation is open, there is no interaction of His-51 with the ribose and Arg-47 is not well-defined in density. The catalytic zinc is in the same location as in the apoenzyme, but the side chain of Glu-68 is not well-defined in density and has a *B* value higher than that of the protein backbone (~2-fold), as compared to other structures.

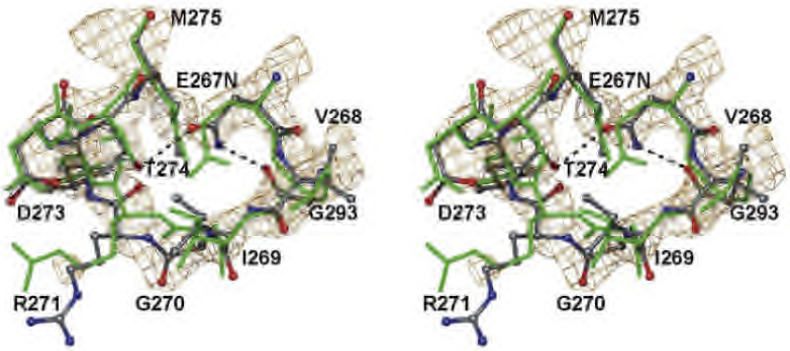
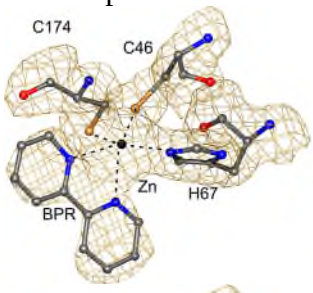


Figure 2. Complex of the apoenzyme with adenosine 5′-diphosphoribose (ADPR). The stereoview is derived from PDB entry **5VKR**. The $2|F_o| - |F_c|$ map is contoured at ~0.25 $e^-/Å^3$.

Structures for two complexes with the zinc chelators, 2,2′-bipyridine and 1,10-phenanthroline, were determined at 1.9 Å after the apoenzyme crystals had been soaked with the chelators (Figures 3 and 4). The structure with bipyridine was not determined before, whereas the structure with 1,10-phenanthroline was determined at 4.5 Å.(18)The tertiary structures of the subunits are very similar to those of the apoenzyme, except for the region between residues 294 and 299, which may be disordered as the electron density is not well-defined. The structure of the complex with bipyridine superimposes onto the apoenzyme structure (PDB entry **1YE3**) with an RMSD of 0.23 Å for α-carbons, and each of the two subunits in the asymmetric unit of the complex with 1,10-phenanthroline superimposes with an RMSD of 0.46 Å. The binding of the chelators is well-defined and indicates that both nitrogens of the chelators are bound to the zinc, and the pentacoordination inferred from spectroscopic studies is confirmed.



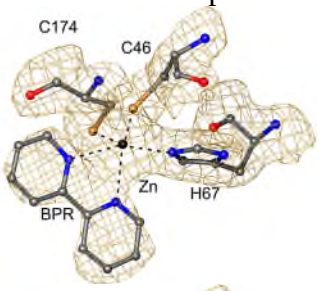
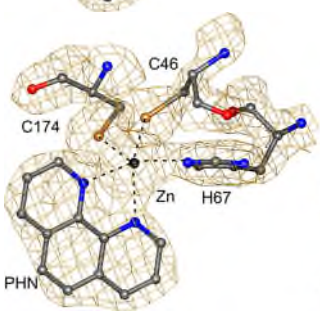


Figure 3. Complex of the apoenzyme with 2,2′-bipyridine (BPR). The stereoview is derived from PDB entry **5VJG**. The $2|F_o| - |F_c|$ map is contoured at ~0.25 $e^-/Å^3$.



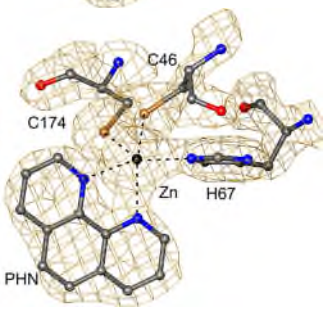


Figure 4. Complex of the apoenzyme with 1,10-phenanthroline (PHN). The stereoview is derived from PDB entry **5VJ5**. The $2|F_o| - |F_c|$ map is contoured at ~0.25 $e^-/Å^3$.

The structure for the enzyme–NADH complex has the dimeric molecule as the asymmetric unit, with two subunits that differ only slightly in conformation, with a water ligated to the catalytic zinc (Figure 5). The protein structure is essentially identical to the atomic-resolution structure of the wild-type enzyme complexed with NAD^+ and 2,3,4,5,6-pentafluorobenzyl alcohol PDB entry **4DWV**(8), as all α-carbons superimpose with an RMSD of 0.16 Å. The two subunits of the enzyme–NADH complex are very similar; the α-carbons of the coenzyme binding domain (residues 176–318) of subunit A superimpose onto subunit B with an RMSD of 0.08 Å, whereas α-carbons of all residues superimpose with an RMSD of 0.14 Å. Both subunits are in the closed

conformation and should be able to bind substrates and be enzymatically active. As discussed below, there appear to be two, alternative positions for the catalytic zinc, but the zinc in the major position has the classical tetrahedral coordination.

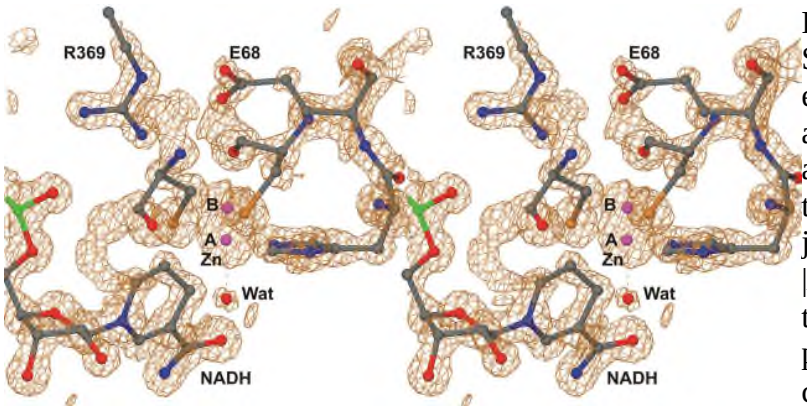


Figure 5. Complex of the holoenzyme with NADH. Stereoview of one active site, derived from PDB entry **4XD2**. Atom coloring is used, gray for carbon and magenta for the zincs in alternative positions, A and B. The zinc in the major position is coordinated to Cys-46, Cys-174, His-67, and a water, "Wat", just as in Figure 1 for the apoenzyme. The $2|F_o| - |F_c|$ map is contoured at ~1.0 $e^-/Å^3$. The graphic in the abstract shows another view of the alternative positions for the zincs, with the $2|F_o| - |F_c|$ map contoured at ~2.0 $e^-/Å^3$.

The structure for the enzyme–NADH–(*R*,*S*)-*N*-1-methylhexylformamide complex was determined at a resolution (1.25 Å) higher than that for the structure described previously with (*R*)-*N*-1-methylhexylformamide 1.57 Å, PDB entry **1P1R**(11) to detect potential water molecules near the catalytic zinc, but there is no significant electron density for such a water. All four subunits of the two homodimers in the asymmetric unit have the closed conformation, typical of ternary complexes with NAD(H) and substrate analogues. For this structure, the racemic *N*-1-methylhexylformamide (an aldehyde analogue) was used for crystallography, and it was expected that the *R* isomer would be bound because it has an affinity 1.7-fold higher than that of the *S* isomer.11 For the structure presented here, however, it appears that both isomers can bind because the electron density is fitted well by the *N*-isopropylformamide portion of the molecule, whereas the density for the C4-C7 tail is weak. Subunits B and D have predominantly the *S* isomer named NWH in the PDB file. Figure 6 illustrates the binding of the *S* isomer in subunit B in the electron density map and shows the binding of the *R* isomer named NMH in PDB entry **1P1R** in green, which is accommodated by alternative rotamers of the side chains of Leu116 and Ile318. The conformations of these residues are indicative of the binding of the two different isomers of *N*-1-methylhexylformamide. Such accommodations are also observed in binding different substrate analogues, such as trifluoroethanol or pentafluorobenzyl alcohol, and the isomers of 3-butylthiolane oxide.(8, 12) It is interesting that the structure of human ADH1C2 complexed with NADH and *N*-1-methylhexylformamide determined at 1.45 Å has the *R* isomer, even though the crystallization medium contained both isomers.(47) For this human ADH, the *R* isomer binds 7-fold more tightly than the *S* isomer.11 The catalytic zinc has one position, in the classical tetracoordination.

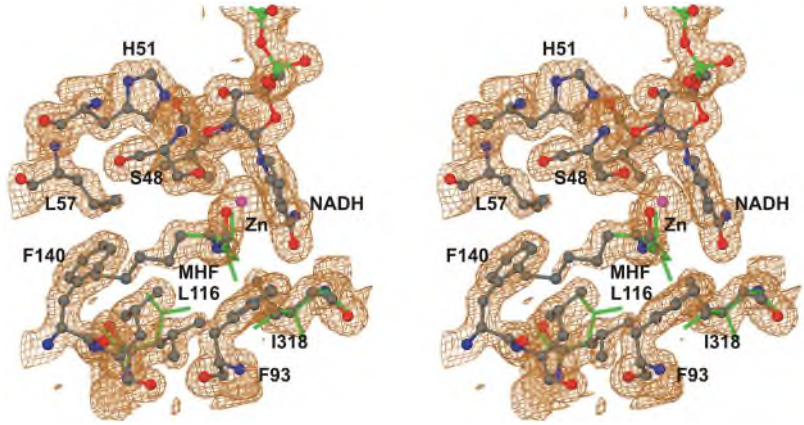
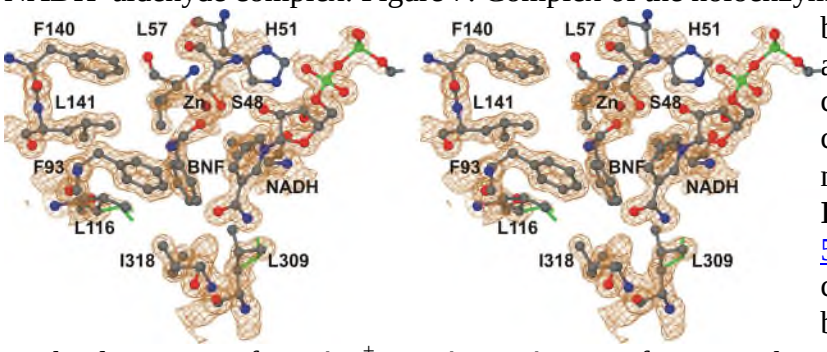


Figure 6. Complex of the holoenzyme with NADH and *N*-1-methylhexylformamide (MHF) based on PDB entry **5VN1**. Subunit B with (*S*)-*N*-1-methylhexylformamide is shown in atom coloring with the $2|F_o| - |F_c|$ electron density map contoured at ~0.35 $e^-/Å^3$. Represented as green sticks are (*R*)-*N*-1-methylhexylformamide and the alternative conformations of Leu116 and Ile318 found in the structure crystallized with (*R*)-*N*-1-methylhexylformamide PDB entry **1P1R**.

The structure of the complex with NADH and *N*-benzylformamide (an aldehyde analogue) has the closed conformation and is very similar to the atomic-resolution structures with NADH and *N*-1-methylhexylformamide PDB entry **1P1R** or NAD^+ and 2,3,4,5,6-pentafluorobenzyl alcohol PDB entry **4DWV** with an RMSD of 0.26 Å for the superposition of α-carbon atoms of one dimer 748 residues on either of the two molecules in the asymmetric unit. The side chain of Leu309 has alternative positions, reflecting differing energetics in the interaction with the benzylformamide, and Leu116 has alternative conformations in subunits A and C (Figure 7). The structures determined at 2.5 Å for the complexes with NADH and *N*-cyclohexylformamide PDB entry **1LDY** or *N*-formylpiperdine PDB entry **1LDE** also differ in the conformation of Leu116.(13) The structure of human ADH1B2 at 1.6 Å has the *N*-benzylformamide in a similar position in the active site, showing that the Thr/Ser48 exchange can be accommodated in either the human or horse enzymes without a large change in affinity for the inhibitor.(47, 51) The structure of horse ADH1E with NAD^+ and pentafluorobenzyl alcohol has alternative

positions for Leu-57 and Leu-309, but not Leu-116.(8) The formamides have the *cis* configuration, with the *re* face of the carbonyl group oriented favorably for direct transfer of a hydride from C4N of the reduced nicotinamide ring at an average distance of 3.48 Å for benzylformamide and 3.55 Å for methylhexylformamide. These atomic-resolution structures provide good analogues of the structure expected for the active enzyme–NADH–aldehyde complex. Figure 7. Complex of the holoenzyme with NADH and *N*-benzylformamide (BNF), based on PDB entry **5VL0**. Subunit A is shown in atom coloring, except for the alternative conformations of Leu-116 and Leu-309, which are colored green. The $2|F_o| - |F_c|$ electron density map is contoured at ~0.7 $e^-/Å^3$.



Identification of NADH in Structures Figures 5–7 show views of the binary and ternary complexes with NADH, which were determined by refinements with relaxed or no restraints on the dictionaries for NAD^+ or NADH. A major feature is the puckered and reduced nicotinamide ring of the NAD; i.e., it is NADH. The enzyme–NADH complex was produced by crystallizing the enzyme with NAD^+, but apparently the enzyme reduced the NAD^+ with 2-methyl-2,4-pentanediol to produce NADH. The diol is a poor substrate; however,(45) the crystals were kept at 5 °C for 1 year, and formation of NADH would be favored as the solution contained excess diol and minimal concentrations of carbonyl compounds or inhibitors. Crystals of the F93A enzyme prepared in the presence of NAD^+, 2,3,4,5,6-pentafluorobenzyl alcohol, and methylpentanediol also contained NADH, apparently because the fluoro alcohol binds relatively weakly while NADH binds tightly PDB entry **1MGO**.(59) Spectroscopic studies show that the enzyme−NAD^+ complex reacts in solution with the diol to form NADH, as do crystals of the ternary enzyme–NAD^+–2,2,2-trifluoroethanol complex that are washed with buffer containing methylpentanediol, which replaces the inhibitory 2,2,2-trifluoroethanol.(60, 61)

The puckering geometry of the nicotinamide rings for structures of ADH complexed with NADH or NADH and formamides, or NAD^+ and fluoro alcohols, is presented in Table 2.(8, 62) The nicotinamide rings have essentially a boat conformation, with C4N directed toward the substrate binding site. Other atomic-resolution (1.0–1.2 Å) structures of the enzyme crystallized with NADH have very similar geometry, but the structures with methylpentanediol, dimethyl sulfoxide, or isobutyramide apparently have partial occupancy of water molecules in close contact (1.7–2.0 Å) with C6N of the nicotinamide ring.(22, 23) There is no clear evidence in the electron density maps of water molecules interacting with the nicotinamide ring in our new structures with NADH alone or with NADH and the aldehyde analogues, *N*-1-methylhexylformamide or *N*-benzylformamide. The nicotinamide ring can be even more puckered, as the enzyme–NAD–pyrazole complexes (PDB entries **1N92** and **1N8K**, with a partial covalent bond of 1.7 Å between pyrazole N2 and nicotinamide C4N) have α-C4 = 21° and α-N1 = 14°.(63) In contrast, in the atomic-resolution (at 1.12 Å) structures with NAD^+ and fluoro alcohols, the nicotinamide rings are only slightly puckered and apparently strained.(8) The puckered nicotinamde rings in the enzyme complexes with NAD^+ and NADH and substrate analogues may represent ground states that could facilitate catalysis of hydride transfer, as nicotinamide C4N moves closer to the reactive carbon in the substrate.(8, 64-67)

Table 2. Nicotinamide Ring Puckering in Complexes of Horse Liver ADH[a]

ligands	PDB entries	α-C4 (deg)	α-N1 (deg)	twist (Å)
NADH,H_2O[b]	**4XD2**	16.0 ± 1.6	12.6±2.2	-0.26±0.03
NADH, MHF[c]	**1P1R**, **5VN1**	18.1 ± 4.2	11.2±2.3	-0.04±0.12
NADH,BNF[d]	**5VL0**	12.5 ± 3.9	13.1±3.2	0.07±0.07
NADH,various[e]	**1HET**, **2JHG**	12.3 ± 2.5	11.0±4.5	-0.31±0.19
NAD^+,FALC[f]	**4DXH**,**4DWV**	4.9 ± 2.3	5.8±2.1	0.06±0.03

a The structures were refined with relaxed or no restraints on the nicotinamide ring. Ring puckering geometry is defined as follows: α-C4, angle between the C3–C4–C5 and C2–C3–C6 planes; α-N1, angle between the C2–N1–C6 and C2–C3–C6 planes; twist, distortion of the boat conformation, defined as the distance between C5 and the C2–C3–C6 plane.(22)

b Weighted average from two subunits as calculated with SHELXL-2013 with no restraints on the nicotinamide ring.

c Average of eight subunits of the enzyme complexed with NADH and *N*-1-methylhexylformamide, an aldehyde analogue and potent inhibitor,(11) calculated with SHELXL-2013 after refinement with REFMAC.

d Average of four subunits of the enzyme complexed with NADH and *N*-benzylformamide calculated with SHELXL-2013 after refinement with REFMAC.

e Average of eight subunits from four structures of wild-type ADH with Zn(II) or Cd(II) at the active sites, complexed with NADH and other ligands, including water, methylpentanediol, dimethyl sulfoxide, or isobutyramide (PDB entries **1HEU**, **2JHF**, **1HET**, and **2JHG**).(22, 23)

f Weighted average of four subunits of the enzyme complexed with NAD^+ and 2,3,4,5,6-pentafluorobenzyl alcohol or 2,2,2-trifluoroethanol calculated with SHELXL-2013 with no restraints on the nicotinamide ring.(62)

The bond distances in the nicotinamide ring (Table 3) are also consistent with the identification of the coenzyme as NADH in the enzyme–NADH and enzyme–NADH–formamide complexes. In particular, the C3N–

C4N and C4N–C5N bonds are longer than the bonds in the complexes with NAD^+. The distances in these complexes also agree with those determined for other structures with NADH.(22, 23) Comparison of bond distances for nicotinamide rings in enzyme complexes is problematic because of different dictionaries for crystallographic refinement procedures, uncertainty about the NADH/NAD^+ ratio in the complex, and the potential effects of binding of the coenzyme and ligands to the enzyme.(8) For the enzyme–NADH structure, the bond distances calculated with SHELXL-2013 with no restraints on the nicotinamide ring for a model of either NAD^+ or NADH in the complex agree within the stated errors, but the distances from refinement with REFMAC5 using the dictionary for NAD^+ with relaxed restraints are somewhat closer to the average of those found in five structures with NADPH or *N*-benzyl-1,4-dihydronicotinamide (see Table 4 of ref 8) We conclude that atomic-resolution crystallography can distinguish NAD^+ from NADH.

Table 3. Bond Distances (angstroms) in the Nicotinamide Rings of NADH or NAD^+ in ADH Complexes

complex	N1–C2	C2–C3	C3–C4	C4–C5	C5–C6	C6–N1
NADH,H_2O[a]	1.36 ±0.021	35 ±0.021	50 ±0.021	43 ±0.021	44 ±0.021	35±0.02
NADH,MHF[b]	1.40 ±0.021	36 ±0.031	49 ±0.051	48 ±0.021	33 ±0.031	42±0.06
NADH,BNF[c]	1.36 ±0.021	35 ±0.021	50 ±0.031	42 ±0.041	36 ±0.051	42±0.02
NADH,various[d]	1.40 ±0.031	35 ±0.021	48 ±0.051	47 ±0.031	41 ±0.041	40±0.03
NAD^+,FALC[e]	1.35 ±0.011	36 ±0.011	42 ±0.011	38 ±0.011	37 ±0.011	39±0.01

a Weighted average of the bond distances and errors for two subunits from refinement with SHELXL-2013 with no restraints on distances or planarity of the nicotinamide ring (PDB entry 4XD2).
b Two structures (1.25 and 1.57 Å resolution) with *N*-1-methylhexylformamide, with an average of eight subunits and the standard deviation from refinement with REFMAC (PDB entries 1P1R and 5VN1).
c Average of four subunits of the structure with NADH and *N*-benzylformamide (PDB entry 5VL0) after restrained refinement with REFMAC and calculation with SHELXL-2103.
d Four structures (1.0–1.2 Å resolution) with an average of eight subunits and the standard deviation in complexes with NADH and various ligands (PDB entries 1HET, 1HEU, 2JHG, and 1JHF) refined with relaxed restraints on the nicotinamide ring.(22, 23)
eWeighted average for four subunits with SHELXL-2013 refinement with no restraints on the nicotinamide ring in complexes with NAD^+ and fluoro alcohols (PDB entries 4DWV and 4DXH).(62)

Catalytic Zinc Coordination For all of the structures described here, except for the enzyme–NADH complex, the electron densities around the catalytic and structural zincs are essentially spherical. The catalytic zinc has tetrahedral coordination (e.g., see Figure 1), but the structures with the two chelators, 2,2′-bipyridine and 1,10-phenanthroline, are pentacoordinated with the two nitrogens binding on adjacent positions of the zinc. The ligation distances listed in Table 4 agree with those found in high-resolution X-ray structures,(68) except that the distances from Cys-46 SG and His-67 NE2 to the zinc in the chelators are somewhat longer than in the other structures and that the zinc–nitrogen distances are also relatively long, suggesting somewhat looser interactions in the pentacoordinated complexes. Three structures have a water ligated to the zinc, where a distance of 2.1–2.3 Å would be consistent with a neutral water. A p*K* value of 9.2, determined from the pH dependence for binding of NAD^+, 2,2′-bipyridine, or 1,10-phenanthroline to the apoenzyme has been attributed to the zinc-bound water.(20, 69-71) The p*K* value is apparently shifted to 7.6 in the enzyme–NAD^+ complex and to 11.2 in the enzyme–NADH complex.(72) The ternary complexes with NADH and *N*-1-methylhexylformamide or *N*-benzylformamide have a carbonyl oxygen ligated to the zinc (Zn–O distance of 2.1 Å), whereas the previously reported structures with NAD^+ and a fluoro alcohol probably have an alcoholate O bound to the zinc, with a Zn–O distance of 2.0 Å.(8) Solvent deuterium isotope effects suggest that the alcohol is deprotonated and participates in a low-barrier hydrogen bond (2.5 Å) to Ser-48 OG.(1, 8) There is no evidence of an additional water near the zinc in these complexes.

Table 4. Coordination Distances (angstroms) from Ligands to Catalytic Zinc in ADH Complexes

E complex	PDB	C46 SG	H67 NE2	C174 SG	ligand O	N1	N8 or N10
apoenzyme, H_2O	1YE3	2.42	2.05	2.21	2.11	–	–
ADPR, H_2O	5VKR	2.28	2.10	2.34	2.35	–	–
2,2′-bipyridine	5VJG	2.42	2.27	2.18	–	2.48	2.60
1,10phenanthroline[a]	5VJ5	2.46	2.30	2.20	–	2.26	2.44
NADH, H_2O[b] “A”	4XD2	2.31	2.06	2.24	2.08	–	–
“B”		2.18	2.24	2.53	(3.35)	–	–
NADH, MHF[c]	1P1R,5VN1	2.29	2.05	2.24	2.16	–	–
NADH, BNF[d]	5VL0	2.31	2.02	2.24	2.09	–	–
NAD^+, FALC[e]	4DWV,4DXH	2.33	2.03	2.28	1.96	–	–

a Distances were averaged for two subunits; the average deviation is 0.025 Å.
b Distances were averaged for two subunits. For the major A position, the average deviation is 0.045 Å and the distance to Glu-68 OE1 is 4.8 Å. For the minor B position, the average deviation is 0.11 Å and the distance to Glu-68 OE1 is 3.7 Å. The alternative zinc position is
c Distances were averaged for eight subunits for structures complexed with NADH and *N*-1-methylhexylformamide; the average standard deviation is 0.021 Å.
d Average for four subunits of the enzyme complexed with NADH and *N*-benzylformamide, with an average standard deviation of 0.015.

closer to Cys-46 SG by ~0.1 Å than is the major position, whereas the alternative position is farther from His-67 NE2 or Cys-174 SG by 0.2–0.3 Å than is the major position. Some movement of the ligands could alter these distances, but refinements could not define alternative positions for the ligands.

e Distances were calculated with SHELXL-2013 refinement with data to 1.1 Å, and weighted averages for four subunits are reported, with an average standard deviation of 0.005 Å.

Of particular interest is the fact that the electron density for the catalytic zinc in the enzyme–NADH binary complex is a prolate ellipsoid (or ovoid), rather than a sphere as for the catalytic and structural zincs in the other structures. Anomalous scattering difference maps show that the density is due to zincs, and not, for instance, a water (see the Supporting Information). We placed two zincs, separated by ~1.3 Å, to illustrate the potential alternative coordination. The major position (80%) is located in the classical coordination with Cys-46 SG, His-67 NE2, Cys-174 SG, and a water molecule, as found in the apoenzyme (Figure 1, PDB entry 1YE3). The alternative zinc position is closer to Glu-68 OE2 (at 3.7 Å), as compared to the distance in the major position (4.8 Å), but the protein ligands were not shifted in the model because the electron density did not justify alternative positions (Table 4). Elongated densities were also observed for horse liver ADH with Cd(II) substituted for Zn(II) in a complex with NADH and 2-methyl-2,4-pentanediol (see Figure 5a of ref 23) and for human ADH3 in complex with NADH.(35, 36) However, in those structures, Glu-68 (or Glu-67 in human ADH3) can also adopt an alternative conformation that brings OE2 into ligation with the alternative position of the metal. In the structure presented here, there is not sufficient electron density to place Glu-68 in an alternative position. Nevertheless, the structure suggests some flexibility in the zinc position, which can be relevant for the mechanism by which the water is replaced with alcohol or aldehyde in the enzyme–coenzyme complexes.

Discussion With the new results on the alternative positions of the catalytic zinc in the ADH–NADH complex, combined with higher-resolution structures of other enzyme complexes and extensive, previously published results, we can address controversial proposals about the role of water and zinc coordination in catalysis and suggest a mechanism that is most consistent with the results.

Does a Water Adducted to the Nicotinamide Ring Participate in Hydrogen Transfer? Meijers and co-workers(22) suggested that a water (as hydroxide) bound to the active site zinc and to C6N of the nicotinamide ring could activate NADH for the enzymatic reaction, but the proposed mechanism does not seem to be chemically necessary, reasonable, or supported by most structural studies. Although their computations support the conclusion that the NADH–hydroxide "adduct" in the X-ray structure is 6-hydroxy-1,4-dihydronicotinamide, it is not clear what this adduct is and how it was formed. The distance between the water oxygen and C6N is between 1.7 and 2.0 Å in six subunits of three different structures (PDB entries 1HET, 1HEU, and 2JHF) and thus is not typical of a C–O bond. They proposed that the water ligated to the catalytic zinc and directed by the hydroxyl group of Ser-48 could react with either the reduced or oxidized nicotinamide ring, but they did not explain why the redox state did not change or how the C6N hydrogen was displaced.

The origin of the electron density is not clear in the structures determined by Meijers and co-workers.(22, 23) Hydration at C6N of the reduced nicotinamide ring occurs at low pH to form 6-hydroxy-1,4,5,6-tetrahydronicotinamide.(73, 74) Glyceraldehyde-3-phosphate dehydrogenase can also catalyze formation of this adduct with NADH, apparently assisted by bound anions.(75) The oxidized nicotinamide ring of NAD^+ can be attacked under basic conditions by various nucleophiles at the C2N, C4N, and C6N atoms,(74) but the source of NAD^+ in the ADH structures is not clear. It may be difficult to assign waters in specific structures because of the presence of alternative positions and occupancies.

In the structure of the enzyme–NADH complex presented here, the water bound to the zinc is 3.40 Å from C6N (3.25 Å to C4N or C5N), and there is no significant electron density for additional atoms attached to the nicotinamide ring. It is not obvious why our studies differ from those of Meijers and co-workers.(22) We crystallized the enzyme with NAD^+ (which was apparently reduced to NADH by the precipitant, methylpentanediol) at pH 7.0, where addition of hydroxide to the nicotinamide ring would be minimized, whereas Meijers and co-workers crystallized the enzyme with NADH with polyethylene glycol 400 and methylpentanediol at pH 8.2, where acid-catalyzed addition of water would be slow.

We suggest that none of the structural evidence supports a catalytic mechanism for the dehydrogenase that involves a covalent NADH–hydroxide adduct. Structures for complexes of ADH with NADH and aldehyde analogues, e.g., with formamides (Figures 6 and 7) or sulfoxides, did not locate such a water.(10-13) Atomic-resolution structures of ternary complexes with NAD^+ and fluoro alcohols also show no evidence of a water near the zinc or the nicotinamide ring.(8) Crystallography could in principle locate a water, as one was found at 3.1 Å from C6N of the NAD^+ in the V203A ADH complex where a cavity is formed by the substitution of Val-203.(62) Computations apparently adequately describe the ADH reaction without invoking a water linked to the coenzyme.(65, 76-78)

Does a Water Bound to Zinc Participate in Proton Transfer in the Central Complex?

Several studies have proposed that a water bound to the zinc, either with the substrate in the second ligation sphere or with both the water and substrate oxygens bound to a pentacoordinated zinc, participates in acid/base catalysis in the ternary complex. Spectroscopic and kinetic studies in which the catalytic zinc was replaced with Co(II) and Cd(II) in horse liver ADH provide information about the metal coordination. An X-ray structure shows that Co(II) can replace the Zn(II) with minimal changes in the coordination of the metal.(79) NMR studies using the paramagnetic effect of Co(II) on the relaxation of the hydrogens of ligands suggested that a water (or hydroxide) binds to the metal whereas substrates and inhibitors bind at distances that are too long to allow direct coordination of the oxygen with the metal, and it was proposed that hydroxide on a tetracoordinated zinc acted as the base for alcohol oxidation.(80) However, a low-resolution X-ray crystallography study suggested that substrates and inhibitors bind directly to the zinc in enzyme–coenzyme complexes that resemble Michaelis complexes.(45) Spectra showed that *trans*-4-(*N*,*N*-dimethylamino)cinnamaldehyde binds to the Co(II) in the complex with 1,4,5,6-tetrahydronicotinamide adenine dinucleotide.(81, 82) The NMR study was extended to ternary complexes with NADH and dimethyl sulfoxide or NAD^+ and 2,2,2-trifluoroethanol, and again it was concluded that the oxygens of the ligands were not directly ligated to the metal.(83) However, other studies concluded that the paramagnetic effects of Co(II) on the relaxation of water or alcohol protons were too small to give reliable determinations of the distance of the protons from the metal.(84, 85) After higher-resolution X-ray studies clearly showed that the oxygens of the ligands are directly ligated to a tetracoordinated zinc and that there is no water near the zinc, we proposed that the oxygen of the substrate is bound to the zinc, and the proton is transferred to or from the substrate through a hydrogen-bonded system, including the hydroxyl group of Ser-48 and the imidazole group of His-51.(21)

EPR measurements of the Co(II) enzyme showed that the zero-field splitting energies were increased in enzyme–coenzyme complexes, including complexes with NAD^+, NADH, NAD^+–pyrazole, and NADH–trifluoroethanol, and suggested a pentacoordinated metal.(24, 25) However, our study shows that the enzyme–NADH complex has a closed conformation with a tetracoordinated zinc, and it is likely that the enzyme–NAD^+ complex was reduced by contaminating alcohols to form the enzyme–NADH complex.(61) Furthermore, the enzyme–NAD^+–pyrazole complex has a closed conformation with a tetracoordinated zinc.(63) Subsequent EPR and magnetic circular dichroism studies suggested that changes in zero-field splitting reflect a highly distorted tetrahedral coordination related to the conformational change in the protein when coenzymes bind.(26) As shown in Table 4, however, the catalytic zincs in the apoenzyme and enzyme–coenzyme complexes have essentially the same coordination of the ligands, even if the environments differ because of the conformational change and the replacement of the water.

The Co(II) enzyme is about as active as the zinc enzyme; however, the p*K* values for the oxidation of benzyl alcohol (k_{cat} and k_{cat}/K_m) are shifted somewhat as compared to the zinc enzyme, and it was concluded that a neutral water ligated to a pentacoordinated metal acted as the base to deprotonate the bound alcohol.(25, 86) However, these kinetic constants are controlled by dissociation of NADH and reaction of the enzyme–NAD^+ complex, respectively, in the ordered mechanism and do not report on the p*K* values in the central complex.(87) The transient oxidation of alcohols (ethanol, 1-propanol, and benzyl alcohol) shows that the hydride transfer rate is most rapid when a group with a p*K* value of ∼6.4 is deprotonated, which can be attributed to the ionization of the alcohol bound to the zinc mediated by the proton relay system.(1, 9, 71, 82, 88-90) The observed rate constant for the transient oxidation of ethanol is decreased to 66% with the substitution of the zinc with Co(II).(91)

The catalytic zinc can also be replaced with Cd(II) to form an enzyme with ∼14% of the maximal turnover activity of the zinc enzyme. NMR studies of the enzyme substituted with ^{113}Cd(II) showed that the resonances are shifted differently in binary complexes with NAD(H) or ternary complexes with NAD^+, but the chemical shift was the same in the complexes with NAD^+ and trifluoroethanol or pyrazole, leading to the conclusion that the substrate analogues did not bind directly to the metal because binding of oxygen or nitrogen atoms should cause different shifts.(92) However, the structures of the zinc enzyme with NAD^+ and either pyrazole or trifluoroethanol determined by high-resolution X-ray crystallography show that the ligands are bound to tetracoordinated zinc.(8, 63) Perturbed angular correlation (PAC) spectroscopy of the enzyme substituted with ^{111}Cd(II) suggested that the apoenzyme and the complex with NAD(H) were tetrahedrally coordinated, but the complexes with NAD^+ and either pyrazole or trifluoroethanol were thought to be pentacoordinated with a water and the ligand bound to the metal.(27, 85) However, X-ray crystallography shows that the apoenzyme and ternary complexes with NAD(H) have the same structures as the zinc enzyme, all tetracoordinated with a water or the oxygen of a ligand binding directly to the metal.(23, 93) A more extensive PAC study of the Cd(II) enzyme with better instrumentation suggested that the geometry is tetracoordinated in the apoenzyme and in complexes with NAD^+, NADH, and NADH–dimethyl sulfoxide, but the apoenzyme and the enzyme–NAD^+ complex have two different pH-dependent

forms that may result when a water bound to the metal ionizes and the Cys46 S–metal–Cys174 S bond angle changes. The enzyme–NADH complex has two pH-independent forms that have different coordination geometries.(28) A subsequent study suggested that the enzyme–NADH–imidazole complex also has two different forms even though X-ray crystallography shows that this complex crystallizes in an open conformation.(29) Some of these PAC results were reanalyzed by optimizing structures of the complexes with a combined quantum chemical and molecular mechanical program (starting with known X-ray structures) and calculating the electric field gradients.(30) It was concluded that all complexes had tetracoordinated Cd(II), but the two spectral forms could be due to different charges of the ligands on the zinc. The apoenzyme could have neutral water or hydroxide; the enzyme–NAD^+ complex could have hydroxide at high pH or one oxygen of the carboxyl group of Glu-68 at low pH, and the enzyme–NADH complex could have either water or the carboxyl group of Glu-68 ligated to the zinc. It was suggested that ∼40% of the Cd(II) was ligated to Glu-68, whereas the percentage should be lower for the Zn(II) enzyme because of the relative sizes of the metals. It is significant that an atomic-resolution structure of the Cd(II) enzyme complexed with NADH shows that the electron density for the Cd(II) is an ellipsoid extending toward Glu-68 and that there is an alternative position for Glu-68 that brings the shortest distance to the metal to 3.0 Å.(23) When dimethyl sulfoxide binds to the metal in this complex with NADH, Glu-68 reverts to the position found in the corresponding zinc enzyme. Additional calculations “quite unexpectedly” suggested that a strained pentacoordinated Cd(II) with water and Glu-68 on opposing sides of the metal could form, but such a species was not obtained with the zinc enzyme.(44) The proposed alternative coordination in the Cd(II)–enzyme–NADH complex is relevant because it appears in this study that the catalytic zinc can move closer to Glu-68. Such a complex could represent a transient in the exchange of water with the substrate, as shown in Scheme 2.

Although the spectroscopic studies suggest that the coordination of the catalytic zinc changes in various complexes, they do not provide strong support for a stable pentacoordinated zinc in the apoenzyme or ternary complexes. We think the X-ray structures that show the catalytic zincs in the apoenzyme and the ternary complexes are tetracoordinated are the definitive evidence of zinc coordination for these forms of the enzyme. Nevertheless, binary enzyme–coenzyme complexes and transient species may have pentacoordinated zinc or the alternative coordination with Glu-68.

Do the Catalytic Zincs in ADHs Become Pentacoordinated?

The results in Figures 3 and 4 and Table 4 show that the catalytic zincs are pentacoordinated in the complexes with 2,2′-bipyridine and 1,10-phenanthroline, with some alteration of the bond distances and angles around the zincs as compared to those around the tetracoordinated zincs. The kinetics of binding of these zinc chelators are relevant. 2,2′-Bipyridine binds most rapidly to horse liver ADH at low pH and is slower at pH values above a p*K* value of 9.2, which can reflect ionization of the zinc-bound water.(20, 94) Equilibrium binding of 2,2′-bipyridine and 1,10-phenanthroline is also tighter below the p*K* of 9.2, apparently because water binds more weakly than hydroxide in the competitive replacement by the chelators. (Our analysis of the binding data for 2,2′-bipyridine and 1,10-phenantroline suggests that at high pH the limiting rate constants for binding are ∼10-fold slower and the equilibrium binding constants are ∼10-fold weaker than at low pH, indicating that formation of zinc hydroxide does not prevent the binding.) In the presence of imidazole, which displaces the water from the zinc,(18, 95) the pH dependence for equilibrium binding of bipyridine is abolished. Chelators bind with rapid association followed by a slower limiting isomerization of ∼200 s^{-1} at high concentrations of the chelator.(19, 20, 94, 96) The limiting rate for bipyridine binding is pH-independent, as is the rate constant for dissociation of the complex, and imidazole abolishes the limiting rate.(20) Water is displaced from the zinc in the enzyme–bipyridine complex (Figure 3), but the mechanism of exchange is not clear; for hydrated metal ions, it involves rapid dissociation of water and rapid chelation.(97) For the zinc in ADH, the limiting kinetics could be due to slow dissociation of water (or hydroxide) and reaction of a tricoordinated zinc, reorganization from a tetracoordinated to a pentacoordinated zinc, or double displacement involving Glu-68 with inversion of a tetracoordinated zinc. In any case, a pentacoordinated zinc can form, and some step in the enzyme mechanism could involve both a water and a substrate oxygen bound adjacently to zinc.

ADHs can slowly oxidize aldehydes to the carboxylic acids,(61, 98) and one or both oxygens of the hydrated aldehyde substrate or the carboxylate product might bind to the zinc. Both monodentate ligation and bidentate ligation are observed for zinc ions in protein structures.(68) However, a structure for *Aeropyrum pernix* ADH (PDB entry **1H2B**) with NAD(H) has just one oxygen of octanoate bound to the zinc.(99) Human ADH3 complexed with NAD (PDB entry **1MAO**) paradoxically binds the methyl group of dodecanoate close to the zinc that is coordinated by Cys44, His-66, Glu67, and Cys173.(34) Perhaps both oxygens of a carboxyl group cannot easily bind to the zinc in ADHs.

The catalytic zinc in human sorbitol dehydrogenase forms a pentacoordinated complex with an inhibitor that mimics the vicinal 1,2-hydroxyl groups of the substrate and ligation to His69 and Cys44 from the protein and a water that is proposed to be the proximal proton donor and/or acceptor in the reaction at C2 of sorbitol PDB entry **1PL6**.(100) The inhibitor approximates an isosteric analogue of 2,2′-bipyridine by having the oxygen and a nitrogen, linked by two carbons, ligated to the zinc. The structure may resemble the reactive ternary complex for this ADH. It is notable that the binding of the inhibitor displaces the carboxyl group of Glu-67 that is ligated to the opposite side of the zinc in the enzyme–NAD^+ complex.

X-ray absorption spectroscopy (XAS) data on the tetrameric ADH from *Thermoanaerobacter brockii* led to the suggestion that binary complexes with $NADP^+$ or NADPH have zinc tetracoordinated with Cys-37, His-59, and oxygen atoms from Glu-60 and Asp-150, whereas the ternary complexes with coenzymes and dimethyl sulfoxide have the coordination expanded to five or six ligands with the addition of oxygen atoms.(32) Model building with an X-ray structure of an enzyme–coenzyme complex suggested that a trigonal bipyramidal coordination could represent a species poised for hydrogen transfer. The XAS studies were extended by studying the transient phase for oxidation of 2-propanol with $NADP^+$ where the coordination number apparently increased to five or six.(31) A mechanism was proposed in which the carboxylate of Glu-60 was replaced stepwise with a water and the substrate oxygen before hydride transfer, to explain two transient intermediates. The results with this enzyme are interesting, but they are not convincing because of the uncertainties in interpreting XAS data, the lack of data in the steady state phase where turnover of central complexes could be observed, the heterogeneity of zinc ligands in the structures of the enzyme–NAD(H) structures (PDB entries **1PL6** and **1YKF**), and the lack of an X-ray structure of a relevant ternary complex. A role for a mobile Glu-60 (homologous to Glu-68 in horse ADH) is intriguing, but the substitution of Glu-60 with an alanine or aspartic acid residue in the *T. brockii* enzyme decreases activity by only ~5-fold.(101) The mechanism with this enzyme may be different from that of the horse or yeast ADHs because the protein ligands to the zinc are different.

How Is the Water Bound to the Zinc Replaced by Substrate?

The catalytic zincs in the horse ADH apoenzyme and holoenzyme complexes with coenzyme and substrate analogues have similar tetracoordinated geometry (e.g., Figures 1, 6, and 7). Transient kinetic studies suggest that the zinc-bound water in the enzyme–NAD^+ complex deprotonates to form zinc hydroxide, with a rate constant of 200–500 s^{-1}, which is coupled to the global protein conformational change and occurs before ligands bind to the zinc to form the ternary complexes.(2) An associative mechanism involving a pentacoordinated zinc with adjacent oxygens is theoretically possible. However, it seems more reasonable that Glu-68 OE2 participates in a double-displacement, interchange mechanism, via a trigonal bipyramidal geometry, to displace the water (or hydroxide) in the first step, and in the second step, the substrate oxygen displaces the Glu-68 and produces the structure observed in the ternary complexes (Scheme 2). The water would dissociate from the enzyme through the hydrophobic substrate binding barrel (illustrated in Figures 6 and 7), and then the substrate would enter the channel and bind to the zinc. Structural studies with yeast ADH, other tetrameric ADHs, and human ADH3, as well as computational studies with horse ADH, support such a mechanism.(2, 3, 33-36, 39, 40, 44) In particular, the structure of the horse ADH–NADH complex (Figure 5) shows an alternative position for the catalytic zinc that is closer to ligating to Glu-68 OE2. This evidence of an inverted coordination in horse ADH is similar to that observed for the complex of human ADH3 complexed with NAD(H) or ADP-ribose where a loose trigonal bipyramidal coordination or alternative positions were modeled for the zinc.(35, 36) Ligand coordination may change more readily in the binary enzyme–coenzyme complexes, as discussed previously for human ADH3 and yeast ADH.(36, 39) Comparison of the structures of the apoenzyme (PDB entry 1YE3) and the enzyme–NADH complex PDB entry **4XD2** supports the suggestion that binding of the coenzyme alters the energetics of the interaction of Glu-68 with Arg-47, because Arg-47 binds the pyrophosphate of the coenzyme (Figure 5), and Glu-68 could then more readily interact with the catalytic zinc.(35, 36) In the structure of the enzyme–NADH complex presented here, the alternative position for the zinc has not moved sufficiently closer to Glu-68 to become coordinated in the inner sphere to OE2, and alternative conformations of the side chains of the other ligands (Cys-46, His-67, and Cys-174) could not be refined, but the elongated electron density for the catalytic zinc indicates the potential dynamics. It appears that a consequence of the conformational change accompanying coenzyme binding is that the zinc becomes more mobile, and it is reasonable that similar effects would occur upon binding of NAD^+. After the second substrate (alcohol or aldehyde) binds to the enzyme–coenzyme complex, the zinc position is restored to that found in various ternary complexes, which seems appropriate for catalysis of the hydride transfer.

The molecular dynamics and combined quantum chemical and molecular mechanical computational studies with horse ADH support the conclusions that only tetracoordinated Zn(II) species are stable, where Glu-68 and water are coordinated alternatively.(44, 102) These computational studies suggest that the enzyme complexes with

NAD^+ or NADH may differ somewhat in the propensity of Glu-68 to ligate the zinc and can be affected by the ionization of the zinc-bound water to form hydroxide, but further studies are required to characterize the dynamics of the exchanges in the apoenzyme and enzyme–coenzyme complexes.

Substitution of the homologous glutamate in yeast ADH (Glu-67) with a glutamine residue decreases the catalytic efficiency for both ethanol oxidation and acetaldehyde reduction by 100-fold.(43) Yeast ADH in the open, apoenzyme conformation (with or without the bound coenzyme) has the glutamate ligated to the zinc, while the ternary complex with NAD^+ and trifluoroethanol has the closed conformation and the classical zinc coordination.(39, 40) Other ADHs also have the homologous glutamate residue ligated to the catalytic zinc in the apoenzyme and some complexes with coenzyme, whereas the ternary complexes with the coenzyme and a substrate analogue have the classical zinc coordination (see the Supporting Information, Table 6S, of ref 39) The differences in coordination in the various apoenzymes could be of crystallization, but we think that the different structures represent energetically accessible states that are relevant for the catalytic mechanism. There may be different mechanisms for ligand exchange in these ADHs, but a common mechanism for these related enzymes is more reasonable.

The exchange of an alcohol via an intermediate coordination with Glu-68 may involve several steps. Glu-68 might displace the zinc hydroxide in the enzyme–NAD^+ complex, or it might be kinetically more rapid to reprotonate the hydroxide through the proton relay system so that a neutral water dissociates.(21) Then, after an alcohol binds and displaces Glu-68, the hydroxyl group of the alcohol would be deprotonated again to facilitate the transfer of hydride from the alkoxide.(1) These steps may involve local conformational changes.(3) We do not think that a pentacoordinated intermediate, with both a water and the substrate bound, is likely because of the limited space to move a water from the zinc to the bulk solvent through the substrate binding site after alcohol is bound, and such complexes were not identified by the computations of Ryde.(44, 103) Release of aldehyde product and replacement with water may also involve intermediate ligation with Glu-68. We note that binding of alcohols and aldehydes to the enzyme–coenzyme complexes (10^5–10^6 M^{-1} s^{-1}) is somewhat slower than diffusion and dissociation of these ligands from the ternary complexes is relatively slow (20–560 s^{-1}), so that various species may isomerize.(1) The mechanism still needs to be studied further to describe the structures of additional intermediate species and the rate constants for their interconversion; new techniques might demonstrate the transient ligation of Glu-67 to the catalytic zinc during the exchange of ligands.

Supporting Information The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.7b00446.

Electron density ($2|F_o| - |F_c|$) and anomalous scattering difference ($|F_o| - |F_c|$) maps for the catalytic and structural zincs in the enzyme–NADH complex (PDF)

Accession Codes

The X-ray coordinates and structure factors have been deposited in the Protein Data Bank as entries 1YE3, 4XD2, 5VJ5, 5VJG, 5VKR, 5VL0, and 5VN1.

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Abbreviations: ADH-alcohol dehydrogenase;ADPR-adenosine 5′-diphosphoribose; MPD-2-methyl-2,4-methylpentanediol;RMSD-root-mean-square deviation;XAS-X-ray absorption spectroscopy.

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The binding of NAD to wild-type horse liver alc. dehydrogenase (ADH) is strongly pH-dependent and is limited by a unimol. step, which may be related to a conformational change of the enzyme-NAD complex. Here, deprotonation during the binding of NAD and inhibitors that trap the enzyme-NAD complex was examd. by transient kinetics with pH indicators, and the formation of complexes was monitored by absorbance and protein fluorescence. Reactions with pyrazole and trifluoroethanol had biphasic proton release, whereas reaction with caprate showed proton release followed by proton uptake. Proton release (200-550 s-1) was a common step that preceded the binding of all inhibitors. At all pH values studied, the rate consts. for proton release or uptake matched those for the formation of ternary complexes, and the most significant quenching of protein fluorescence (or perturbation of adenine absorbance at 280 nm) was obsd. for enzyme species involved in the deprotonation steps. Kinetic simulations of the combined transient data for the multiple signals indicated that all inhibitors bound faster and tighter to the unprotonated enzyme-NAD complex, which had a pK of ∼7.3. The results suggested that rate-limiting deprotonation of the enzyme-NAD complex is coupled to the conformational change and controls the formation of ternary complexes.>> More from SciFinder ®
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A review. As shown by x-ray crystallog., horse liver alc. dehydrogenase undergoes a global conformational change upon binding of NAD or NADH, involving a rotation of the catalytic domain relative to the coenzyme binding domain and the closing up of the active site to produce a catalytically efficient enzyme. The conformational change requires a complete coenzyme and is affected by various chem. or mutational substitutions that can increase the catalytic turnover by altering the kinetics of the isomerization and rate of dissocn. of coenzymes. The binding of NAD is kinetically limited by a unimol. isomerization (corresponding to the conformational change) that is controlled by deprotonation of the catalytic zinc-water to produce a neg.-charged zinc-hydroxide, which can attract the pos.-charged nicotinamide ring. The deprotonation is facilitated by His-51 acting through a H-bonded network to relay the proton to solvent. Binding of NADH also involves a conformational change, but the rate is very fast. After the enzyme binds NAD and closes up, the substrate displaces the hydroxide bound to the catalytic Zn; this exchange may involve a double displacement reaction where the carboxylate group of a Glu residue 1st displaces the hydroxide (inverting the tetrahedral coordination of the Zn), and then the exogenous ligand displaces the Glu residue. The resulting enzyme-NAD-alcoholate complex is poised for H transfer, and small conformational fluctuations may bring the reactants together so that the hydride ion is transferred by quantum mech. tunneling. In the process, the nicotinamide ring may become puckered, as seen in structures of complexes of the enzyme with NADH. The conformational changes of alc. dehydrogenase demonstrate the importance of protein dynamics in catalysis.>> More from SciFinder ®
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Plapp, Bryce V.; Ramaswamy, S.Biochemistry (2012), 51 (19), 4035-4048CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Crystal structures of horse liver alc. dehydrogenase complexed with NAD and the unreactive substrate analogs, 2,2,2-trifluoroethanol (TFE) or 2,3,4,5,6-pentafluorobenzyl alc. (PFB), were detd. at 100 K at 1.12 or 1.14 Å resoln., providing ests. of at. positions with overall errors of ∼0.02 Å, the geometry of ligand binding, descriptions of alternative conformations of amino acid residues and waters, and evidence of a strained nicotinamide ring. The 4 independent subunits from the 2 homodimeric structures differed only slightly in the peptide backbone conformation. Alternative conformations for amino acid side-chains were identified for 50 of the 748 residues in each complex, and Leu-57 and Leu-116 adopted different conformations to accommodate the different alcs. at the active site. Each fluoroalc. occupied one position, and the F atoms of the alcs. were well-resolved. These structures closely resembled the expected Michaelis complexes with the pro-R H atoms of the methylene C atoms of the alcs. directed toward the re face of C4N of the nicotinamide rings with a C-C distance of 3.40 Å. The O atoms of the alcs. were ligated to the catalytic Zn at a distance expected for a Zn alkoxide (1.96 Å) and participated in a low-barrier H-bond (2.52 Å) with the OH group of Ser-48 in a proton relay system. As detd. by x-ray refinement with no restraints on bond distances and planarity, the nicotinamide rings in the 2 complexes were slightly puckered (quasi-boat conformation, with torsion angles of 5.9° for C4N and 4.8° for N1N relative to the plane of the other atoms) and had bond distances that were somewhat different compared to those found for NAD(P). It appeared that the nicotinamide ring was strained toward the transition state on the path to alc. oxidn.>> More from SciFinder ®
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9.Participation of Histidine-51 in Catalysis by Horse Liver Alcohol Dehydrogenase
LeBrun, Laurie A.; Park, Doo-Hong; Ramaswamy, S.; Plapp, Bryce V.Biochemistry (2004), 43 (11), 3014-3026CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Histidine-51 in horse liver alc. dehydrogenase (ADH) is part of a hydrogen-bonded system that appears to facilitate deprotonation of the hydroxyl group of water or alc. ligated to the catalytic zinc. The contribution of His-51 to catalysis was studied by characterizing ADH with Gln substituted for His-51 (H51Q). The steady-state kinetic consts. for ethanol oxidn. and acetaldehyde redn. at pH 8 are similar for wild-type and H51Q enzymes. In contrast, the H51Q substitution significantly shifts the pH dependencies for steady-state and transient reactions and decreases by 11-fold the rate const. for the transient oxidn. of ethanol at pH 8. Modest substrate deuterium isotope effects indicate that hydride transfer only partially limits the transient oxidn. and turnover. Transient data show that the H51Q substitution significantly decreases the rate of isomerization of the enzyme-NAD+ complex and becomes a limiting step for ethanol oxidn. Isomerization of the enzyme-NAD+ complex is rate limiting for acetaldehyde redn. catalyzed by the wild-type enzyme, but release of alc. is limiting for the H51Q enzyme. X-ray crystallog. of doubly substituted His51Gln:Lys228Arg ADH complexed with NAD+ and 2,3- or 2,4-difluorobenzyl alc. shows that Gln-51 isosterically replaces histidine in interactions with the nicotinamide ribose of the coenzyme and that Arg-228 interacts with the adenosine monophosphate of the coenzyme without affecting the protein conformation. The difluorobenzyl alcs. bind in one conformation. Results indicate that His-51 is a participant but is not essential for proton transfers assocd. with the mechanism of ADH.>> More from SciFinder ®
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10.Refined crystal structure of liver alcohol dehydrogenase-NADH complex at 1.8 A resolution
Al-Karadaghi S; Cedergren-Zeppezauer E S; Hovmoller S
Acta crystallographica. Section D, Biological crystallography (1994), 50 (Pt 6), 793-807 ISSN:0907-4449.The crystal structure of the ternary complex of horse liver alcohol dehydrogenase (LADH) with the coenzyme NADH and inhibitor dimethyl sulfoxide (DMSO) has been refined by simulated annealing with molecular dynamics and restrained positional refinement using the program X-PLOR. The two subunits of the enzyme were refined independently. The space group was P1 with cell dimensions a = 51.8, b = 44.5, c = 94.6 A, alpha = 104.8, beta = 102.3 and gamma = 70.6 degrees. The resulting crystallographic R factor is 17.3% for 62 440 unique reflections in the resolution range 10.0-1.8 A. A total of 472 ordered solvent molecules were localized in the structure. An analysis of secondary-structure elements, solvent content and NADH binding is presented.>> More from SciFinder ®
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11.Venkataramaiah, T. H. and Plapp, B. V. (2003) Formamides mimic aldehydes and inhibit liver alcohol dehydrogenases and ethanol metabolism J. Biol. Chem. 278, 36699– 36706 DOI: 10.1074/jbc.M305419200[Crossref], [PubMed], [CAS]
11.Formamides Mimic Aldehydes and Inhibit Liver Alcohol Dehydrogenases and Ethanol Metabolism
Venkataramaiah, Thulasiram H.; Plapp, Bryce V.Journal of Biological Chemistry (2003), 278 (38), 36699-36706CODEN: JBCHA3; ISSN:0021-9258. (American Society for Biochemistry and Molecular Biology)
Formamides are unreactive analogs of the aldehyde substrates of alc. dehydrogenases and are useful for structure-function studies and for specific inhibition of alc. metab. They bind to the enzyme-NADH complex and are uncompetitive inhibitors against varied concns. of alc. Fourteen new branched chain and chiral formamides were prepd. and tested as inhibitors of purified Class I liver alc. dehydrogenases: horse (EqADH E), human (HsADH1C*2), and mouse (MmADH1). In general, larger, substituted formamides, such as N-1-ethylheptylformamide, are better inhibitors of HsADH1C*2 and MmADH1 than of EqADH, reflecting a few differences in amino acid residues that change the sizes of the active sites. In contrast, the linear, alkyl (Pr and n-butyl) formamides are better inhibitors of EqADH and MmADH1 than of HsADH1C*2, probably because water disrupts van der Waals interactions. These enzymes are also inhibited strongly by sulfoxides and 4-substituted pyrazoles. The structure of EqADH complexed with NADH and (R)-N-1-methylhexylformamide was detd. by x-ray crystallog. at 1.6 Å resoln. The structure resembles the expected Michaelis complex with NADH and aldehyde, and shows for the first time that the reduced nicotinamide ring of NADH is puckered, as predicted for the transition state for hydride transfer. Metab. of ethanol in mice was inhibited by several formamides. The data were fitted with kinetic simulation to a mechanism that describes the non-linear progress curves and yields ests. of the in vivo inhibition consts. and the rate consts. for elimination of inhibitors. Some small formamides, such as N-isopropylformamide, may be useful inhibitors in vivo.>> More from SciFinder ®
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12.Cho, H., Ramaswamy, S., and Plapp, B. V. (1997) Flexibility of liver alcohol dehydrogenase in stereoselective binding of 3-butylthiolane 1-oxides Biochemistry 36, 382– 389 DOI: 10.1021/bi9624604[ACS Full Text ACS Full Text], [CAS]
12.Flexibility of Liver Alcohol Dehydrogenase in Stereoselective Binding of 3-Butylthiolane 1-Oxides
Cho, Heeyeong; Ramaswamy, S.; Plapp, Bryce V.Biochemistry (1997), 36 (2), 382-389CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Thiolane 1-oxides are analogs of the carbonyl substrates that bind to the alc. dehydrogenase-NADH complex and are potent uncompetitive inhibitors against alc. [Chadha, V. K., et al. (1985) J. Med. Chem. 28, 36-40]. The four stereoisomers of 3-butylthiolane 1-oxide (BTO) were sepd. by chiral phase chromatog. CD and 1H-NMR spectra identified the enantiomeric pairs. 1H-NMR chem. shifts were assigned on the basis of COSY spectra of both diastereoisomers and confirmed by HMQC spectra. Coupling

consts. were detd. through one-dimensional decoupling expts. NMR with chiral shift reagents, Eu(hfc)3 [europium tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorate]] or (R)-(-)-N-(3,5-dinitrobenzoyl)-α-methylbenzylamine, detd. that the most inhibitory isomer is either 1S,3R or 1R,3S. The chem. shifts of protons in the thiolane 1-oxide ring were influenced by the whole structure and were not correlated with the computed Mulliken charges. X-ray crystallog. at 2.1 and 1.66 Å resoln. of the ternary enzyme complexes with NADH demonstrated that the abs. configuration of the most inhibitory (Kii = 0.31 μM) stereoisomer is 1S,3R and the next best inhibitor (Kii = 0.73 μM) is 1S,3S. The thiolane 1-oxide rings bind in the same position, in the substrate binding site, but the geometry of the complexes suggests that the sulfoxides are not transition state analogs. Significantly, the Bu groups of the two isomers are accommodated differently by flexible amino acid side chains adopting alternative rotameric conformations.>> More from SciFinder ®
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13.Binding of Formamides to Liver Alcohol Dehydrogenase
Ramaswamy, S.; Scholze, Michael; Plapp, Bryce V.Biochemistry (1997), 36 (12), 3522-3527CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Amides are analogs of aldehydes and potent inhibitors of liver alc. dehydrogenases. They can be used for structural studies and for inhibiting the metab. of alcs. that form toxic products. We studied N-alkyl amides that bind to the enzyme-NADH complex and act as uncompetitive inhibitors against varied concns. of ethanol (millimolar Kii values, at pH 8 and 25°): N-propylacetamide (16), δ-valerolactam (1.6), N-formylpiperidine (0.14), N-isobutylformamide (0.028), N-(cyclohexylmethyl)formamide (0.011), and N-cyclohexylformamide (0.0087). The lower affinity of δ-valerolactam and N-propylacetamide can be explained by steric hindrance with Phe93 of the enzyme. Replacing Phe93 with Ala in the S48T/F93A mutated enzyme, which resembles the natural α-isoenzyme of primates, improved binding of δ-valerolactam by 210-fold. The structures of horse liver enzyme complexed with NADH and N-cyclohexylformamide or N-formylpiperidine were detd. by x-ray crystallog. at 2.5 Å resoln. In both complexes, the carbonyl oxygens of the inhibitors bind to the catalytic zinc and form a hydrogen bond to the hydroxyl group of Ser48 of the enzyme. The six-membered rings bind in overlapping, but rotated, positions that optimize hydrophobic interactions. The binding modes of the unreactive formamides appear to resemble the Michaelis complexes of the analogous substrates, with the re face of the carbonyl carbon suitably positioned to accept a hydrogen from NADH.>> More from SciFinder ®
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14.pH, isotope, and substituent effects on the interconversion of aromatic substrates catalyzed by hydroxybutyrimidylated liver alcohol dehydrogenase
Dworschack, Robert T.; Plapp, Bryce V.Biochemistry (1977), 16 (12), 2716-25CODEN: BICHAW; ISSN:0006-2960.The mechanism of H transfer catalyzed by horse liver alc. dehydrogenase with amidinated amino groups was studied with steady-state kinetics. Product inhibition studies with the modified and native enzymes are consistent with an ordered mechanism. Primary 2H isotope effects obtained for oxidn. of pentadeuterioethanol and benzyl alc.-1.1-2H2 and for redn. of a series of para-substituted benzaldehydes indicate that the turnover nos. reflect the rates of H transfer with hydroxybutyrimidylated enzyme. Isotope effects were not obsd. for native enzyme. The magnitudes and signs of the ρ values obtained for oxidn. of p-substituted benzyl alcs. and for redn. of a series of p-substituted benzaldehydes catalyzed by hydroxybutyrimidylated enzyme suggest that interconversion of central complexes occurs via concerted hydride and proton transfer in which only a small amt. of charge develops in the transition state. The pH effects for both enzymes can be explained by a coherent model that is consistent with the structure of the enzyme as detd. by x-ray crystallog. This model postulates that a H2O (or OH-) mol. coordinated to the Zn2+ acts as a proton donor (or acceptor) and that the state of protonation of histidine-51 modulates the rate of transfer of H in the central complexes.>> More from SciFinder ®
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Vallee, Bert L.; Coombs, Thomas L.Journal of Biological Chemistry (1959), 234 (), 2615-20CODEN: JBCHA3; ISSN:0021-9258.cf. C.A. 52, 7393c; following abstr. The complexes of 1,10-phenanthroline (OP) with ionic Zn and the Zn in alc. dehydrogenase of horse liver were studied by the method of continuous variations. The formation of [ZnOP1] ++, [ZnOP2)++, and [ZnOP3]++ complexes was confirmed and their extinction coeffs. at 3275 A. were established. One mole of OP interacts with each of the 2 Zn atoms of horse-liver alc. dehydrogenase (LADH) to form a mixed complex [(LADH)Z2.OP2]which has absorption max. at 2972, 3287, and 3450 A. This mixed complex, demonstrated spectrophotometrically, represents the enzymically inactive but dissociable enzyme-inhibitor complex postulated from kinetic studies. The extinction coeff. of the complex is 1700 ± 100 and the dissocn. const. is 3.3 ± 0.5 × 10-5M. This const. is close to that derived from kinetic data and its magnitude supports the validity of the mechanisms of the interaction of OP with the Zn atoms of alc. dehydrogenase of horse liver previously suggested. Consequently, OP can be used simultaneously to det. the Zn of the enzyme and the directly correlated disappearance of enzymic activity. The approach described provides a means of establishing the intrinsic dissocn. consts. of such systems as these. With a series of chelating agents of different degree of binding strengths, the true value of the const. is susceptible to detn. by a series of successive approximations.>> More from SciFinder ®
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16.Role of zinc in alcohol dehydrogenase. IV. Kinetics of the instantaneous inhibition of horse-liver alcohol dehydrogenase by 1,10-phenanthroline
Vallee, Bert L.; Williams, Robert J. P.; Hoch, Frederic L.Journal of Biological Chemistry (1959), 234 (), 2621-6CODEN: JBCHA3; ISSN:0021-9258.cf. C.A. 52, 17393b; preceding abstr. The kinetics were studied of the instantaneous, reversible inhibition of horse liver alc. dehydrogenase by 1,10-phenanthroline (OP). The data indicate that diphosphopyridine nucleotide (DPN) and its reduced form (DPNH) interact with the enzyme at or near a Zn atom, whereas EtOH and AcH do not. The inhibition is mediated through the formation of an enzyme-inhibitor complex in which 1 mol. of OP is bound to each Zn atom. This finding is consistent with the results of studies with spectrophotometric rather than enzymic material. The kinetically detd. values of KOP, the dissocn. const. of this complex, extrapolated to DPN = 0 or DPNH = 0, are 7.0 × 10-5M and 4.0 × 10-6M, resp. These values compare with 3.1M × 10-5 obtained by spectrophotometric detns. of the dissocn. consts. This discrepancy, together with certain anomalies in the inhibition kinetics when EtOH or AcH are varied, is the basis for several model schemes of enzyme reaction, including the assumption that liver alc. dehydrogenase exists in oxidn.-redn. forms which interconvert through the oxidn.-redn. reaction between DPN and DPNH. The inhibition kinetics imply that [(LADH)Zn2] and [(YADH)Zn4] have different modes of action (LADH and YADH are liver and yeast alc. dehydrogenases, resp.).>> More from SciFinder ®
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DeTraglia, Michael C.; Schmidt, Jack; Dunn, Michael F.; McFarland, James T.Journal of Biological Chemistry (1977), 252 (10), 3493-500CODEN: JBCHA3; ISSN:0021-9258.Sp. rate consts. for the assocn. and dissocn. of liver alc. dehydrogenase-coenzyme complexes were measured within the pH range 6-10.5. Dissocn. rates were measured. The rate const. for NAD and NADH assocn. showed the same pH rate profile with pKa≃9.5. Furthermore, the pKa for o-phenanthroline assocn. to liver alc. dehydrogenase was 8.1. Since x-ray studies indicated that o-phenanthroline displaces the H2O bound to Zn2+ at the active site,. The pKa of 8.1 is most likely assocd. with ionization of this H2O mol. Thus, ionization of H2O bound to Zn2+ apparently does not control the rate of nucleotide assocn. The dissocn. rate const. for (enzyme) E-NADS showed a pKa = 8.0. Y contrast, the rate const. for E-NADH dissocn. exhibited complex behavior not interpretable as assocd. with ionization of a single group. Agreement between equil.-binding consts. for nucleotide and Kdissocn./kassocn. ratios was quite good. Furthermore, agreement with rate consts. detd. from steady-state kinetic expts. was good except for the rate of NADH assocn. Liver alc. dehydrogenase rapidly lost Zn2+ at pH values >10.>> More from SciFinder ®
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18.X-ray investigation of the binding of 1,10-phenanthroline and imidazole to horse-liver alcohol dehydrogenase

Boiwe, Torne; Branden, Carl Ivar
European Journal of Biochemistry (1977), 77 (1), 173-9CODEN: EJBCAI; ISSN:0014-2956.The binding of 2 inhibitor mols., imidazole (I) and 1,10-phenanthroline (II), to liver alc. dehydrogenase (III) was studied by crystallog. methods. X-ray data for the I-III complex were collected to 0.29-nm resoln. and for the II-III complex to 0.45-nm resoln. In both cases, only 1 peak was found in the difference electron-d. maps close to the active Zn. The peak corresponding to II overlaps the site of the d. of the Zn-bound H2O in apo-III and the I d. partially overlaps this d. No addnl. peaks can be discerned close to the Zn which would correspond to new positions of bound H2O. Thus, it is concluded that both of these inhibitors bind to the catalytic Zn and that upon binding they displace the H2O that is firmly bound to this Zn in apo-III. No structural changes can be seen in the remaining part of the mol.>> More from SciFinder ®
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19.Limiting rates of ligand association to alcohol dehydrogenase
Frolich, Marianne; Creighton, Donald J.; Sigman, David S.Archives of Biochemistry and Biophysics (1978), 189 (2), 471-80CODEN: ABBIA4; ISSN:0003-9861.Detailed stopped-flow kinetic studies of the assocn. of 2,2-bipyridine, 1,10-phenanthroline, and 5-chloro-1,10-phenanthroline to Zn at the active site of horse liver alc. dehydrogenase showed that a limiting rate const. of ∼200 s-1 restricts the binding of the bidentate chelating agents to the free enzyme. The formation of the enzyme-ligand complexes was followed by the characteristic absorption spectra of the resulting complexes or by the displacement of the fluorescent dye, auramine O. Monodentate ligands, on binding to the free enzyme or enzyme-NAD(H) complexes, did not have a comparable limiting rate. In analogy with simple inorg. systems, these observations were interpreted in terms of the rate-limiting dissocn. of an inner sphere water mol. following the rapid formation by the bidentate ligand of an outer sphere complex. The displacement of a water mol. from Zn by 1,10-phenanthroline was obsd. in crystallog. studies which also established that the Zn in the enzyme-1,10-phenanthroline complex is pentacoordinate. Monodentate ligands, which are substrate analogs, did not exhibit limiting rates because displacement of water was not required for their addn. to a coordinate position which is apparently vacant in the free enzyme. If a water mol. remains bound to Zn in the kinetically competent ternary complex, it may play an essential role in the proton transfer reaction accompanying catalysis.>> More from SciFinder ®
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20.The role of zinc-bound water in liver alcohol dehydrogenase catalysis
Evans, Susan A.; Shore, Joseph D.Journal of Biological Chemistry (1980), 255 (4), 1509-14CODEN: JBCHA3; ISSN:0021-9258.To elucidate the role of Zn-bound water in liver alc. dehydrogenase catalysis, chelation by 1,10-phenanthroline and 2,2-bipyridine was studied. The rate consts. for assocn. of both chelating agents to the active center Zn were pH-dependent with a pKa of 9.2 and preferential binding to a protonated form. The binary complex dissocn. rate consts. were pH-independent for both chelating agents. In the presence of satg. NAD, the pKa for the equil. binding of 2,2-bipyridine was perturbed to 7.6, similar to the functional group previously shown to be involved in NAD binding. The presence of satg. imidazole resulted in pH-independent 2,2-bipyridine binding. These studies provide compelling evidence that the ionizing enzyme functional group involved in coenzyme binding, proton liberation, and conformational states is Zn-bound water. The limiting rate of chelation by 2,2-bipyridine was pH-independent, and no limiting rate was obsd. in the presence of satg. imidazole. These results indicate that the limiting rate of chelation is due to the rate of dissocn. of Zn-bound water. The implications of this regarding the role of Zn in catalytic turnover of liver alc. dehydrogenase are discussed.>> More from SciFinder ®
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Eklund, Hans; Plapp, Bryce V.; Samama, Jean Pierre; Braenden, Carl Ivar
Journal of Biological Chemistry (1982), 257 (23), 14349-58CODEN: JBCHA3; ISSN:0021-9258.Horse liver alc. dehydrogenase was crystd. from an equil. mixt. contg. predominantly NAD and p-bromobenzyl alc. X-ray diffractometer data to a resoln. of 2.9 Å were collected and used to compute electron d. maps with phases calcd. from the isomorphous enzyme-NADH-DMSO complex, which was refined to an R value of 25.6%. The electron d. maps were readily interpreted in a graphics display system. Both subunits of the dimer bound coenzyme and alc. in essentially the same manner; there was no evidence of asymmetry between subunits. The bromophenyl group was accommodated in a large hydrophobic pocket that had the side chain of leucine-116 rotated into a different position than in the complex with DMSO. The alc. O atom was directly ligated to the catalytic Zn atom. The Zn was tetracoordinate and there was no room for a water mol. to make the Zn pentacoordinate. A H-bonded system formed with the OH groups of the alc., serine-48, and nicotinamide ribose(2'), and the imidazole of histidine-51 may provide a proton relay system that links the buried alc. to solvent. The insertion of the coenzyme OH group into this system appeared to install the catalytically active species. The obsd. structure had the pro-R H atom on C1 of the alc. pointing away from C4 of the nicotinamide ring. This was probably a nonproductive complex that easily became productive by a rapid rotation of the alc. to put the pro-R H atom within 3 Å of C4 of the nicotinamide ring and in position for a direct transfer of H. A model of the productive complex readily explains the stereospecificity of hydride transfer obsd. for EtOH.>> More from SciFinder ®
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Meijers, Rob; Morris, Richard J.; Adolph, Hans W.; Merli, Angelo; Lamzin, Victor S.; Cedergren-Zeppezauer, Eila S.Journal of Biological Chemistry (2001), 276 (12), 9316-9321CODEN: JBCHA3; ISSN:0021-9258. (American Society for Biochemistry and Molecular Biology)
At. (1 Å) resoln. x-ray structures of horse liver alc. dehydrogenase in complex with NADH revealed the formation of an adduct in the active site between a metal-bound water and NADH. Furthermore, a pronounced distortion of the pyridine ring of NADH was obsd. A series of quantum chem. calcns. on the water-nicotinamide adduct showed that the puckering of the pyridine ring in the crystal structures can only be reproduced when the water is considered a hydroxide ion. These observations provide fundamental insight into the enzymic activation of NADH for hydride transfer.>> More from SciFinder ®
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23.Meijers, R., Adolph, H. W., Dauter, Z., Wilson, K. S., Lamzin, V. S., and Cedergren-Zeppezauer, E. S. (2007) Structural evidence for a ligand coordination switch in liver alcohol dehydrogenase Biochemistry 46, 5446– 5454 DOI: 10.1021/bi6023594[ACS Full Text ACS Full Text], [CAS]
23.Structural Evidence for a Ligand Coordination Switch in Liver Alcohol Dehydrogenase
Meijers, Rob; Adolph, Hans-Werner; Dauter, Zbigniew; Wilson, Keith S.; Lamzin, Victor S.; Cedergren-Zeppezauer, Eila S.Biochemistry (2007), 46 (18), 5446-5454CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
The use of substrate analogs as inhibitors provides a way to understand and manipulate enzyme function. Here we report two 1 Å resoln. crystal structures of liver alc. dehydrogenase in complex with NADH and two inhibitors: DMSO and isobutyramide. Both structures present a dynamic state of inhibition. In the DMSO complex structure, the inhibitor is caught in transition on its way to the active site using a flash-freezing protocol and a cadmium-substituted enzyme. One inhibitor mol. is partly located in the first and partly in the second coordination sphere of the active site metal. A hydroxide ion bound to the active site metal lies close to the pyridine ring of NADH, which is puckered in a twisted boat conformation. The cadmium ion is coordinated by both the hydroxide ion and the inhibitor mol., providing structural evidence of a coordination switch at the active site metal ion. The structure of the isobutyramide complex reveals the partial formation of an adduct between the isobutyramide inhibitor and NADH. It provides evidence of the contribution of a shift from the keto to the enol tautomer during aldehyde redn. The different positions of the inhibitors further refine the knowledge of the dynamics of the enzyme mechanism and explain how the crowded active site can facilitate the presence of a substrate and a metal-bound hydroxide ion.>> More from SciFinder ®
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24.Coordination environment of the active-site metal ion of liver alcohol dehydrogenase

Makinen, Marvin W.; Yim, Moon B.Proceedings of the National Academy of Sciences of the United States of America (1981), 78 (10), 6221-5CODEN: PNASA6; ISSN:0027-8424.The coordination environment of the catalytically active metal ion of horse liver alc. dehydrogenase (EC 1.1.1.1) (I) was investigated by ESR methods with use of the active-site-specific Co2+-reconstituted enzyme. The ESR absorption spectrum of the metal-substituted enzyme is characteristic of a rhombically distorted environment. The spectrum of the enzyme-NAD complex shows approx. axial symmetry of the metal ion site, indicating that coenzyme binding induces a structural alteration in the active site region. This environment is not further altered by binding of the competitive inhibitor pyrazole. To assign the coordination no. of the active-site metal ion, the zero-field splitting was detd. on the basis of the temp. dependence of the spin-lattice relaxation of Co2+. The zero-field splitting energies are ∼9 cm-1 for the free Co2+-reconstituted enzyme and ∼46 and ∼47 cm-1 for the enzyme-NAD and enzyme-NAD-pyrazole complexes, resp. These values are compatible with a tetracoordinate metal ion in the active site of free I, but a pentacoordinate metal ion in the binary enzyme-NAD complex and in the ternary enzyme-NAD-inhibitor complex and, therefore, presumably in the catalytically active ternary enzyme-NAD-alc. complex formed in the course of alc. oxidn.>> More from SciFinder ®
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25.Neutral metal-bound water is the base catalyst in liver alcohol dehydrogenase
Makinen, Marvin W.; Maret, Wolfgang; Yim, Moon B.Proceedings of the National Academy of Sciences of the United States of America (1983), 80 (9), 2584-8CODEN: PNASA6; ISSN:0027-8424.The catalytic role of the active site metal-water complex in horse liver alc. dehydrogenase (EC 1.1.1.1) is investigated on the basis of comparative anal. of the pH dependence of steady-state kinetic parameters of the native and active site-specific Co2+-reconstituted enzyme and on the basis of assignment of the coordination environment of the Co2+ by ESR methods. The pH dependence of the kinetic parameters for the oxidn. of benzyl alc. reveals 2 ionizations (pK1 ≈6.7; pK2 ≈10.6) that govern kcat and belong to the ternary enzyme-NAD-alc. complex and 2 ionizations (pK1' ≈7.5; pK2' ≈8.9) that govern kcat/Km and belong to the binary enzyme-NAD complex. The ionizations pK2 and pK2' decrease by 0.5-1 pKa unit on replacement of the active site Zn2+ by Co2+. A similar metal ion dependence of pK2 and pK2' is obsd. for the oxidn. of 2-PrOH. These ionizations are attributed to a metal-bound water mol. The zero-field splitting energy of Co2+ in the binary enzyme-NADH complex and the ternary enzyme-NADH-CF3CH2OH complex is ≈22 cm-1, indicative of a pentacoordinate species. Binding of a water mol. to the metal ion as the 5th ligand in the ternary enzyme-NADH-CF3CH2OH complex is confirmed on the basis of magnetic interactions of H217O with Co2+. Thus, the active site metal ion in catalytically competent ternary enzyme-coenzyme-substrate complexes is pentacoordinate and is ligated by a neutral water mol. in the physiol. pH range. The neutral metal-bound water mol. may serve as the base catalyst for proton abstraction in alc. oxidn.>> More from SciFinder ®
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26.Magnetic Circular Dichroism and Electron Paramagnetic Resonance Studies of Cobalt-Substituted Horse Liver Alcohol Dehydrogenase
Werth, Mark T.; Tang, Syou-Fen; Formicka, Grazyna; Zeppezauer, Michael; Johnson, Michael K.Inorganic Chemistry (1995), 34 (1), 218-28CODEN: INOCAJ; ISSN:0020-1669. (American Chemical Society)
The ground and excited state properties of Co(II) substituted for Zn(II) at the catalytic (c) and the noncatalytic (n) sites of horse liver alc. dehydrogenase EE isoenzyme have been investigated by parallel EPR and UV/visible variable-temp. magnetic CD (VTMCD) spectroscopies. Samples were investigated as prepd. and after formation of a ternary complex with NAD+ and the potent inhibitor pyrazole. In accord with the structural role proposed for the noncatalytic metal, the spectroscopic properties of Co(II) at the noncatalytic site were unperturbed by formation of the ternary complex. The EPR spectra were readily analyzed in terms of a S = 3/2 spin Hamiltonian using anisotropic intrinsic g-values in the range characteristic of tetrahedral Co(II), i.e. g = 2.1-2.4; E/D ≈ 0.33, 0.05 (with D < 0), and 0 (with D > 0) for Co(c)Zn(n)-HLADH, Co(c)Zn(n)-HLADH/NAD+/pyrazole, and Zn(c)Co(n)-HLADH, resp. VTMCD studies facilitated resoln. and assignment of S → Co(II) charge transfer bands (300-400 nm) and the components of the 4A2 → 4T1(P) tetrahedral d-d band (500-800 nm) that are split by spin-orbit coupling and low-symmetry distortions. The splittings of the highest energy d-d band are indicative of a much more distorted coordination environment for Co(II) at the catalytic site than the noncatalytic site. This is also reflected in the magnitude of ground state zero-field splitting, Δ, detd. by anal. of the temp. dependence of discrete MCD bands, |Δ| = 33, 56, and 7 cm-1 for Co(c)Zn(n)-HLADH, Co(c)Zn(n)-HLADH/NAD+/pyrazole, and Zn(c)Co(n)-HLADH, resp. MCD magnetization data are rationalized in terms of the EPR-detd. ground state effective g-values, ground state zero-field splitting, and the polarization of the electronic transitions. The zero-field splittings for the samples with Co(II) at the catalytic site detd. by VTMCD are quite different from those detd. by EPR from the temp. dependence of the spin relaxation (Makinen, M. W.; Yim, M. B. Proc. Natl. Acad. Sci. U.S.A. 1981 78, 6221-6225), and the origin of this discrepancy is discussed. In accord with x-ray crystallog. studies, the EPR and VTMCD data are rationalized in terms of a highly distorted tetrahedral coordination environment for Co(II) at the catalytic site (two cysteines, one histidine, and one H2O for Co(c)Zn(n)-HLADH and two cysteines, one histidine and one pyrazole for Co(c)Zn(n)-HLADH/NAD+/pyrazole) and a more regular tetrahedral environment for Co(II) at the noncatalytic site (four cysteines).>> More from SciFinder ®
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27.Structural information concerning the catalytic metal site in horse liver alcohol dehydrogenase, obtained by perturbed angular correlation spectroscopy on cadmium-111
Andersson, Inger; Bauer, Rogert; Demeter, Istvan
Inorganica Chimica Acta (1982), 67 (2), 53-9CODEN: ICHAA3; ISSN:0020-1693.Perturbed angular correlation spectra on horse liver alc. dehydrogenase, measured on 111Cd inserted specifically in the catalytic site, were obtained under various conditions. Spectra were obtained at pH 6-9 and in the presence of NAD and(or) pyrazole or trifluoroethanol. No ionization at the metal site could be detected at pH 6-9, irresp. of NAD presence. Thus, a H2O mol. ligated to Cd(II) must have a pK of ionization >9.5. NAD, pyrazole, and trifluoroethanol affect the spectra of Cd incorporated in the catalytic site of alc. dehydrogenase. This is consistent with a 4-coordinated, nearly tetrahedral metal geometry, both with and without the coenzyme bound to the enzyme. The effect of NAD on the metal coordination can best be explained by a redn. of the 2 cysteine metal bond lengths. Both pyrazole and trifluoroethanol enter as a 5th ligand not displacing the solvent ligand to the metal.>> More from SciFinder ®
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28.Cd-substituted horse liver alcohol dehydrogenase: catalytic site metal coordination geometry and protein conformation
Hemmingsen, L.; Bauer, R.; Bjerrum, M. J.; Zeppezauer, M.; Adolph, H. W.; Formicka, G.; Cedergren-Zeppezauer, E.Biochemistry (1995), 34 (21), 7145-53CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
The coordination geometry of the catalytic site in Cd-substituted horse liver alc. dehydrogenase (LADH) has been investigated as a function of pH using the method of perturbed angular correlation of γ-rays (PAC). LADH in soln. fully loaded with cadmium, including radioactive 111mCd in the catalytic site [Cd2(111mCd)Cd2LADH], was studied over the pH range 7.9-11.5. Anal. of the PAC spectra showed the ionization of a group with a pKa of 11. This pKa value is about 2 pH units higher than that of native zinc-contg. LADH. A pKa of 9.6 was found for the binary complex of Cd2(111mCd)Cd2LADH with NAD+. This value is also about 2 pH units higher than that of the binary complex of native zinc-contg. enzyme and NAD+. No pH dependency was detected for the binary complex of Cd2(111mCd)Cd2LADH with NADH within the pH range measured (pH 8.3-11.5). Assuming that metal-coordinated water is the ionizing group, the authors conclude that the larger ionic radius of Cd(II) relative to Zn(II) in the catalytic site causes the elevated pKa values of metal-bound water. Interpretation of nuclear quadrupole interaction (NQI) parameters derived from PAC spectra is based on the use of the angular overlap model, using the coordinates for the catalytic zinc site from the 1.8 Å resoln. crystal structure of the ternary complex between LADH, NADH, and DMSO as a model. Within the limits of uncertainty of the x-ray data, good agreement is obtained between the calcd. and measured NQI values for LADH at low pH and for the binary complex of LADH with NADH. However, the high-pH form of LADH and both the low- and high-pH forms of the binary complex with NAD+ do not agree with this coordination geometry. In these cases the authors could only achieve agreement between data and model if a local conformational change involving the metal ligands Cys46 and Cys174 was introduced, decreasing the S-Cd-S angle by approx. 20°. Such a local change in conformation might be triggered by the presence of a neg. charged solvent metal ligand causing electrostatic repulsion. The catalytic site is flexible in Cd2(111mCd)Cd2LADH, but rigid in coenzyme complexes. The binary complex between NADH and LADH in soln. exists in two mol. populations having different coordination geometries. This is

manifested as two sep., sharply distinguished NQIs, whereas for the ternary complex between DMSO, NADH, and LADH only one form is detected.>> More from SciFinder ®
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29.The protein conformation of Cd-substituted horse liver alcohol dehydrogenase and its metal-site coordination geometry in binary and ternary inhibitor complexes
Hemmingsen, Lars; Bauer, Rogert; Bjerrum, Morten Jannik; Adolph, Hans Werner; Zeppezauer, Michael
European Journal of Biochemistry (1996), 241 (2), 546-551CODEN: EJBCAI; ISSN:0014-2956. (Springer)
The coordination geometry of the metal at the active site in Cd-substituted horse liver alc. dehydrogenase (LADH) was investigated for the binary complexes of LADH with imidazole, isobutyramide, decanoic acid, and Cl-, and for the ternary complexes of LADH with NADH and imidazole, NADH and isobutyramide, NAD and decanoic acid and NAD and Cl-, by perturbed angular correlation of γ-rays (PAC). The spectral results were consistent with a flexible structure around the metal for the binary complexes with inhibitors. For ternary complexes, a rigid structure was obsd. An exception was the ternary complex between LADH, NADH, and imidazole, in which the metal site was still flexible. Comparing with available structures detd. by x-ray crystallog., a correlation was found between open structures and flexible metal sites, and between closed structures and rigid metal sites. This indicated that the PAC technique could be applied to distinguish the 2 conformations in soln. The spectral parameters, ωo and η, of the expts., except for the complexes with imidazole, fell into 2 groups: one with low ωo and one with high ωo (η was relatively const. in all expts.). The authors clarified that the low ωo values were connected with the presence of a neg. charged solvent ligand. Using an angular-overlap approach to interpret the results, the low ωo values were found to be compatible with a coordination geometry where the S-Cd-S (Cys-174 and Cys-46 coordinate to the metal) angle was ∼110°, whereas high ωo values were compatible with an S-Cd-S angle of 130°. The presence of a neg. charged metal ligand, might trigger the movement of the S atom of Cys-174. Since it is believed that alcs. coordinate to the metal as alcoholate ions this could be important for catalysis.>> More from SciFinder ®
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Ryde, U.; Hemmingsen, L.JBIC, Journal of Biological Inorganic Chemistry (1997), 2 (5), 567-579CODEN: JJBCFA; ISSN:0949-8257. (Springer-Verlag)
The structure of eleven complexes of cadmium-substituted alc. dehydrogenase with or without coenzyme and with different non-protein cadmium ligands has been estd. by combined quantum chem. and mol. mech. geometry optimizations. The geometry of the optimized complexes is similar to the crystal structure of cadmium-substituted alc. dehydrogenase, indicating that the method behaves well. The optimized structures do not differ significantly (except for the metal bond lengths) from those of the corresponding zinc complexes, which shows that cadmium is a good probe of zinc coordination geometries. The elec. field gradients at the cadmium nucleus have been calcd. quantum chem. at the MP2 level with a large cadmium basis set, and they have been used to interpret exptl. data obtained by perturbed angular correlation of γ-rays. The exptl. and calcd. field gradients (all three eigenvalues) differ by less than 0.35 a.u. (3.4·1021 Vm-2), the av. error is 0.11 a.u., and the av. relative error in the two largest eigenvalues of the field gradients is 9%. Calcd. field gradients of four-coordinate structures agree better with the exptl. results than do those of any five-coordinate model. Thus, the results indicate that the catalytic metal ion remains four-coordinate in all examd. complexes. Two measurements are best explained by a four-coordinate cadmium ion with Glu-68 as the fourth ligand, indicating that Glu-68 probably coordinates intermittently to the catalytic metal ion in horse liver alc. dehydrogenase under physiol. conditions.>> More from SciFinder ®
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31.Active site electronic structure and dynamics during metalloenzyme catalysis
Kleifeld, Oded; Frenkel, Anatoly; Martin, Jan M. L.; Sagi, Irit
Nature Structural Biology (2003), 10 (2), 98-103CODEN: NSBIEW; ISSN:1072-8368. (Nature Publishing Group)
Zinc-dependent enzymes play important roles in many cellular processes. Assignment of their reaction mechanisms is often a subject of debate because the zinc ion is silent in several spectroscopic techniques. The authors have combined time-resolved x-ray absorption spectroscopy, pre-steady state kinetics and computational quantum chem. to study the active site zinc ion of bacterial alc. dehydrogenase during single substrate turnover. The authors detect a series of alternations in the coordination no. and structure of the catalytic zinc ion with concomitant changes in metal-ligand bond distances. These structural changes are reflected in the effective charge of the metal ion. The present work emphasizes the flexibility of catalytic zinc sites during catalysis and provides novel mechanistic insights into alc. dehydrogenase catalysis.>> More from SciFinder ®
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32.Spectroscopic studies of inhibited alcohol dehydrogenase from Thermoanaerobacter brockii: Proposed structure for the catalytic intermediate state
Kleifeld, Oded; Frenkel, Anatoly; Bogin, Oren; Eisenstein, Miriam; Brumfeld, Vlad; Burstein, Yigal; Sagi, Irit
Biochemistry (2000), 39 (26), 7702-7711CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Thermoanaerobacter brockii alc. dehydrogenase (TbADH) catalyzes the reversible oxidn. of secondary alcs. to the corresponding ketones using NADP+ as the cofactor. The active site of the enzyme contains a zinc ion that is tetrahedrally coordinated by four protein residues. The enzymic reaction leads to the formation of a ternary enzyme-cofactor-substrate complex; and catalytic hydride ion transfer is believed to take place directly between the substrate and cofactor at the ternary complex. Although crystallog. data of TbADH and other alc. dehydrogenases as well as their complexes are available, their mode of action remains to be detd. It is firmly established that the zinc ion is essential for catalysis. However, there is no clear agreement about the coordination environment of the metal ion and the competent reaction intermediates during catalysis. We used a combination of X-ray absorption, CD, and fluorescence spectroscopy, together with structural anal. and modeling studies, to investigate the ternary complexes of TbADH that are bound to a transition-state analog inhibitor. Our structural and spectroscopic studies indicated that the coordination sphere of the catalytic zinc site in TbADH undergoes conformational changes when it binds the inhibitor and forms a pentacoordinated complex at the zinc ion. These studies provide the first active site structure of bacterial ADH bound to a substrate analog. Here, we suggest the active site structure of the central intermediate complex and, more specifically, propose the substrate-binding site in TbADH.>> More from SciFinder ®
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33.Structure of human χχ alcohol dehydrogenase: a glutathione-dependent formaldehyde dehydrogenase
Yang, Zhong-Ning; Bosron, William F.; Hurley, Thomas D.Journal of Molecular Biology (1997), 265 (3), 330-343CODEN: JMOBAK; ISSN:0022-2836. (Academic)
The crystal structure of the human class III χχ alc. dehydrogenase (ADH) in a binary complex with NAD+γ was solved to 2.7 Å resoln. by mol. replacement with human class I β1β1 ADH. χχ ADH catalyzes the oxidn. of long-chain alcs. such as ω-hydroxy fatty acids as well as S-hydroxymethyl-glutathione, a spontaneous adduct between formaldehyde and glutathione. There are two subunits per asym. unit in the χχ ADH structure. Both subunits display a semi-open conformation of the catalytic domain. This conformation is half-way between the open and closed conformations described for the horse EE ADH enzyme. The semi-open conformation and key changes in elements of secondary structure provide a structural basis for the ability of χχ ADH to bind S-hydroxymethyl-glutathione and 10-hydroxydecanoate. Direct coordination of the catalytic zinc ion by Glu68 creates a novel environment for the catalytic zinc ion in χχ ADH. This new configuration of the catalytic zinc is similar to an intermediate for horse EE ADH proposed through theor. computations and is consistent with the spectroscopic data of the Co(II)-substituted χχ enzyme. The position for residue His47 in the χχ ADH structure suggests His47 may function both as a catalytic base for proton transfer and in the binding of the adenosine phosphate of NAD(H). Modeling of

substrate binding to this enzyme structure is consistent with prior mutagenesis data which showed that both Asp57 and Arg115 contribute to glutathione binding and that Arg115 contributes to the binding of ω-hydroxy fatty acids and identifies addnl. residues which may contribute to substrate binding.>> More from SciFinder ®
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34.Human Glutathione-Dependent Formaldehyde Dehydrogenase. Structures of Apo, Binary, and Inhibitory Ternary Complexes
Sanghani, Paresh C.; Robinson, Howard; Bosron, William F.; Hurley, Thomas D.Biochemistry (2002), 41 (35), 10778-10786CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
The human glutathione-dependent formaldehyde dehydrogenase is unique among the structurally studied members of the alc. dehydrogenase family in that it follows a random bi bi kinetic mechanism. The structures of an apo form of the enzyme, a binary complex with substrate 12-hydroxydodecanoic acid, and a ternary complex with NAD+ and the inhibitor dodecanoic acid were detd. at 2.0, 2.3, and 2.3 Å resoln. by X-ray crystallog. using the anomalous diffraction signal of zinc. The structures of the enzyme and its binary complex with the primary alc. substrate, 12-hydroxydodecanoic acid, and the previously reported binary complex with the coenzyme show that the binding of the first substrate (alc. or coenzyme) causes only minor changes to the overall structure of the enzyme. This is consistent with the random mechanism of the enzyme where either of the substrates binds to the free enzyme. The catalytic-domain position in these structures is intermediate to the closed and open conformations obsd. in class I alc. dehydrogenases. More importantly, two different tetrahedral coordination environments of the active site zinc are obsd. in these structures. In the apoenzyme, the active site zinc is coordinated to Cys44, His66 and Cys173, and a water mol. In the inhibitor complex, the coordination environment involves Glu67 instead of the solvent water mol. The coordination environment involving Glu67 as the fourth ligand likely represents an intermediate step during ligand exchange at the active site zinc. These observations provide new insight into metal-assisted catalysis and substrate binding in glutathione-dependent formaldehyde dehydrogenase.>> More from SciFinder ®
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35.Human Glutathione-Dependent Formaldehyde Dehydrogenase. Structural Changes Associated with Ternary Complex Formation
Sanghani, Paresh C.; Bosron, William F.; Hurley, Thomas D.Biochemistry (2002), 41 (51), 15189-15194CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Human glutathione-dependent formaldehyde dehydrogenase plays an important role in the metab. of glutathione adducts such as S-(hydroxymethyl)glutathione and S-nitrosoglutathione. The role of specific active site residues in binding these physiol. important substrates and the structural changes during the catalytic cycle of glutathione-dependent formaldehyde dehydrogenase was examd. by detg. the crystal structure of a ternary complex with S-(hydroxymethyl)glutathione and the reduced coenzyme to 2.6 Å resoln. The formation of the ternary complex caused the movement of the catalytic domain toward the coenzyme-binding domain. This represents the first observation of domain closure in glutathione-dependent formaldehyde dehydrogenase in response to substrate binding. A water mol. adjacent to the 2'-ribose hydroxyl of NADH suggests that the alc. proton is relayed to solvent directly from the coenzyme, rather than through the action of the terminal histidine residue as obsd. in the proton relay system for class I alc. dehydrogenases. S-(Hydroxymethyl)glutathione is directly coordinated to the active site zinc and forms interactions with the highly conserved residues Arg114, Asp55, Glu57, and Thr46. The active site zinc has a tetrahedral coordination environment with Cys44, His66, and Cys173 as the three protein ligands in addn. to S-(hydroxymethyl)glutathione. This is in contrast to zinc coordination in the binary coenzyme complex where all of the ligands were contributed by the enzyme and included Glu67 as the fourth protein ligand. This change in zinc coordination is accomplished by an ∼2.3 Å movement of the catalytic zinc.>> More from SciFinder ®
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36.Structure-Function Relationships in Human Glutathione-Dependent Formaldehyde Dehydrogenase. Role of Glu-67 and Arg-368 in the Catalytic Mechanism
Sanghani, Paresh C.; Davis, Wilhelmina I.; Zhai, LanMin; Robinson, Howard
Biochemistry (2006), 45 (15), 4819-4830CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
The active-site zinc in human glutathione-dependent formaldehyde dehydrogenase (FDH) undergoes coenzyme-induced displacement and transient coordination to a highly conserved glutamate residue (Glu-67) during the catalytic cycle. The role of this transient coordination of the active-site zinc to Glu-67 in the FDH catalytic cycle and the assocd. coenzyme interactions were investigated by studying enzymes in which Glu-67 and Arg-368 were substituted with Leu. Structures of FDH·ADP ribose (ADP-ribose) and E67L·NAD(H) binary complexes were detd. Steady-state kinetics, isotope effects, and presteady-state anal. of the E67L enzyme show that Glu-67 is crit. for capturing the substrates for catalysis. The catalytic efficiency (V/Km) of the E67L enzyme in reactions involving S-nitrosoglutathione (GSNO), S-hydroxymethylglutathione (HMGSH) and 12-hydroxydodecanoic acid (12-HDDA) were 25 000-, 3000-, and 180-fold lower, resp., than for the wild-type enzyme. The large decrease in the efficiency of capturing GSNO and HMGSH by the E67L enzyme results mainly because of the impaired binding of these substrates to the mutant enzyme. In the case of 12-HDDA, a decrease in the rate of hydride transfer is the major factor responsible for the redn. in the efficiency of its capture for catalysis by the E67L enzyme. Binding of the coenzyme is not affected by the Glu-67 substitution. A partial displacement of the active-site zinc in the FDH·ADP-ribose binary complex indicates that the disruption of the interaction between Glu-67 and Arg-368 is involved in the displacement of active-site zinc. Kinetic studies with the R368L enzyme show that the predominant role of Arg-368 is in the binding of the coenzyme. An isomerization of the ternary complex before hydride transfer is detected in the kinetic pathway of HMGSH. Steps involved in the binding of the coenzyme to the FDH active site are also discerned from the unique conformation of the coenzyme in one of the subunits of the E67L·NAD(H) binary complex.>> More from SciFinder ®
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37.Structure of Escherichia coli AdhP (ethanol-inducible dehydrogenase) with bound NAD
Thomas, Leonard M.; Harper, Angelica R.; Miner, Whitney A.; Ajufo, Helen O.; Branscum, Katie M.; Kao, Lydia; Sims, Paul A.Acta Crystallographica, Section F: Structural Biology and Crystallization Communications (2013), 69 (7), 730-732CODEN: ACSFCL; ISSN:1744-3091. (International Union of Crystallography)
The crystal structure of AdhP, a recombinantly expressed alc. dehydrogenase from Escherichia coli K-12 (substrain MG1655), was detd. to 2.01 Å resoln. The structure, which was solved using mol. replacement, also included the structural and catalytic zinc ions and the cofactor NAD. The crystals belonged to space group P21, with a 68.18, b 118.92, c 97.87 Å, β 106.41°. The final R factor and R free were 0.138 and 0.184, resp. The structure of the active site of AdhP suggested a no. of residues that may participate in a proton relay, and the overall structure of AdhP, including the coordination to structural and active-site zinc ions, is similar to those of other tetrameric alc. dehydrogenase enzymes.>> More from SciFinder ®
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38.Tetrameric NAD-dependent alcohol dehydrogenase
Karlsson, Andreas; El-Ahmad, Mustapha; Johansson, Kenth; Shafqat, Jawed; Jornvall, Hans; Eklund, Hans; Ramaswamy, S.Chemico-Biological Interactions (2003), 143-144 (), 239-245CODEN: CBINA8; ISSN:0009-2797. (Elsevier Science Ireland Ltd.)
A review and discussion. Three-dimensional crystal structures of EtOH-induced, tetrameric alc. dehydrogenase (ADH) from Escherichia coli have recently been detd. in the absence and presence of NAD. The structure of E. coli ADH is similar to those of the dimeric mammalian alc. dehydrogenases, but it has a deletion of 21 residues located at the surface of the catalytic domain. The catalytic Zn2+ ions have 2 different types of coordination, which have also been obsd. in the class III dimeric mammalian enzyme. Comparison of the structures has provided new insights into the relation between tetrameric and dimeric ADHs and has provided a link to the structure of tetrameric yeast ADH.>> More from SciFinder ®
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There is no corresponding record for this reference. 40.Plapp, B. V., Charlier, H. A., Jr., and Ramaswamy, S. (2016) Mechanistic implications from structures of yeast alcohol dehydrogenase complexed with coenzyme and an alcohol Arch. Biochem. Biophys. 591, 35– 42 DOI: 10.1016/j.abb.2015.12.009[Crossref], [PubMed], [CAS]
40.Mechanistic implications from structures of yeast alcohol dehydrogenase complexed with coenzyme and an alcohol
Plapp, Bryce V.; Charlier, Henry A., Jr.; Ramaswamy, S.Archives of Biochemistry and Biophysics (2016), 591 (), 35-42CODEN: ABBIA4; ISSN:0003-9861. (Elsevier B.V.)
Yeast alc. dehydrogenase I is a homotetramer of subunits with 347 amino acid residues, catalyzing the oxidn. of alcs. using NAD+ as coenzyme. A new X-ray structure was detd. at 3.0 Å where both subunits of an asym. dimer bind coenzyme and trifluoroethanol. The tetramer is a pair of back-to-back dimers. Subunit A has a closed conformation and can represent a Michaelis complex with an appropriate geometry for hydride transfer between coenzyme and alc., with the oxygen of 2,2,2-trifluoroethanol ligated at 2.1 Å to the catalytic zinc in the classical tetrahedral coordination with Cys-43, Cys-153, and His-66. Subunit B has an open conformation, and the coenzyme interacts with amino acid residues from the coenzyme binding domain, but not with residues from the catalytic domain. Coenzyme appears to bind to and dissoc. from the open conformation. The catalytic zinc in subunit B has an alternative, inverted coordination with Cys-43, Cys-153, His-66 and the carboxylate of Glu-67, while the oxygen of trifluoroethanol is 3.5 Å from the zinc. Subunit B may represent an intermediate in the mechanism after coenzyme and alc. bind and before the conformation changes to the closed form and the alc. oxygen binds to the zinc and displaces Glu-67.>> More from SciFinder ®
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41.Crystal structure of the alcohol dehydrogenase from the hyperthermophilic archaeon Sulfolobus solfataricus at 1.85 Å resolution
Esposito, Luciana; Sica, Filomena; Raia, Carlo Antonio; Giordano, Antonietta; Rossi, Mose; Mazzarella, Lelio; Zagari, Adriana
Journal of Molecular Biology (2002), 318 (2), 463-477CODEN: JMOBAK; ISSN:0022-2836. (Elsevier Science Ltd.)
The crystal structure of a medium-chain NAD(H)-dependent alc. dehydrogenase (ADH) from an archaeon was solved by multiwave-length anomalous diffraction, using a selenomethionine-substituted enzyme. The protein (SsADH), extd. from the hyperthermophilic organism, S. solfataricus, was a homotetramer with crystallog. 222 symmetry. Despite the low level of sequence identity, the overall fold of the monomer was similar to that of the other homologous ADHs of known structure. However, a significant difference was the orientation of the catalytic domain relative to the coenzyme-binding domain that resulted in a larger interdomain cleft. At the bottom of this cleft, the catalytic Zn(II) ion was coordinated tetrahedrally and lacked the Zn-bound water mol. that is usually found in ADH apo-form structures. The 4th coordination position was indeed occupied by a Glu residue, as found in bacterial tetrameric ADHs. Other differences were found in the architecture of the substrate pocket whose entrance was more restricted than in other ADHs. SsADH is the 1st tetrameric ADH x-ray structure contg. a 2nd Zn(II) ion playing a structural role. This latter metal cation showed a peculiar coordination, with a Glu residue replacing one of the 4 Cys ligands that are highly conserved throughout the structural Zn-contg. dimeric ADHs.>> More from SciFinder ®
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42.Structural study of a single-point mutant of Sulfolobus solfataricus alcohol dehydrogenase with enhanced activity
Esposito, Luciana; Bruno, Ilaria; Sica, Filomena; Raia, Carlo Antonio; Giordano, Antonietta; Rossi, Mose; Mazzarella, Lelio; Zagari, Adriana
FEBS Letters (2003), 539 (1-3), 14-18CODEN: FEBLAL; ISSN:0014-5793. (Elsevier Science B.V.)
Alc. dehydrogenase from Sulfolobus solfataricus (SsADH) is the only enzyme from Archaea among the structurally studied members of the medium-chain ADH family described so far. Here, the authors present the 3-dimensional structure of the apo form of mutant N249Y which exhibits increased catalytic activity when compared to the wild-type enzyme. The substitution, located in the coenzyme binding domain, decreases the affinity for the NAD(H) cofactor. The rearrangement of segments 248-250 and 270-275, induced by the mutation, suggests an explanation for the lower coenzyme affinity. This study also highlights the role in SsADH catalysis of the flexible loops located at the interface between the catalytic and the coenzyme domains.>> More from SciFinder ®
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43.Carboxyl groups near the active site zinc contribute to catalysis in yeast alcohol dehydrogenase
Ganzhorn, Axel J.; Plapp, Bryce V.Journal of Biological Chemistry (1988), 263 (11), 5446-54CODEN: JBCHA3; ISSN:0021-9258.The importance of carboxyl groups near the active site Zn for the catalytic function of alc. dehydrogenase I from Saccharomyces cerevisiae was examd. by site-directed mutagenesis and steady state kinetics. Aspartate-49 was changed to asparagine (Asn) and glutamate-68 to glutamine (Gln) (residue numbering as for horse liver enzyme). The catalytic efficiencies (Vmax/Km) for EtOH oxidn. and acetaldehyde (AcH) redn. were decreased by factors of 1000 with the Asn-49 mutant and 100 with the Gln-68 enzyme. For the Asn-49 mutant, dissocn. consts. for coenzymes increased 7-fold, and Km and Ki values for substrates and substrate analogs increased by factors of 20-50. The turnover nos. were reduced 50-fold for EtOH oxidn. and 15-fold for AcH redn. Product and dead-end inhibition studies and kinetic isotope effects showed that the mechanism with NAD and EtOH was rapid equil. random, in contrast to the ordered mechanism of wild-type enzyme. Alc. dehydrogenase I and the Asn-49 mutant had similar CD spectra and 2 Zn atoms/subunit, but slightly different UV absorption and fluorescence spectra. The Gln-68 mutant resembled the wild-type enzyme in most kinetic consts., but the turnover no. for EtOH oxidn. decreased 35-fold, and the dissocn. const. for NAD and Km for AcH increased by factors of 4 and 50, resp. The pK values for Vmax and Vmax/Km for EtOH oxidn. were shifted from 7.7 (wild-type) to 6.8 in the Gln-68 and 6.2 in the Asn-49 mutant. The altered electrostatic environment near the active site Zn apparently decreases activities by hindering isomerizations of enzyme-substrate complexes.>> More from SciFinder ®
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Ryde, Ulf
Protein Science (1995), 4 (6), 1124-32CODEN: PRCIEI; ISSN:0961-8368. (Cambridge University Press)
Theor. computations (mol. dynamics and combined quantum chem. and mol. mech. geometry optimizations) were performed on horse liver alc. dehydrogenase. The results provided evidence that Glu-68, a highly conserved residue located 0.47 nm from the catalytic Zn, may intermittently coordinate to the Zn. Structures with Glu-68 coordinated to the Zn were almost as stable as structures with Glu-68 at the crystal position and the barrier between the 2 configurations of Glu-68 was so low that it could readily be bypassed at room temp. There was a cavity behind the Zn that appeared to be tailored to allow such coordination of Glu-68 to the Zn. It was suggested that Glu-68 may facilitate the exchange of ligands in the substrate site by coordinating to the Zn when the old ligand dissocs.>> More from SciFinder ®
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Plapp, Bryce V.; Eklund, Hans; Branden, Carl Ivar
Journal of Molecular Biology (1978), 122 (1), 23-32CODEN: JMOBAK; ISSN:0022-2836.Horse liver alc. dehydrogenase was crystd. at pH 7 and 5° from an equil. mix. contg. predominantly NAD and p-bromobenzyl alc. and lower concns. of NADH and p-bromobenzaldehyde. Most of the enzyme was complexed with NAD and p-bromobenzyl alc. A ternary complex with NAD and trifluoroethanol and complexes with NAD or NADH and 2-methyl-2,4-pentanediol were also crystd. These complexes formed triclinic crystals that were isomorphous with the enzyme-NADH-Me2SO complex, which was solved to a resoln. of 4.5 Å. Electron d. difference maps, showed that both subunits of the dimeric enzyme bound p-bromobenzyl alc. or trifluoroethanol in a substrate binding pocket close to the position occupied by Me2SO. These complexes appeared to have the same protein and coenzyme structure as the complex with NADH and Me2SO. As Me2SO appeared to be ligated to the catalytic Zn atom, it was not possible to see a direct connection between the alcs. and Zn in these difference maps. However, maps computed from the differences in amplitudes between the NAD-alc. complexes and NAD(H)-methylpentanediol complexes and the phases from the NADH-Me2SO complex showed continuous electron d. from the bromo or

trifluoromethyl positions of the substrates to Zn. A provisional model of the active site was constructed. The O atom of either alc. was directly ligated to Zn (2 Å), whereas C 1 of the alc. was 3.5 Å from C 4 of the nicotinamide ring.>> More from SciFinder ®
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Abdallah, Mohammed A.; Biellmann, Jean F.; Nordstroem, Bo; Braenden, Carl I.European Journal of Biochemistry (1975), 50 (3), 475-81CODEN: EJBCAI; ISSN:0014-2956.Coenzyme NAD analogs, 8-bromoadenosine diphosphoribose (I) and nicotinamide 8-bromoadenine dinucleotide (II), were prepd. and complexed with liver alc. dehydrogenase. The complexes were studied by crystallog. methods. X-ray data for the complex formed by alc. dehydrogenase and I and for the alc. dehydrogenase-II complex showed that adenosine diphosphoribose has a conformation very similar to the corresponding moiety of NAD when NAD is bound to lactate and malate dehydrogenase. I had the same anti conformation of the adenine ring with respect to ribose as did adenosine diphosphoribose and NAD. This is in contrast to the syn conformation found in 8-bromoadenosine. The overcrowding at the 8-position is relieved in I by having the ribose moiety in the 2'-endo conformation instead of the usual 3'-endo position as in adenosine diphosphoribose and NAD.>> More from SciFinder ®
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Gibbons, Brian J.; Hurley, Thomas D.Biochemistry (2004), 43 (39), 12555-12562CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Formamides are aldehyde analogs that have demonstrated potent and selective inhibition of human alc. dehydrogenase isoenzymes. The αα, β1β1, γ2γ2, and σσ isoforms have all been found to be strongly inhibited by substituted formamides. In this paper, the structure of the αα isoform of human alc. dehydrogenase complexed with N-cyclopentyl-N-cyclobutylformamide was detd. by x-ray crystallog. to 2.5 Å resoln., the β1β1 isoform of human alc. dehydrogenase complexed with N-benzylformamide and with N-heptylformamide was detd. to 1.6 and 1.65 Å resoln., resp., and the structure of the γ2γ2 isoform complexed with N-1-methylheptylformamide was detd. to 1.45 Å resoln. These structures provide the first substrate-level view of the local structural differences that give rise to the individual substrate preferences shown by these highly related isoenzymes. Consistent with previous work, the carbonyl oxygen of the inhibitors interacts directly with the catalytic zinc and the hydroxyl group of Thr-48 (Ser-48 for γ2γ2) of the enzyme. The benzene ring of N-benzylformamide and the carbon chains of N-heptylformamide and N-1-methylheptylformamide interact with the sides of the hydrophobic substrate pocket whose size and shape is dictated by residue exchanges between the β1β1 and γ2γ2 isoenzymes. In particular, the exchange of Ser for Thr at position 48 and the exchange of Val for Leu at position 141 in the γ2γ2 isoenzyme create an environment with stereoselectivity for the R-enantiomer of the branched N-1-methylheptylformamide inhibitor in this isoenzyme. The primary feature of the αα isoform is the Ala for Phe-93 exchange that enlarges the active site near the catalytic zinc and creates the specificity for the branched N-cyclopentyl-N-cyclobutylformamide inhibitor, which shows the greatest selectivity for this unique isoenzyme of any of the formamide inhibitors.>> More from SciFinder ®
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Zeppezauer, Eila; Soderberg, Bengt O.; Branden, Carl I.; Akeson, Ake; Theorell, Hugo
Acta Chemica Scandinavica (1947-1973) (1967), 21 (4), 1099-1101CODEN: ACSAA4; ISSN:0001-5393.Crystals of horse liver alc. dehydrogenase (LADH) were obtained suitable for a crystal-structure detn. of LADH. A stock suspension of microcrystals of homogeneous LADH maintained at -20° in a 30% EtOH-H2O soln. was centrifuged at -20°, the residual crystals were dissolved in 0.5M Tris-HCl buffer (pH 9.4) at 4°, and the soln. was dialyzed first against 0.05M Tris-HCl buffer (pH 9.4) at 4° for 24 hrs. and then for another 24 hrs. against this buffer at pH 8.4 contg. EtOH 5%. Denatured protein was removed by centrifugation, the remaining soln. was dild. with buffer to a final protein concn. of 0.5 or 1% and transferred to dialysis bags contg. 1 ml. protein soln., and each bag was placed in a bottle contg; 20 ml. buffer at pH 8.4 and EtOH to a concn. just below the point of pptn. Binary and ternary complexes were prepd. by the addn. of excess coenzyme and inhibitor to the protein soln.; the complexes were dialyzed during crystn. against buffer contg. excess coenzyme and inhibitor; successive small addns. of alc. were made until pptn. began (where the coenzyme was employed in the form of NAD, rather than the NADH with which EtOH was used, MeOH was employed for crystn.), and crystal growth was allowed to proceed for several days before further growth, without the formation of new crystals, could be achieved by successively increasing the alc. concn. another 5-10%. X-ray emposures of these crystals at 4° enabled extensions of diffraction patterns to a resolution of at least 2.5 A., but there was a sharp decrease in the intensities of reflections beyond 2.8 A. Results observed here and elsewhere indicated that the whole coenzyme was required for a conformational change Different types of crystals were obtained from different cryst. solns.>> More from SciFinder ®
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The Journal of biological chemistry (1970), 245 (7), 1727-35 ISSN:0021-9258.There is no expanded citation for this reference.>> More from SciFinder ®
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Yonetani, T.; Theorell, H.Archives of Biochemistry and Biophysics (1964), 106 (1), 243-51CODEN: ABBIA4; ISSN:0003-9861.cf. CA 60, 1999b. A graphic method was devised to analyze the multiple inhibition of an enzyme by 2 competitive inhibitors. This method not only distinguishes whether 2 inhibitors interact with the same site or different sites of the enzyme, but also gives an interaction const.(α) between 2 inhibitors in the enzyme-inhibitor complex. This method was applied to the multiple inhibition of liver alc. dehydrogenase by coenzyme-competitive inhibitor pairs among o-phenanthroline, adenosine diphosphate ribose, and adenosine 5'-mono- and diphosphates. The present kinetic analysis quant. reconfirmed the previous conclusion that o-phenanthroline and adenosine diphosphate ribose interact independently with different sites of the enzyme.>> More from SciFinder ®
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Schindler, John F.; Berst, Kristine B.; Plapp, Bryce V.Journal of Medicinal Chemistry (1998), 41 (10), 1696-1701CODEN: JMCMAR; ISSN:0022-2623. (American Chemical Society)
Human alc. dehydrogenase (HsADH) comprises class I (α, β, and γ), class II (π), and class IV (σ) enzymes. Selective inhibitors of the enzymes could be used to prevent the metab. of alcs. that form toxic products. Formamides are unreactive analogs of aldehydes and bind to the enzyme-NADH complex [Ramaswamy, S.; Scholze, M.; Plapp, B. V. Biochem. 1997, 36, 3522-3527]. They are uncompetitive inhibitors against varied concns. of alc., and this makes them effective even with satg. concns. of alcs. Mol. modeling led to the design and synthesis of a series of cyclic, linear, and disubstituted formamides. Evaluation of 23 compds. provided structure-function information and selective inhibitors for the enzymes, which have overlapping but differing substrate specificities. Monosubstituted formamides are good inhibitors of class I and II enzymes, and disubstituted formamides are selective for the α enzyme. Selective inhibitors, with Ki values at pH 7 and 25 °C of 0.33-0.74 μM, include N-cyclopentyl-N-cyclobutylformamide for HsADH α, N-benzylformamide for HsADH β1, N-1-methylheptylformamide for HsADH γ2, and N-heptylformamide for HsADH σ and HsADH β1.>> More from SciFinder ®

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52.The finer things in X-ray diffraction data collection
Pflugrath, J. W.Acta Crystallographica, Section D: Biological Crystallography (1999), D55 (10), 1718-1725CODEN: ABCRE6; ISSN:0907-4449. (Munksgaard International Publishers Ltd.)
X-ray diffraction images from two-dimensional position-sensitive detectors can be characterized as thick or thin, depending on whether the rotation-angle increment per image is greater than or less than the crystal mosaicity, resp. The expectations and consequences of the processing of thick and thin images in terms of spatial overlap, satd. pixels, X-ray background and I/σ(I) are discussed. The d*TREK software suite for processing diffraction images is briefly introduced, and results from d*TREK are compared with those from another popular package.>> More from SciFinder ®
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53.Kabsch, W. (2010) XDS Acta Crystallogr., Sect. D: Biol. Crystallogr. 66, 125– 132 DOI: 10.1107/S0907444909047337[Crossref], [PubMed], [CAS]
53.Software XDS for image rotation, recognition and crystal symmetry assignment
Kabsch, Wolfgang
Acta Crystallographica, Section D: Biological Crystallography (2010), 66 (2), 125-132CODEN: ABCRE6; ISSN:0907-4449. (International Union of Crystallography)
The usage and control of recent modifications of the program package XDS for the processing of rotation images are described in the context of previous versions. New features include automatic detn. of spot size and reflecting range and recognition and assignment of crystal symmetry. Moreover, the limitations of earlier package versions on the no. of correction/scaling factors and the representation of pixel contents have been removed. Large program parts have been restructured for parallel processing so that the quality and completeness of collected data can be assessed soon after measurement.>> More from SciFinder ®
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54.Overview of the CCP4 suite and current developments
Winn, Martyn D.; Ballard, Charles C.; Cowtan, Kevin D.; Dodson, Eleanor J.; Emsley, Paul; Evans, Phil R.; Keegan, Ronan M.; Krissinel, Eugene B.; Leslie, Andrew G. W.; McCoy, Airlie; McNicholas, Stuart J.; Murshudov, Garib N.; Pannu, Navraj S.; Potterton, Elizabeth A.; Powell, Harold R.; Read, Randy J.; Vagin, Alexei; Wilson, Keith S.Acta Crystallographica, Section D: Biological Crystallography (2011), 67 (4), 235-242CODEN: ABCRE6; ISSN:0907-4449. (International Union of Crystallography)
A review. The CCP4 (Collaborative Computational Project, No. 4) software suite is a collection of programs and assocd. data and software libraries which can be used for macromol. structure detn. by X-ray crystallog. The suite is designed to be flexible, allowing users a no. of methods of achieving their aims. The programs are from a wide variety of sources but are connected by a common infrastructure provided by std. file formats, data objects and graphical interfaces. Structure soln. by macromol. crystallog. is becoming increasingly automated and the CCP4 suite includes several automation pipelines. After giving a brief description of the evolution of CCP4 over the last 30 years, an overview of the current suite is given. While detailed descriptions are given in the accompanying articles, here it is shown how the individual programs contribute to a complete software package.>> More from SciFinder ®
https://chemport.cas.org/services/resolver?origin=ACS&resolution=options&coi=1%3ACAS%3A528%3ADC%252BC3MXktFWqt70%253D&md5=c407e4d47bef46864be336d60147c17d
55.Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models Acta Crystallogr., Sect. A: Found. Crystallogr. 47, 110– 119 DOI: 10.1107/S0108767390010224[Crossref], [PubMed], [CAS]
55.Improved methods for building protein models in electron density maps and the location of errors in these models
Jones T A; Zou J Y; Cowan S W; Kjeldgaard M
Acta crystallographica. Section A, Foundations of crystallography (1991), 47 (Pt 2) (), 110-9 ISSN:0108-7673.Map interpretation remains a critical step in solving the structure of a macromolecule. Errors introduced at this early stage may persist throughout crystallographic refinement and result in an incorrect structure. The normally quoted crystallographic residual is often a poor description for the quality of the model. Strategies and tools are described that help to alleviate this problem. These simplify the model-building process, quantify the goodness of fit of the model on a per-residue basis and locate possible errors in peptide and side-chain conformations.>> More from SciFinder ®
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56.Crystal structure refinement with SHELXL
Sheldrick, George M.Acta Crystallographica, Section C: Structural Chemistry (2015), 71 (1), 3-8CODEN: ACSCGG; ISSN:2053-2296. (International Union of Crystallography)
The improvements in the crystal structure refinement program SHELXL have been closely coupled with the development and increasing importance of the CIF (Crystallog. Information Framework) format for validating and archiving crystal structures. An important simplification is that now only one file in CIF format (for convenience, referred to simply as 'a CIF') contg. embedded reflection data and SHELXL instructions is needed for a complete structure archive; the program SHREDCIF can be used to ext. the and files required for further refinement with SHELXL. Recent developments in SHELXL facilitate refinement against neutron diffraction data, the treatment of H atoms, the detn. of abs. structure, the input of partial structure factors and the refinement of twinned and disordered structures. SHELXL is available free to academics for the Windows, Linux and Mac OS X operating systems, and is particularly suitable for multiple-core processors.>> More from SciFinder ®
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57.MolProbity: all-atom structure validation for macromolecular crystallography
Chen, Vincent B.; Arendall, W. Bryan, III; Headd, Jeffrey J.; Keedy, Daniel A.; Immormino, Robert M.; Kapral, Gary J.; Murray, Laura W.; Richardson, Jane S.; Richardson, David C.Acta Crystallographica, Section D: Biological Crystallography (2010), 66 (1), 12-21CODEN: ABCRE6; ISSN:0907-4449. (International Union of Crystallography)
MolProbity is a structure-validation web service that provides broad-spectrum solidly based evaluation of model quality at both the global and local levels for both proteins and nucleic acids. It relies heavily on the power and sensitivity provided by optimized hydrogen placement and all-atom contact anal., complemented by updated versions of covalent-geometry and torsion-angle criteria. Some of the local corrections can be performed automatically in MolProbity and all of the diagnostics are presented in chart and graphical forms that help guide manual rebuilding. X-ray crystallog. provides a wealth of biol. important mol. data in the form of at. three-dimensional structures of proteins, nucleic acids and increasingly large complexes in multiple forms and states. Advances in automation, in everything from crystn. to data collection to phasing to model building to refinement, have made solving a structure using crystallog. easier than ever. However, despite these improvements, local errors that can affect biol. interpretation are widespread at low resoln. and even high-resoln. structures nearly all contain at least a few local errors such as Ramachandran outliers, flipped branched protein side chains and incorrect sugar puckers. It is crit. both for the crystallographer and for the end user that there are easy and reliable methods to diagnose and correct these sorts of errors in structures. MolProbity is the authors' contribution to helping solve this problem and this article reviews its general capabilities, reports on recent enhancements and usage, and presents evidence that the resulting improvements are now beneficially affecting the global database.>> More from SciFinder ®
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Harris, Mark; Jones, T. Alwyn
Acta Crystallographica, Section D: Biological Crystallography (2001), D57 (8), 1201-1203CODEN: ABCRE6; ISSN:0907-4449. (Munksgaard International Publishers Ltd.)
A publicly available web-based interface is presented for producing high-quality ray-traced images and movies from the mol. modeling program O. The interface allows the user to select O-plot files and set parameters to create std. input files for the popular ray-tracing renderer POV-Ray, which can then produce publication-quality still images or simple movies. To ensure ease of use, this service were made available to the O user community via the World Wide Web.>> More from SciFinder ®
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There is no corresponding record for this reference. 60.Bignetti, E., Rossi, G. L., and Zeppezauer, E. (1979) Microspectrophotometric measurements on single crystals of coenzyme containing complexes of horse liver alcohol dehydrogenase FEBS Lett. 100, 17– 22 DOI: 10.1016/0014-5793(79)81122-9[Crossref], [PubMed], [CAS]
60.Microspectrophotometric measurements on single crystals of coenzyme containing complexes of horse liver alcohol dehydrogenase
Bignetti, Enrico; Rossi, Gian Luigi; Zeppezauer, Eila
FEBS Letters (1979), 100 (1), 17-32CODEN: FEBLAL; ISSN:0014-5793.Microspectrometric measurements of single crystals contg. liver alc. dehydrogenase-coenzyme-substrate complexes were performed as a rapid method to detect NADH present in the lattice, follow the binding of the chromophoric substrate, trans-4-N,N-dimethylaminocinnamaldehyde, in the active site, and to test for substrate conversion in crystals of complexes used for x-ray anal. The findings confirmed the need for a direct method for recognizing the nature of enzyme complexes actually present within a crystal before a crystallog. study is undertaken. The microspectrometric measurements also can be used to explore the conditions from which one can obtain a desired redox state of an enzyme complex in the cryst. state.>> More from SciFinder ®
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61.Alternative pathways and reactions of benzyl alcohol and benzaldehyde with horse liver alcohol dehydrogenase
Shearer, Gretchen L.; Kim, Keehyuk; Lee, Kang Man; Wang, C. Kathy; Plapp, Bryce V.Biochemistry (1993), 32 (41), 11186-94CODEN: BICHAW; ISSN:0006-2960.Liver alc. dehydrogenase catalyzes the reaction of NAD+ and benzyl alc. to form NADH and benzaldehyde by a predominantly ordered reaction. However, enzyme-alc. binary and abortive ternary complexes form at high concns. of benzyl alc., and benzaldehyde is slowly oxidized to benzoic acid. Steady-state and transient kinetic studies, equil. spectrophotometric measurements, product anal., and kinetic simulations provide ests. of rate consts. for a complete mechanism with the following reactions: (1) E ⇆ E-NAD+⇆E-NAD+-RCH2OH h E-NADH-RCHO h E-NADH h E; (2) E-NADH hE-NADH-RCH2OH h E-RCH2OH h E; (3) E-NAD+ h E-NAD+-RCHO h E-NADH-RCOOH h E-NADH. The internal equil. const. for hydrogen transfer detd. at 30°C and pH 7 is about 5:1 in favor of E-NAD+-RCH2OH and has a complex pH dependence. Benzyl alc. binds weakly to free enzyme (Kd = 7 mM) and significantly decreases the rates of binding of NAD+ and NADH. The reaction of NAD+ and benzyl alc. is therefore kinetically ordered, not random. High concns. of benzyl alc. (> 1 mM) inhibit turnover by formation of the abortive E-NADH-RCH2OH complex, which dissocs. at 0.3 s-1 as compared to 6.3 s-1 for E-NADH. The oxidn. of benzaldehyde by E-NAD+ (Km = 15 mM, V/E = 0.4 s-1) is inefficient relative to the oxidn. of benzyl alc. (Km = 28 μM, V/E = 3.1 s-1) and leads to a dismutation (2RCHO → RCH2OH + RCOOH) as E-NADH reduces benzaldehyde. The results provide a description of final product distributions for the alternative reactions catalyzed by the multifunctional enzyme.>> More from SciFinder ®
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62.Effects of Cavities at the Nicotinamide Binding Site of Liver Alcohol Dehydrogenase on Structure, Dynamics and Catalysis
Yahashiri, Atsushi; Rubach, Jon K.; Plapp, Bryce V.Biochemistry (2014), 53 (5), 881-894CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
A role for protein dynamics in enzymic catalysis of hydrogen transfer has received substantial scientific support, but the connections between protein structure and catalysis remain to be established. Valine residues 203 and 207 are at the binding site for the nicotinamide ring of the coenzyme in liver alc. dehydrogenase (ADH) and have been suggested to facilitate catalysis with "protein-promoting vibrations" (PPV). We find that the V207A substitution has small effects on steady-state kinetic consts. and the rate of hydrogen transfer; the introduced cavity is empty and is tolerated with minimal effects on structure (detd. at 1.2 Å for the complex with NAD+ and 2,3,4,5,6-pentafluorobenzyl alc., or PFB). Thus, no evidence is found to support a role for Val-207 in the dynamics of catalysis. The protein structures and ligand geometries (including donor-acceptor distances) in the V203A enzyme complexed with NAD+ and 2,3,4,5,6-pentafluorobenzyl alc. or 2,2,2-trifluoroethanol (detd. at 1.1 Å) are very similar to those for the wild-type enzyme, except that the introduced cavity accommodates a new water mol. that contacts the nicotinamide ring. The structures of the V203A enzyme complexes suggest, in contrast to previous studies, that the diminished tunneling and decreased rate of hydride transfer (16-fold, relative to that of the wild-type enzyme) are not due to differences in ground-state ligand geometries. The V203A substitution may alter the PPV and the reorganization energy for hydrogen transfer, but the protein scaffold and equil. thermal motions within the Michaelis complex may be more significant for enzyme catalysis.>> More from SciFinder ®
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Rubach, Jon K.; Plapp, Bryce V.Biochemistry (2003), 42 (10), 2907-2915CODEN: BICHAW; ISSN:0006-2960. (American Chemical Society)
Amino acid residues Thr-178, Val-203, and Val-292, which interact with the nicotinamide ring of the coenzyme bound to alc. dehydrogenase (ADH), may facilitate hydride transfer and H tunneling by orientation and dynamic effects. Here, the T178S, T178V, V203A, V292A, V292S, and V292T substitutions of horse liver ADH significantly altered the steady state and transient kinetics of the enzyme. The V292A, V292S, and V292T enzymes had decreased affinity for coenzyme (NAD by 30-50-fold and NADH by 35-75-fold) as compared to wild-type ADH. The substitutions in the nicotinamide binding site decreased the rate const. of hydride transfer for benzyl alc. oxidn. by 3-fold (for V292T ADH) to 16-fold (for V203A ADH). The modest effects suggested that catalysis does not depend critically on individual residues and that several residues in the nicotinamide binding site contribute to catalysis. The x-ray crystal structures of the V292T ADH·NAD·pyrazole and wild-type ADH·NAD·4-iodopyrazole ternary complexes were very similar. Only subtle changes in the V292T enzyme caused the large changes in coenzyme binding and the small change in hydride transfer. In these complexes, one pyrazole N atom bound to the catalytic Zn atom, and the other N atom formed a partial covalent bond with the C4 atom of the nicotinamide ring, which adopted a boat conformation that was postulated to be relevant for hydride transfer. The results provided an exptl. basis for evaluating the contributions of dynamics to hydride transfer.>> More from SciFinder ®
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64.Combining Electronic Structure Methods with the Calculation of Hydrogen Vibrational Wavefunctions: Application to Hydride Transfer in Liver Alcohol Dehydrogenase
Webb, Simon P.; Agarwal, Pratul K.; Hammes-Schiffer, Sharon
Journal of Physical Chemistry B (2000), 104 (37), 8884-8894CODEN: JPCBFK; ISSN:1089-5647. (American Chemical Society)
This paper presents an application of a computational approach combining electronic structure methods with the calcn. of hydrogen vibrational wave functions. This application is directed at elucidating the nature of the nuclear quantum mech. effects in the oxidn. of benzyl alc. catalyzed by liver alc. dehydrogenase (LADH). The hydride transfer from the benzyl alc. substrate to the NAD+ cofactor is described by a 148-atom model of the active site. The hydride potential energy curves and the assocd. hydrogen vibrational wave functions are calcd. for structures along min. energy paths and straight-line reaction paths obtained from electronic structure calcns. at the semiempirical PM3 and ab initio RHF/3-21G levels. The results indicate that, for these levels of theory, the hydride transfer is adiabatic and hydrogen tunneling does

not play a crit. role along the min. energy path. In contrast, nonadiabatic effects and hydrogen tunneling are shown to be important along the more relevant straight-line reaction paths. The secondary hydrogens were found to be significantly coupled to the transferring hydride near the transition state. In addn., the puckering of the NAD+ ring was found to be a dominant contribution to the reaction coordinate near the transition state. Further from the transition state, the reaction coordinate is a mixt. of many heavy-atom modes, including the donor-acceptor distance and the distance between the substrate and the neighboring zinc and serine residue. These results imply that hydrogen tunneling in LADH is strongly impacted by the puckering of the NAD+ ring (which modulates the asymmetry of the hydride potential energy curve) and the distance between the donor and acceptor carbons (which modulates the barrier of the hydride potential energy curve).>> More from SciFinder ®
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65.Hydride Transfer in Liver Alcohol Dehydrogenase: Quantum Dynamics, Kinetic Isotope Effects, and Role of Enzyme Motion
Billeter, Salomon R.; Webb, Simon P.; Agarwal, Pratul K.; Iordanov, Tzvetelin; Hammes-Schiffer, Sharon
Journal of the American Chemical Society (2001), 123 (45), 11262-11272CODEN: JACSAT; ISSN:0002-7863. (American Chemical Society)
The quantum dynamics of the hydride transfer reaction catalyzed by liver alc. dehydrogenase (LADH) are studied with real-time dynamical simulations including the motion of the entire solvated enzyme. The electronic quantum effects are incorporated with an empirical valence bond potential, and the nuclear quantum effects of the transferring hydrogen are incorporated with a mixed quantum/classical mol. dynamics method in which the transferring hydrogen nucleus is represented by a three-dimensional vibrational wave function. The equil. transition state theory rate consts. are detd. from the adiabatic quantum free energy profiles, which include the free energy of the zero point motion for the transferring nucleus. The nonequil. dynamical effects are detd. by calcg. the transmission coeffs. with a reactive flux scheme based on real-time mol. dynamics with quantum transitions (MDQT) surface hopping trajectories. The values of nearly unity for these transmission coeffs. imply that nonequil. dynamical effects such as barrier recrossings are not dominant for this reaction. The calcd. deuterium and tritium kinetic isotope effects for the overall rate agree with exptl. results. These simulations elucidate the fundamental nature of the nuclear quantum effects and provide evidence of hydrogen tunneling in the direction along the donor-acceptor axis. An anal. of the geometrical parameters during the equil. and nonequil. simulations provides insight into the relation between specific enzyme motions and enzyme activity. The donor-acceptor distance, the catalytic zinc-substrate oxygen distance, and the coenzyme (NAD+/NADH) ring angles are found to strongly impact the activation free energy barrier, while the donor-acceptor distance and one of the coenzyme ring angles are found to be correlated to the degree of barrier recrossing. The distance between VAL-203 and the reactive center is found to significantly impact the activation free energy but not the degree of barrier recrossing. This result indicates that the exptl. obsd. effect of mutating VAL-203 on the enzyme activity is due to the alteration of the equil. free energy difference between the transition state and the reactant rather than nonequil. dynamical factors. The promoting motion of VAL-203 is characterized in terms of steric interactions involving THR-178 and the coenzyme.>> More from SciFinder ®
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66.Dynamic Structures of Horse Liver Alcohol Dehydrogenase (HLADH): Results of Molecular Dynamics Simulations of HLADH-NAD+-PhCH2OH, HLADH-NAD+-PhCH2O-, and HLADH-NADH-PhCHO
Luo, Jia; Bruice, Thomas C.Journal of the American Chemical Society (2001), 123 (48), 11952-11959CODEN: JACSAT; ISSN:0002-7863. (American Chemical Society)
Mol. dynamics simulations of the oxidn. of benzyl alc. by horse liver alc. dehydrogenase (HLADH) have been carried out. The following three states have been studied: HLADH·PhCH2OH·NAD+ (MD1), HLADH·PhCH2O-·NAD+ (MD2), and HLADH·PhCHO·NADH (MD3). MD1, MD2, and MD3 simulations were carried out on one of the subunits of the dimeric enzyme covered in a 32-Å-radius sphere of TIP3P water centered on the active site. The proton produced on ionization of the alc. when HLADH·PhCH2OH·NAD+ → HLADH·PhCH2O-·NAD+ is transferred from the active site to solvent water via a hydrogen bonding network consisting of Ser48 hydroxyl, ribose 2'- and 3'-hydroxyl groups, and His51. Hydrogen bonding of the 3'-OH of ribose to Ile269 carbonyl maintains this proton in position to be transferred to water. Mol. dynamic simulations have been employed to track water1287 from the TIP3 water pool to the active site, thus exhibiting the mode of entrance of water to the active site. With time the water1287 accumulates in two different positions in order to accept the proton from the ribose 3'-OH and from His51. There can be identified two structural substates for proton passage. In the first substate the imidazole Ne2 of His51 is adjacent to the nicotinamide ribose C2'-OH and hydrogen bonding distances for proton transfer through the hydrogen bonded relay series PhCH2OH···Ser48-OH···Ribose2'-OH···His51···OH2 (path 1) av. 2.0, 2.0, and 2.1 Å and (for His51···OH2) minimal distances less or equal to 2.5 Å. The structure for path 1 is present 20% of the time span. And in the second substate, there are two possible proton passages:path 1 as before and path 2. Path 2 involves the hydrogen-bonded relay series PhCH2OH···Ser48-OH···Ribose2'-OH···Ribose3'-OH···His51···OH2 with the av. bonding distances being 2.0, 2.0, 2.1, and 2.0 Å and (for His51····OH2) minimal distances less or equal to 2.5 Å (20% probability of the time span), resp. During the mol. dynamics simulation the NAD+ ribose conformations have stabilized at the C2'-endo-C3'-exo or the C2'-endo conformations. With the C2'-endo conformation the first and second substates are able to persist for different time spans, while with the C2'-endo-C3'-exo conformation the only possible pathway involves the first substate. For both first and second substates the fluctuation of the distances between the ribose-OH protons and Nε2 of His51 imidazole ring is partially contributed by the windshield wiper motion of the His51 imidazole ring. Since the imidazole of His-51 contributes only about 10-fold to activity, as estd. from the decrease in activity upon substitution with a Gln, there must be an alternate route for the proton to pass to solvent without going through this histidine. A third pathway involves ribose C3'-OH and Ile-269. In MD2, near attack conformers (NACs) for hydride transfer from PhCH2O- to NAD+ represent ∼60% of E·S conformers. The mol. dynamic study of MD3 at mildly basic pH reveals that reactive ground state conformers (NACs) for hydride transfer from NADH to PhCHO amt. to 12 mol % of conformers. In MD3, anisotropic bending of the dihydronicotinamide ring of NADH (av. value of αc = 4.0° and αn = 0.5°, resp.) is obsd.>> More from SciFinder ®
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67.Evaluation of the factors influencing reactivity and stereospecificity in NAD(P)H dependent dehydrogenase enzymes
Almarsson, Orn; Bruice, Thomas C.Journal of the American Chemical Society (1993), 115 (6), 2125-38CODEN: JACSAT; ISSN:0002-7863.Calcns. of AM1 potential energies for information of N-(1,β-ribosyl)-1,4-dihydronicotinamide (3H) and N-(1,β-ribosyl)nicotinamide (3), as defined by the nicotinamide ring deformation angles αC and αN and the torsion angles Xn and Xam, have been carried out. Xn Is the angle of rotation of the nicotinamide ring relative to the ribose ring and the conformers are designated as syn (nicotinamide-CONH2 and ribose ring O are close) and anti (nicotinamide-CONH2 and ribose ring O are distant). The angles αC and αN reflect the degree of bending of nicotinamide N and C4 out of the plane with C2, C3, C5, and C6 to provide quasi-boat and quasi-half-chair conformations. With the 1,4-dihydronicotinamide ring in a quasi-boat geometry, the relationship of unshared electron pairs on the ribose ether O and nicotinamide ring N is described as periplanar or antiperiplanar. The quasi-boat conformations may then be described as anti antiperiplanar (1a), sn antiperiplanar (1b), anti periplanar (1c), and syn periplanar (1d). The AM1 potential energies expended in the deformation of the 1,4-dihydropyridine ring to quasi-boat or quasi-half-chair conformations (with αC + αN ≤ 20° and αC ≥ αN) are less than the gain in the accompanying stabilization of the transition-state in the redn. of a carbonyl function by transfer of the pseudoaxial hydrogen at C4. With αC = 15° and αN = 5°, the potential energies of 3 as a function of Xn is raised relative to its global min. by an av. value of 16 kcal/mol. To bend the flat 1,4-dihydronicotinamide of 3H such that αC = 15° and αN = 5° costs 1.8 kcal/mol, while the activation enthalpy is decreased by 6 kcal/mol compared with the flat nicotinamide. In the bent conformation, the initial state [3H + H2O:O···HImH+] and immediate product state [3 + H3COH··ImH] potential energies are not greatly different. Energetically, there is little difference as to whether HR or HS are the transferred entities at the pseudoaxial positions. From structures deposited in the Brookhaven database, Xn = -87° to -140° (conformation 1a) for A-specific and Xn = 45° to 66° (conformation 1b) for B-specific dehydrogenases. The mismatch in the preferred values of Xn for unbound (AM1) and enzyme bound (Brookhaven database) reduced and oxidized cofactors has little influence on the equil. consts. for cofactor binding which favors the reduced form. CHARMm mol. dynamics simulations show the deformation of the αC + αN of 3H to A- and B-sides is isoenergetic when 3H is not enzyme bound. The same expts. with dogfish muscle lactate dehydrogenase show the motion of the NAD+ and NADH torsional angles Xn and Xam, as well as the puckering angle αC for C4, are quite flexible as predicted by AM1 calcns. Mol. dynamics simulations with lobster D-glyceraldehyde 3-phosphate dehydrogenase, L casei dihydrofolate reductase and porcine heart malate dehydrogenase also show the puckering angle αC for C4 of NADH to be quite flexible. With the various dehydrogenases the puckering motion of NADH is anisotropic, such that the C4 predominantly bends toward the substrate binding site. For each enzyme, steric hindrance by hydrophobic residues on the distal face of the cofactor are responsible for this anisotropic movement. In the dogfish lactate dehydrogenase pyruvate-NADH complex, the transferable pseudoaxial HR at C4 comes within van der Waals contact with the carbonyl carbon of the substrate. Further, dynamics calcns. indicate that the protonated imidazole

group of HIS 193 is required not only as a general-acid catalyst but also for the correct alignment of pyruvate. The important catalytic features of the dogfish lactate dehydrogenase were examd. by AM1 calcns. using the x-ray coordinates for the enzyme around the active site and by mol. dynamics calcns. using the entire enzyme. These features are the following: (i) in the ground state, approach of all reactants to within van der Waals radii; (ii) in the transition state, proton transfer from imidazolium cation (ImH2+) of HIS 193 to substrate carbonyl oxygen is almost complete while hydrogen transfer from 1,4-dihydronicotinamide to carbonyl carbon is about midway. The late transition state for proton transfer from ImH2+ is facilitated by the repulsive pos. charges of ImH2+ and the arginine guanidinium cation substituent of ARG 106. It is proposed that the major driving forces in the catalysis are the steric compression in the ground state, the lateness of proton transfer which provides a large partial pos. charge on the carbonyl carbon, and the preequil. puckering of the 1,4-dihydronicotinamide ring to quasi-boat conformation with transfer of the pseudoaxial hydrogen. The favoring of A- or B-side hydrogen transfer is detd. by shielding of one side of the 1,4-dihydronicotinamide ring, which prevents access of substrate to and dynamic deformation at the unblocked side. An earlier proposal that dehydrogenases evolved in such a manner to level their dynamics by having NADH assume the weaker reductant anti antiperiplanar conformation (1a) in HR transfer (A-side) and the stronger reductant syn antiperiplanar conformation (1b) in HS transfer (B-side) cannot be correct on the basis of the AM1 potential energies of these conformations (i.e., 1a and 1b should be comparable reducing agents). In L. casei dihydrofolate reductase, the weakly polar interactions of oxygen functions of THR 45, ILE 13, and ALA 97 with the hydrogens of C2-H, C4-H, and C6-H of NADPH do not represent hydrogen bonds previously thought to be important in the activation of the cofactor. In the dehydrogenases in general, the amino acid oxygen functionalities which point inwards toward the nicotinamide ring are involved in hydrogen bonds to water mols. in the apoenzyme thereby heat of formation keeping the cofactor binding site open.>> More from SciFinder ®
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Harding, Marjorie M.Acta Crystallographica, Section D: Biological Crystallography (2001), D57 (3), 401-411CODEN: ABCRE6; ISSN:0907-4449. (Munksgaard International Publishers Ltd.)
The geometry of metal-ligand interactions in proteins is examd. and compared with information for small-mol. complexes from the Cambridge Structural Database (CSD). The paper deals with the metals Ca, Mg, Mn, Fe, Cu, Zn and with metal-donor atom distances, coordination nos. and extent of distortion from ideal geometry (octahedral, tetrahedral etc.). It assesses the agreement between geometry found in all metalloprotein structures in the Protein Data Bank (PDB) detd. at resoln. ≤ 1.6 Å with that predicted from the CSD for ligands which are analogs of amino-acid side chains in proteins (Harding, 1999 and 2000). The agreement is reasonably good for these structures but poorer for many detd. at lower resoln. (examd. to 2.8 Å resoln.). For metal-donor distances, the predictions from the CSD, with minor adjustments, provide good targets either for validation or for restraints in refinement of structures where only poorer resoln. data is available. These target distances are tabulated and the use of restraints is recommended. Validation of angles or the use in refinement of restraints on angles at the metal atom is more difficult because of the inherent flexibility of these angles. A much simplified set of parameters for angle restraints with quite large std. deviations is provided. (Despite the flexibility of the angles, acceptable and preferred coordination nos. and shapes are well established and a summary table is provided.). An unusual and perhaps biochem. important feature of Zn coordination with carboxylate seen in the CSD examples is also clearly present in metalloprotein structures. With metals like Ca, carboxylate coordination is monodentate or bidentate (two M-O bonds of nearly equal length). In Zn carboxylates a continuous range between monodentate and bidentate coordination is found, with one Zn-O bond of normal length and another of any length between this and a van der Waals contact.>> More from SciFinder ®
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Shore, J. D.; Gutfreund, H.; Brooks, R. L.; Santiago, D.; Santiago, P.Biochemistry (1974), 13 (20), 4185-91CODEN: BICHAW; ISSN:0006-2960.The liberation of protons during turnover of liver alc. dehydrogenase was studied using transient and inhibition kinetics and direct titrimetric detns. Proton release occurred prior to, and uncoupled from, the catalytic H-transferring step. The addn. of satg. concns. of NAD to the enzyme resulted in a pH-dependent release of protons, with 0.5 proton/equiv liberated at pH 7.6. Formation of a ternary complex of enzyme, NAD, and trifluoroethanol resulted in liberation of 1 proton/equiv of enzyme in the pH range 5.5-8.5. Trifluoroethanol binding to binary complex was dependent on an enzyme functional group with a pKa of 7.6, with tight binding to the unprotonated form. Caprate binding showed the reverse pH dependence but with the same pKa, and caused uptake of protons liberated due to NAD binding. The rate consts. for proton release in burst reactions and from formation of ternary enzyme-NAD-trifluoroethanol complex, 250-280 sec-1, were compatible with a functional group with a pKa of 7.6. A scheme was developed indicating that protons were released from the enzyme during turnover as a result of perturbation of the pKa of a functional group on the enzyme from 9.6 to 7.6, and direct binding of the alc. hydroxyl group to the basic form of the perturbed functional group. This scheme accounts for the required stoichiometry of proton liberation and is compatible with a concerted hydride transfer mechanism of catalysis.>> More from SciFinder ®
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Kvassman, Jan; Pettersson, Goesta
European Journal of Biochemistry (1979), 100 (1), 115-23CODEN: EJBCAI; ISSN:0014-2956.The transient-state kinetics of ligand-displacement reactions were analyzed. Methods based on this anal. were used to obtain reliable ests. of on-velocity and off-velocity consts. for coenzyme binding to liver alc. dehydrogenase at different pH values between 6 and 10. The rate of NADH dissocn. from the enzyme showed no pronounced dependence on pH. The rate of NAD dissocn. was controlled by a group with a pKa of 7.6, agreeing with the pKa reported to regulate the binding of certain inhibitory substrate analogs to the enzyme-NAD complex. Crit. expts. were performed to test a recent proposal that on-velocity consts. for the binding of NADH and NAD are controlled by proton equil. exhibiting different pKa values. The results showed that assocn. rates for NADH and NAD exhibit the same pH dependence corresponding to a pKa of 9.2. Titrimetric evidence is presented indicating that the latter effect of pH derives from ionization of a group which affects the anion-binding capacity of the coenzyme-binding site.>> More from SciFinder ®
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CRC critical reviews in biochemistry (1987), 21 (4), 349-89 ISSN:0045-6411.The article deals with the structure and function of liver alcohol dehydrogenase and reviews mainly literature published after 1979, i.e., summarizes progress made in the field since Klinman presented her review on alcohol dehydrogenases. The emphasis will be on high-resolution crystallographic data, results obtained with metal-substituted enzyme derivatives, and on the mechanism and pH dependence of the catalytic reaction.>> More from SciFinder ®
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Andersson, Pia; Kvassman, Jan; Lindstroem, Anders; Olden, Bertil; Pettersson, Goesta
European Journal of Biochemistry (1981), 113 (3), 425-33CODEN: EJBCAI; ISSN:0014-2956.Equil. consts. for coenzyme binding to liver alc. dehydrogenase have been detd. over the pH range 10-12 by pH-jump stop-flow techniques. The binding of NADH or NAD requires the protonated form of an ionizing group (distinct from Zn(II)-bound water) with a pKa of 10.4. Complex formation with NADH exhibits an addnl. dependence on the protonation state of an ionizing group with a pKa of 11.2. The binding of trans-N,N-dimethylaminocinnamaldehyde to the enzyme-NADH complex is prevented by ionization of the latter group. Thus the pKa-11.2-dependence of NADH binding most likely derives from ionization of the water mol. bound at the catalytic Zn(II) of the enzyme subunit. The pKa value of 11.2 thus assigned to Zn(II)-bound water in the enzyme-NADH complex appears to be typical for an aquo ligand in the inner-sphere ligand field provided by the Zn(II)-binding amino acid residues in

liver alc. dehydrogenase. Thus the pKa of metal-bound water in Zn(II)-contg. enzymes can be assumed to correlate primarily with the no. of neg. charged protein ligands coordinated by the active-site Zn(II).>> More from SciFinder ®
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Oppenheimer, Norman J.; Kaplan, Nathan O.Biochemistry (1974), 13 (23), 4675-85CODEN: BICHAW; ISSN:0006-2960.The α-O2'-6B-cyclotetrahydronicotinamide adenine dinucleotide structure of the primary acid product of βNADH was detd. This structure is based on high frequency PMR at 220 MHz and CD. The acid product is a C-6 substituted tetrahydronicotinamide. The reaction is stereospecific, generating only 1 diastereomeric form characterized by an axial C-4A proton and an equatorial substitution on the B side of the C-6 position. Both αNADH and βNADH yielded the identical primary acid product. The acid product has an α configuration and a static 3'-endo-ribose conformation, and only the 3'-ribose OH of the acid product was free to be acylated. Blocking the 2'-OH of βNADH, followed by incubation in dil. acid, generated a compd. with spectral properties of a 6-hydroxytetrahydronicotinamide. A reaction scheme is discussed for the acid-catalyzed chem. of βNADH. Two pathways may be possible for the quant. rearrangement of βNADH to the primary acid product: 1 through β-6-hydroxytetrahydronicotinamide adenine dinucleotide and the other through the acid-catalyzed anomerization of βNADH to a αNADH. The causes of the stereospecificity of the cyclization reaction are discussed as are the possible implications of the acid-catalyzed chem. on the nature of the transition state complex of βNAD and βNADH in the active site of dehydrogenases.>> More from SciFinder ®
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Oppenheimer, Norman J.; Kaplan, Nathan O.Biochemistry (1974), 13 (23), 4685-94CODEN: BICHAW; ISSN:0006-2960.High-frequency PMR studies at 220 MHz of the modified nicotinamide coenzyme formed from βNADH by glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) established that the enzyme catalyzed the nonspecific addn. of water across the 5-6 double bond of the dihydronicotinamide ring of βNADH to form a β-6-hydroxy-1,4,5,6-tetrahydronicotinamide adenine dinucleotide, (6HTN)AD. Formation of (6HTN)AD in D2O led to a random incorporation of D into the C-5 position with no evidence for exchange of the C-5 proton. Hydroxylation at the C-6 position occurred with the generation of unequal populations of the 2 diastereomers: 65% A-side OH and 35% B-side OH. The proton absorptions for each of the diastereomeric forms of the 6-hydroxytetrahydronicotinamide ring were assigned and the coupling consts. detd. by computer anal. Mechanisms for the formation of (6HTN)AD by glyceraldehyde 3-phosphate dehydrogenase are discussed involving catalysis by either the polybasic anion bound in the active site or by acidic amino acid residues in close proximity to the 5-6 double bond of βNADH.>> More from SciFinder ®
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76.Canonical Variational Theory for Enzyme Kinetics with the Protein Mean Force and Multidimensional Quantum Mechanical Tunneling Dynamics. Theory and Application to Liver Alcohol Dehydrogenase
Alhambra, Cristobal; Corchado, Jose; Sanchez, Maria Luz; Garcia-Viloca, Mireia; Gao, Jiali; Truhlar, Donald G.Journal of Physical Chemistry B (2001), 105 (45), 11326-11340CODEN: JPCBFK; ISSN:1089-5647. (American Chemical Society)
We present a theor. framework for the calcn. of rate consts. of enzyme-catalyzed reactions that combines variational optimization of the dynamical bottleneck for overbarrier reactive events and multidimensional quantum mech. tunneling dynamics for through-barrier reactive events, both in the presence of the protein environment. The theory features a two-zone, three-stage procedure called ensemble-averaged variational transition state theory with multidimensional tunneling (EA-VTST/MT) with the transmission coeff. based on the equil. secondary-zone (ESZ) approxn. for including the effects of the protein on a catalytic reaction center, called the primary zone. The dynamics is calcd. by canonical variational theory with optimized multidimensional tunneling contributions, and the formalism allows for Boltzmann averaging over an ensemble of reactant and transition state conformations. In the first stage of the calcns., we assume that the generalized transition states can be well described by a single progress coordinate expressed in primary-zone internal coordinates; in subsequent steps, the transmission coeff. is averaged over a set of primary-zone reaction paths that depend on the protein configuration, and each reaction path has its own reaction coordinate and optimized tunneling path. We also present a simpler approxn. to the transmission coeff. that is called the static secondary-zone (SSZ) approxn. We illustrate both versions of this method by carrying out calcns. of the reaction rate consts. and kinetic isotope effects for oxidn. of benzyl alcoholate to benzaldehyde by horse liver alc. dehydrogenase. The potential energy surface is modeled by a combined generalized hybrid orbital/quantum mech./mol. mech./semiempirical valence bond (GHO-QM/MM/SEVB) method. The multidimensional tunneling calcns. are microcanonically optimized by employing both the small-curvature tunneling approxn. and version 4 of the large-curvature tunneling approxn. We find that the variation of the protein mean force as a function of reaction coordinate is quant. significant, but it does not change the qual. conclusions for the present reaction. We obtain good agreement with expt. for both kinetic isotope effects and Swain-Schaad exponents.>> More from SciFinder ®
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Villa, Jordi; Warshel, Arieh
Journal of Physical Chemistry B (2001), 105 (33), 7887-7907CODEN: JPCBFK; ISSN:1089-5647. (American Chemical Society)
A review with 169 refs., considering the advances made in using computer simulations to elucidate the catalytic power of enzymes. It is shown that some current approaches, and in particular the empirical valence bond approach, allow one to describe enzymic reactions by rigorous concepts of current chem. physics and to est. any proposed catalytic contribution. This includes evaluation of activation free energies, nonequil. solvation, quantum mech. tunneling, entropic effects, and other factors. The ability to evaluate activation free energies for reactions in water and proteins allows one to simulate the rate acceleration in enzymic reactions. It is found that the most important contribution to catalysis comes from the redn. of the activation free energy by electrostatic effects. These effects are found to be assocd. with the pre-organized polar environment of the enzyme active site. The use of computer simulations as effective tools for examg. different catalytic proposals is illustrated by 2 examples. (1) The popular proposal that enzymes catalyze reactions by special dynamical effects is considered. It is shown that this proposal is not supported by any consistent simulation study. It is also shown that the interpretation of recent expts. as evidence for dynamic contributions to catalysis is unjustified. Obviously, all chem. reactions involve motion, but unless this motion provides non-Boltzmann probability for reaching the transition state there are not dynamic effects. Vibrationally enhanced tunneling is shown to be a well-understood phenomenon that does not lead to special catalytic effects. Similarly, it is shown that nonequil. solvation effects do not constitute dynamic contributions to catalysis. (2) The effectiveness of simulation approaches is also demonstrated in studies of entropic contributions to catalysis. It is found that the corresponding contributions are smaller than previously thought.>> More from SciFinder ®
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Cui, Qiang; Elstner, Marcus; Karplus, Martin
Journal of Physical Chemistry B (2002), 106 (10), 2721-2740CODEN: JPCBFK; ISSN:1089-5647. (American Chemical Society)

The proton and hydride transfers in horse liver alc. dehydrogenase (LADH) were studied with a potential surface obtained by use of the self-consistent-charge-d.-functional-tight-binding (SCC-DFTB) QM/MM method implemented in the CHARMM program; a correction for solvent shielding was introduced by use of a continuum model. The proton transfers were found to proceed in a virtually concerted fashion before the hydride transfer. The calcns. also showed that a radical mechanism, suggested as a possibility in the literature for the H transfer between the substrate and NAD+, is very unlikely. The energetics of the reaction and pKa's of residues involved in catalysis indicate that the chem. steps of LADH, as characterized by the calcd. value of kcat, are slow for a pH below 5.5, and the hydride transfer is hardly affected for pH between 5.5 and 8.1. These results are compared with the exptl. measured pH dependence of kcat for LADH, although a quant. comparison is difficult because the chem. steps are only partially rate-limiting in the expts. A perturbation anal. of the QM/MM energies suggest that a no. of charged residues close to the active site (i.e., Asp 49, Glu 68, and Arg 369), as well as the phosphate groups of NAD+, make important contributions to the energetics of the proton and hydride transfer reactions; mutation expts. to test these predictions would be of interest. Ser 48 interacts with the substrate via a short hydrogen bond, which leads to an inverse solvent isotope effect, in accord with expt. The overall calcd. barrier and endothermicity and the effect of the double mutation (F93W,V203A) on the hydride transfer are in qual. agreement with measurements; i.e., the hydride transfer barrier is higher in the mutant, presumably due mainly to the fact that the av. distance between the donor and acceptor is larger. In accord with the study of Alhambra et al. (Alhambra, C.; Corchado, J. C.; Sanchez, M. L.; Gao, J.; Truhlar, D. G. J. Am. Chem. Soc. 2000, 122, 8197.), hydride tunneling was shown to be very important for the calcd. magnitude of kinetic isotope effects and the Swain-Schaad exponents, although the abs. contribution of tunneling to the rate const. is calcd. to be small (a factor of 2) at room temp. It was shown that the secondary Swain-Schaad exponent can be affected by the variation in the position of the transition state upon secondary isotopic substitution. The exponent, therefore, does not necessarily reflect the magnitude of tunneling; i.e., one has to be cautious in using the secondary Swain-Schaad exponent to est. the magnitude of tunneling. Equil. effects of protein "dynamics" on the hydride transfer in LADH were studied by potential of mean force calcns. at different temps. To obtain a clearer description of the origin of the calcd. variation, an anal. was made with the QM and MM atoms maintained at different temps. by use of Nose-Hoover thermostats. It was found that the barrier is correlated primarily with the temp. of the QM region, while the temp. of the MM atoms has a larger effect on the exothermicity. The variation of the barrier with the structures accessible by mol. dynamics simulations was found to be small (<1 kcal/mol). The effect arises mainly from the change in the position of residues that directly interact with the reacting groups, such as Ser 48.>> More from SciFinder ®
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79.Schneider, G., Eklund, H., Cedergren Zeppezauer, E., and Zeppezauer, M. (1983) Crystal structures of the active site in specifically metal-depleted and cobalt-substituted horse liver alcohol dehydrogenase derivatives Proc. Natl. Acad. Sci. U. S. A. 80, 5289– 5293 DOI: 10.1073/pnas.80.17.5289[Crossref], [PubMed], [CAS]
79.Crystal structures of the active site in specifically metal-depleted and cobalt-substituted horse liver alcohol dehydrogenase derivatives
Schneider, Gunter; Eklund, Hans; Cedergren-Zeppezauer, Eila; Zeppezauer, Michael
Proceedings of the National Academy of Sciences of the United States of America (1983), 80 (17), 5289-93CODEN: PNASA6; ISSN:0027-8424.Two derivs. of horse liver alc. dehydrogenase (LADH) in which the active site is specifically metal-depleted [H4Zn(n)2LADH] or specifically Co-substituted [Co(c)2Zn(n)2LADH] were studied by crystallog. methods. (In these formulas, n identifies the noncatalytic Zn2+ and c identifies the catalytic metal ion.). X-ray data were collected for H4Zn(n)2LADH to 2.7-Å resoln. and for Co(c)2Zn(n)2LADH to 2.4-Å resoln. Difference Fourier maps demonstrate clearly that catalytic Zn2+ is removed in H4Zn(n)2LADH, whereas noncatalytic Zn2+ is still present. A 2.5-Å shift in the S position of cysteine-46 and a slight torsion of the imidazole ring of histidine-67 are the only changes in protein structure detected when compared to the native Zn enzyme. The structure of Co(c)2Zn(n)2LADH is essentially the same as that of the native enzyme. Each Co is bound to the ligands cysteine-46, cysteine-174, histidine-67, and a water mol. in a distorted tetrahedral geometry. A slight change in the position of histidine-67 was found. No further structural changes were obsd.>> More from SciFinder ®
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80.Nuclear magnetic resonance studies of substrate interaction with cobalt substituted alcohol dehydrogenase from liver
Sloan, Donald L.; Young, J. Maitland; Mildvan, Albert S.Biochemistry (1975), 14 (9), 1998-2008CODEN: BICHAW; ISSN:0006-2960.The role of Zn in liver alc. dehydrogenase was studied by replacement of 1.3 and 3.5 of the 4 Zn(II) with Co(II) and measuring the effects of the paramagnetic Co(II) on the relaxation rates of the protons of H2O, EtOH and isobutyramide. Water relaxation studies at 8, 24, 100, and 200 MHz indicated 2 classes of bound Co(II). The ∼2 readily replaced Co(II) ions retained 1 fast exchanging water proton in their inner coordination spheres, whereas the ∼2 slowly exchanging Co(II) coordinated no detectable water protons, indicating that the former replaced Zn(II) at the catalytic sites and the latter replaced Zn(II) at the structural sites. EtOH acetaldehyde, and isobutyramide bound with appropriate affinities to the Co(II) substituted alc. dehydrogenases decreasing the no. of fast exchanging protons at the catalytic Co(II) site by ≥54%. Coenzyme binding caused smaller changes in the water relaxation rate which may be due to local conformation changes. The paramagnetic effects of Co(II) at the catalytic site on the relaxation rates of the Me protons of isobutyramide at 100 and 220 MHz indicated that this analog binds at a site 9.1 Å from the catalytic Co(II). This distance decreased to 6.9 Å when NADH was bound, and a Co(II) to methyne proton distance of 6.6 Å was detd. indicating a conformation change leading to the formation of a 2nd sphere enzyme-CO(II)-isobutyramide complex in which a hydroxyl or water ligand intervenes between the metal and the substrate analog. Similar behavior was observed in the enzyme-EtOH complexes. The role of the catalytic Co(II) thus appears to be the activation of a hydroxyl or water ligand which polarizes the aldehyde carbonyl group by H bonding. The role of the structural Co(II), which is more distant from isobutyramide (9-11 Å), may be that of a template for protein conformation changes. The arrangement of coenzyme, metal, and substrate at the active site in soln. is discussed.>> More from SciFinder ®
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81.Active-site-specific reconstituted cobalt(II) horse liver alcohol dehydrogenase. Changes of the spectra of the substrate trans-4-(N,N-dimethylamino)-cinnamaldehyde and of the catalytic cobalt ion upon ternary complex formation with NADH and 1,4,5,6-tetrahydronicotinamide-adenine dinucleotide
Dietrich, Helmut; Maret, Wolfgang; Wallen, Leif; Zeppezauer, Michael
European Journal of Biochemistry (1979), 100 (1), 267-70CODEN: EJBCAI; ISSN:0014-2956.Horse liver alc. dehydrogenase (EC 1.1.1.1), specifically reconstituted with Co(II) in the catalytic metal-binding sites formed ternary complexes with the chromophoric substrate, trans-4-(N,N-dimethylamino)cinnamaldehyde, and NADH or 1,4,5,6-tetrahydronicotinamide adenine dinucleotide (H2NADH). Control expts. with enzyme depleted of metal in the catalytic sites demonstrated that the presence of a metal ion is an abs. requirement for binding of the substrate. The spectra of both the chromophore and the metal ion were changed significantly upon complex formation. The red shift of the visible absorption band of the substrate mol. was 14 nm larger for both the complexes with NADH and H2NADH as compared to the native zinc enzyme; this was taken as evidence for direct coordination of the substrate's carbonyl O to the catalytic Co. The greatly increased intensity of the metal's visible d-d band upon substrate binding added support to this conclusion. Since binding of the coenzyme as well as of the analog, which must precede the binding of the substrate, led to considerable red shifts of the d-d band at 650 nm (28 nm for NADH and 21 nm for H2NADH), a distortion of the coordination sphere of the catalytic metal ion was triggered prior to substrate binding. Presumably the coenzyme-triggered conformation change of the protein involving the catalytic metal ion results in a modulation of the Lewis acid strength of the metal which then is able to coordinate and activate the substrate.>> More from SciFinder ®
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82.Active-site cobalt(II)-substituted horse liver alcohol dehydrogenase: characterization of intermediates in the oxidation and reduction processes as a function of pH
Sartorius, Christian; Gerber, Martin; Zeppezauer, Michael; Dunn, Michael F.Biochemistry (1987), 26 (3), 871-82CODEN: BICHAW; ISSN:0006-2960.Substitution of Co(II) for the catalytic site Zn(II) of horse liver alc. dehydrogenase (LADH) has previously been shown to yield an active enzyme deriv., CoIIE, with characteristic Co(II) charge-transfer and d-d electronic transitions that are sensitive to the events which take place during catalysis. Here, UV-visible spectroscopy and rapid-scanning stopped-flow (RSSF) kinetic methods were used to detect and identify intermediates in the LADH catalytic mechanism. In the presence of the inhibitor isobutyramide, the presteady-state phase of alc. (RCH2OH) oxidn. at a pH of >7 was characterized by the formation and decay of an intermediate with max. at wavelengths of 570, 640, and 672 nm for both arom. and aliph. alcs. (benzyl, p-nitrobenzyl, and anisyl alcs., EtOH, and MeOH). By comparison with the spectrum of the stable ternary complex formed with NAD and 2,2',2"-trifluoroethoxide ion (TFE-) [CoIILADH(NAD,TFE-)], the intermediate which formed was proposed to be the alkoxide ion (RCH2O-) complex, CoIILADH(NAD, RCH2O-). The timing of NADH formation indicated that intermediate decay was limited by the interconversion of ternary complexes, i.e.,

CoIILADH(NAD, RCH2O-) .dblharw. CoIILADH(NADH,RCHO). From competition expts., it was inferred that at pH values of <5, NAD and alc. formed a CoIILADH(NAD, RCH2OH) ternary complex. Rapid-scanning stopped-flow studies carried out as a function of pH indicated that the apparent pKa values for the ionization of alc. within the ternary complex, i.e., CoIILADH(NAD,RCH2OH) .dblharw. CoIILADH(NAD,RCH2O-) + H+, fell in the range 5-7.5. Pyrazole was used as a dead-end inhibitor to det. the single-turnover time course for the redn. of benzaldehyde, p-nitrobenzaldehyde, anisaldehyde, and acetaldehyde; at pH >7, all showed evidence for the formation and decay of an intermediate. By means of spectral comparisons with CoIILADH(NAD, TFE-) and with the intermediate formed during alc. oxidn., the intermediate was identified as the same CoIILADH(NAD,RCH2O-) ternary complex detected during alc. oxidn.>> More from SciFinder ®
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83.A nuclear magnetic resonance study of cobalt II alcohol dehydrogenase: substrate analog-metal interactions

Drysdale, Beth-Ellen; Hollis, Donald P.Archives of Biochemistry and Biophysics (1980), 205 (1), 267-79CODEN: ABBIA4; ISSN:0003-9861.Two models for the active site of liver alc. dehydrogenase (EC 1.1.1.1) have been proposed. X-ray diffraction studies (Plapp, B.V. et al., 1978) on the native enzyme indicate that substrates are directly coordinated to the active site Zn(II), whereas NMR studies (Sloan, D.L. et al., 1975) on the Co(II) enzyme indicate that substrates are not bound directly to the metal. It was unclear whether the basis for this difference was structural or tech. Therefore, this NMR study has been done with well-characterized Zn and Co enzymes. To facilitate comparison with x-ray diffraction data, the substrate analogs chosen were Me2SO and trifluoroethanol. Binding of either analog to the Zn enzyme in the presence of the appropriate cofactor produced unique changes in the T1 and T2 relaxation rates of the 1H and 19F nuclei. Similar results were obtained when Co enzyme was used for T1 measurements, but relaxation was more rapid due to the presence of the paramagnetic ion. The distances between the analog nuclei and the catalytic site Co(II) were calcd. to be 8.9 and 10.5 Å for the Co enzyme-NADH-Me2SO and the Co enzyme-NAD-trifluoroethanol complexes, resp. The distances are comparable and the magnitudes indicate that the functional groups are not directly coordinated to the active site Co(II). These values are in good agreement with those previously reported by D.L. Sloan et al. (1975) for the Co enzyme-NADH-isobutyramide complex, and are consistent with their model in which a metal water ligand forms a bridge between the substrate and the metal. Therefore, there must be a structural basis for the differences obsd. in magnetic resonance vs. x-ray diffraction studies.>> More from SciFinder ®
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84.Metal ion substitution at the catalytic site of horse-liver alcohol dehydrogenase: results from solvent magnetic relaxation studies. 1. Copper(2+) and cobalt(2+) ions

Andersson, Inger; Maret, Wolfgang; Zeppezauer, Michael; Brown, Rodney D., III; Koenig, Seymour H.Biochemistry (1981), 20 (12), 3424-32CODEN: BICHAW; ISSN:0006-2960.The influence of the paramagnetic ions, Cu2+ and Co2+, substituted for Zn2+ at the catalytic sites of native alc. dehydrogenase from horse liver (EC 1.1.1.1), on the nuclear magnetic spin-lattice relaxation rates of solvent water and substrate (MeOD) protons was studied as a function of magnetic field strength. For Cu2+, the data could best be fit to a model in which the resulting blue Cu center (type I) of the enzyme was characterized by inner sphere-coordinated water or substrate, both more strongly bound in the binary complex of protein with coenzyme, and displaced from the ternary complex with pyrazole. In the binary complex with pyrazole, a pentacoordinated species was indicated; thus the coordination no. was reduced upon formation of the enzyme-pyrazole-coenzyme ternary complex. Although the Cu2+-enzyme was able to bind coenzyme, thereby distorting its metal-binding site, it could not discriminate significantly between alc. substrates and water. The resulting relatively weak binding of alc. was sufficient to explain the obsd. absence of enzymic activity of the Cu2+-substituted protein under the usual exptl. conditions. This is the 1st example of a blue Cu protein for which the Cu2+ is accessible to solvent. The correlation times for the paramagnetic dipolar interaction between the solvent protons and Cu2+ were unusually short, presumably due to strong spin-orbit interaction of the electronic spins of Cu2+ with SH ligands. The magnetic spin-lattice relaxation rates of both solvent water protons and solvent MeOH Me protons were also measured for solns. of the native enzyme and the enzyme with Zn2+ removed from the catalytic sites and with Co2+ specifically substituted for Zn2+ at the catalytic site. No paramagnetic contribution from Co2+ to the magnetic relaxation rate of the solvent water and MeOH protons could be detected, despite attempts to enhance the detection of paramagnetic effects by altering a variety of exptl. parameters, including temp. and ionic content of the solvent, and by the addn. of coenzyme and inhibitors. There were small differences in the diamagnetic contributions to the relaxation rates of the native, demetalized, and Co2+-substituted enzymes that changed sign with magnetic field; these small variations could readily be mistaken for true paramagnetic effects when anal. of the relaxation data was limited to the high values of magnetic field strength usually used for measurements of relaxation enhancement. As a result, previous high-field data require reinterpretation: any paramagnetic effects that may be present are small and not easily separable from a variety of small diamagnetic effects that depend on solvent compn. The reason appears to be an unusually short correlation time for the interaction between solvent protons and Co2+, due to a strong spin-orbit interaction of the electronic spin of Co2+, as was found for the Cu2+-substituted enzyme.>> More from SciFinder ®
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86.The pH variation of steady-state kinetic parameters of site-specific cobalt(2+)-reconstituted liver alcohol dehydrogenase. A mechanistic probe for the assignment of metal-linked ionizations

Maret, Wolfgang; Makinen, Marvin W.Journal of Biological Chemistry (1991), 266 (31), 20636-44CODEN: JBCHA3; ISSN:0021-9258.To identify ionizations of the active site metal-bound water in horse liver alc. dehydrogenase (EC 1.1.1.1), the pH, solvent isotope, temp., and anion dependences of the steady-state kinetic parameters kcat and kcat/KM have been evaluated under initial velocity conditions for the native and the active site-specific Co2+-reconstituted enzyme,. In the oxidn. of benzyl alc., a bell-shaped pattern of four prototropic equil. was obsd. under conditions of satg. concns. of NAD. It is shown that the ionizations governing kcat (pK1 ≃ 6.7, pK2 ≃ 10.6) belong to the ternary enzyme-NAD-alc. complex, whereas the ionizations governing kcat.KM (pK1' ≃ 7.5, pK2' ≃ 8.9) belong to the binary enzyme-NAD complex. The ionizations pK1 and pK1' are not influenced by metal substitution and are ascribed to His-51 on the basis of exptl. ests. of their assocd. enthalpies of ionization. On the other hand, pK2 and pK2' are significantly decreased (ΔpKα ≃ 1.0) in the Co2+-enzyme and are attributed to the active site metal-bound water mol. The shape of the pH profiles requires that the metal ion coordinates a neutral water mol. in the ternary enzyme-NAD-alc. complex under physiol. conditions. The possible catalytic role of the water mol. within a pentacoordinate metal ion complex in the active site is discussed.>> More from SciFinder ®
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Brooks, Robert L.; Shore, Joseph D.; Gutfreund, H.Journal of Biological Chemistry (1972), 247 (8), 2382-3CODEN: JBCHA3; ISSN:0021-9258.The pH and temp. dependence of catalytic H transfer in the liver alc. dehydrogenase reaction mechanism was studied with transient kinetic techniques. The rate const. for this reaction was dependent on a group with a pK of 6.4, indicating the possible involvement of a histidine residue. Thermodynamic values detd. at pH 7.5 were 14.5 kcal per mole for ΔG‡, 13.0 kcal per mole for ΔH‡, and -4.8 entropy units for ΔS‡.>> More from SciFinder ®
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89.Effect of pH on the process of ternary complex interconversion in the liver alcohol dehydrogenase reaction

Kvassman, Jan; Pettersson, Gosta

European Journal of Biochemistry (1978), 87 (2), 417-27CODEN: EJBCAI; ISSN:0014-2956.Kinetic relations referring to multiple-turnover conditions were derived for the slowest exponential transient appearing in 2-substrate enzyme reactions proceeding by an ordered ternary-complex mechanism and applied to the liver alc.

dehydrogenase (I) reaction. All essential features of the transient-state kinetics of the I system can be qual. and quant. explained in view of the compulsory-order mechanism in the proposed scheme. Transient-state rate parameters for benzyl alc./benzaldehyde catalysis by liver I were detd. at different pH. The interpretation of such rate parameters is critically discussed with ref. to their informative value for the purpose of detn. of rate consts. (k and k') for the process of ternary-complex interconversion in the proposed scheme. The apparent rate const. (k') for hydride transfer from benzyl alc. to NAD is dependent on a proton dissocn. step with a pKa of 6.4, whereas the rate const. (k) for hydride transfer from NADH to benzaldehyde exhibits no corresponding dependence on proton assocn. The asym. pH dependence of the forward and reverse rate of ternary-complex interconversion during I catalysis appears to reflect an obligatory step of alc./alcoholate ion equilibration occurring at the ternary-complex level. The obsd. pKa 6.4 dependence of the transient rate of alc. oxidn. may be attributed to a coupled acid-base system involving minimally the enzyme-bound alc. and the protein residues serine-48 and histidine-51.>> More from SciFinder ®
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Sekhar, V. Chandra; Plapp, Bryce V.Biochemistry (1988), 27 (14), 5082-8CODEN: BICHAW; ISSN:0006-2960.The binding of NAD to liver alc. dehydrogenase was studied by stopped-flow techniques at pH 6.1-10.9 at 25°. Varying the concns. of NAD and a substrate analog used to trap the enzyme-NAD complex gave satn. kinetics. The same max. rate consts. were obtained with or without the trapping agent and by following the reaction with protein fluorescence or absorbance of a ternary complex. The data fit a mechanism with diffusion-controlled assocn. of enzyme and NAD, followed by an isomerization with a forward rate const. of 500 s-1 at pH 8. The isomerization may be related to the conformational change detd. by x-ray crystallog. of free enzyme and enzyme-coenzyme complexes. Overall bimol. rate consts. for NAD binding show a bell-shaped pH dependence with apparent pK values at 6.9 and 9.0. Acetimidylation of ε-NH2 groups shifts the upper pK to ≥11, suggesting that lysine-228 is responsible for the pK of 9.0. Formation of the enzyme-imidazole complex abolishes the pK value of 6.9, suggesting that a H-bonded system extending from the Zn-bound H2O to histidine-51 is responsible for this pK value. The rates of isomerization of the binary complex and of pyrazole binding were maximal at pH below a pK of ∼8, which is attributable to the H-bonded system. Acetimidylation of lysines or displacement of Zn-H2O with imidazole had little effect on the rate of isomerization of the enzyme-NAD complex. Rate consts. from a computer simulation suggest that the isomerization partially controls the transient phase of 1-propanol oxidn.>> More from SciFinder ®
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91.Role of metal in liver alcohol dehydrogenase catalysis. Spectral and kinetic studies with cobalt-substituted enzyme
Shore, Joseph D.; Santiago, David
Journal of Biological Chemistry (1975), 250 (6), 2008-12CODEN: JBCHA3; ISSN:0021-9258.The kinetic and spectral properties of native and totally Co-substituted liver alc. dehydrogenase were compared. Based on titrimetric detns. of enzyme active site concn., the turnover no. at pH 7.0 for the Co-contg. enzyme was the same as for the native enzyme. At pH 10, however, the turnover no. was slower for the Co-substituted enzyme, 3.14 sec-1 as compared with 4.05 for the native enzyme. A comparison between native and totally Co-substituted enzyme showed a blue-shifted enzyme-NADH double difference spectrum and a splitting and red-shifted enzyme-NAD-pyrazole double difference spectrum in the near uv. The 655-nm peak of the Co-substituted enzyme was perturbed by the formation of enzyme-NADH binary complex, enzyme-NAD-pyrazole ternary complex, and enzyme-NAD-trifluoroethanol ternary complex, but not by enzyme-NAD binary complex formation. At pH 7.0, the only observable step in the reaction sequence with a significantly different rate const. for the Co-contg. enzyme was the catalytic H-transferring step. The rate const. for this step is 92 sec-1 for totally Co-substituted enzyme as compared with 138 sec-1 for native liver alc. dehydrogenase. The results of this study indicate that Zn is involved in catalysis and in binding interactions with alc. and NADH.>> More from SciFinder ®
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Bobsein, Barrett R.; Myers, Rollie J.Journal of Biological Chemistry (1981), 256 (11), 5313-16CODEN: JBCHA3; ISSN:0021-9258.113Cd NMR was obsd. for Cd(II)-substituted horse liver alc. dehydrogenase (I) and its complexes with coenzymes and several substrate analogs. Compared to free enzyme, the catalytic Cd(II) resonance is shielded by 41-42 ppm in both I-NADH and I-NAD. In ternary complexes of I-NAD with either trifluoroethanol or pyrazole, this resonance narrows and is deshielded by 75 ppm. The I-NADH-butyramide complex gives only 3 ppm of deshielding relative to the I-NADH resonance. At pH 10.3, the catalytic resonance of unbound I is broadened and slightly deshielded. No other resonances are dependent on pH in the range 8-10. These data are the most consistent with a 2nd sphere coordination of the substrate analogs to the catalytic metal ion. The obsd. difference between complexes of the alc. analogs and the aldehyde analog would then be explained as the presence of a hydroxide vs. a water mol., resp., in the 1st coordination sphere. The data also show that the pKa of the coordinated water on the Zn(II) in native I is close to 9.2 as previously assumed, whereas the pKa of the Zn(II)-bound water in the I-NAD complex is most likely >9 and not 7.6 as previously assumed.>> More from SciFinder ®
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Biochemistry (1985), 24 (25), 7503-10CODEN: BICHAW; ISSN:0006-2960.Three crystal structures were detd. for active site-specific Cd(II)-substituted horse liver alc. dehydrogenase (LADH) and its complexes. Intensities were collected for the free, orthorhombic enzyme to 2.4-Å resoln. and for a triclinic binary complex with NADH to 2.7-Å resoln. A ternary complex was crystd. from an equil. mixt. of NAD and p-bromobenzyl alc. The microspectrophotometric anal. of these single crystals showed the protein-bound coenzyme to be largely NADH, which proves the complex to consist of CdII-LADH, NADH, and p-bromobenzyl alc. Intensity data for this abortive ternary complex were collected to 2.9-Å resoln. The coordination geometry in the free Cd(II)-substituted enzyme was highly similar to that of the native enzyme. Cd(II) is bound to cysteine (Cys)-46, Cys-174, histidine-67, and a water mol. in a distorted tetrahedral geometry. Binding of coenzyme induced a conformational change similar to that in the native enzyme. The interactions between the coenzyme and the protein in the binary and ternary complexes were highly similar to those in the native ternary complexes. The substrate bound directly to Cd(II) in a distorted tetrahedral geometry. No large, significant structural changes compared to the native ternary complex with coenzyme and p-bromobenzyl alc. were found. The implications of these results for the use of active site-specific Cd(II)-substituted horse liver alc. dehydrogenase as a model system for the native enzyme are discussed.>> More from SciFinder ®
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European Journal of Biochemistry (1980), 108 (1), 303-12CODEN: EJBCAI; ISSN:0014-2956.The mechanistic and structural origin of the pKa 9.2 dependence of coenzyme assocn. and anion binding to liver alc. dehydrogenase was investigated by titrimetric and spectrophotometric binding studies involving ligands (imidazole, 1,10-phenanthroline, and 2,2'-bipyridine) which combine to the catalytic Zn of the enzyme subunit with displacement of Zn-bound water. Imidazole abolishes the pKa 9.2 dependence of NADH binding to the enzyme. The pH dependence of ADP-ribose and Pt(CN)42- binding is similarly abolished by imidazole, as well as by 1,10-phenanthroline and 2,2'-bipyridine. Thus, the pKa 9.2 dependence of coenzyme and anion binding most likely derives from ionization of Zn-bound water. Evidence is presented showing that the protonation state of the pKa 9.2 group also regulates ligand binding to the catalytic Zn ion, which lends strong support to the conclusion that this ionizing group can be identified as Zn-bound water. The pKa 9.2 dependence of bipyridine binding is abolished by ADP-ribose and Pt(CN)42-, indicating that complex formation at the anion-binding subsite of the coenzyme-binding site perturbs the pKa of Zn-bound water to a value >10. The cooperative interrelation between ionization of Zn-bound water and complex formation at the anion-binding subsite is proposed to be attributable to a competition between the Zn-bound OH- and external

anionic ligands for salt bridge formation with the guanidinium group of arginine-47. This explains why ionization of Zn-bound water affects the anion-binding capacity of the enzyme and why complex formation at the anion-binding subsite affects the pKa of Zn-bound water. The kinetics of complex formation with bipyridine are consistent with a 2-step binding mechanism in which a pKa 9.2 dependent rapid pre-equil. between enzyme and ligand is followed by a pH independent rate-limiting formation of the chromophoric binary complex. Desorption of Zn-bound water, therefore, is likely to take place in the primary assocn. step rather than in the subsequent rate-limiting step. This renders the possibility less likely that productive ternary complex formed during catalysis may contain a Zn-bound penta-coordinate water mol.>> More from SciFinder ®
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Eklund, Hans; Samama, Jean-Pierre; Wallen, Leif
Biochemistry (1982), 21 (20), 4858-66CODEN: BICHAW; ISSN:0006-2960.Pyrazole (I) is a strong inhibitor of liver alc. dehydrogenase (II) in combination with oxidized coenzyme, NAD. Three different complexes of I with II were studied by crystallog. methods: (1) the binary complex with I to 3.2 Å resoln., (2) the ternary complex with NAD-I to 2.9 Å resoln., and (3) the ternary complex with NAD-4-iodopyrazole to 2.9 Å resoln. Crystals of the binary complex are isomorphous to the apoenzyme, and I binds to the active-site Zn in a way analogous to imidazole. Crystals of the 2 ternary complexes are isomorphous with the ternary II-NADH-DMSO complex. One of the N atoms of the I ring is directly bound to the active-site Zn with a Zn-N bond distance of 2.1 Å. The other N is 2 Å from the C4 atom of the nicotinamide ring of the coenzyme. The I atom in 4-iodopyrazole is located in the hydrophobic substrate cleft. The effect of substitutions on the I ring are discussed in relation to the structure of the II active site and substrate pocket. I derivs. with long alkyl chains bound in the 4-position are outstanding inhibitors, and this property is related to the topog. of the hydrophobic substrate cleft. The conformation of NAD in the ternary complexes is essentially the same as that of NADH in the NADH-DMSO complex.>> More from SciFinder ®
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Gilleland, Martha J.; Shore, Joseph D.Biochemical and Biophysical Research Communications (1970), 40 (1), 230-5CODEN: BBRCA9; ISSN:0006-291X.The binding rates of 2 Zn chelators, bipyridyl and pyrazole, to liver alc. dehydrogenase (LADH) have been studied by stopped-flow methods. The pseudo first-order rate const. for binding bipyridyl to liver alc. dehydrogenase is 228/sec at extrapolated infinite concns., while that for binding pyrazole to enzyme-NAD complex is 238/sec. From these data it is postulated that any coenzyme or substrate which binds to the Zn of LADH cannot be bound at a rate >230/sec.>> More from SciFinder ®
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Holyer, R. H.; Hubbard, C. D.; Kettle, S. F. A.; Wilkins, R. G.Inorganic Chemistry (1965), 4 (7), 929-35CODEN: INOCAJ; ISSN:0020-1669.The kinetics of formation and dissocn. of metal complexes of 1,10-phenanthroline, 2,2'-bipyridine, and some related ligands were measured by the stopped-flow method. The 2nd-order formation rate const. for the mono complex increases in the sequence Ni2+ < Co2+ < Fe2+ ∼ Mn2+ < Zn2+ < Cu2+ ∼ Cd2+ < Ag2+ < Hg2+. Although most attention was directed to the 1:1 complexes, some of the reactions of the higher species were examd. also. Agreement between thermodn. data obtained from kinetic and equil. studies was good. The results are discussed in terms of current ideas on the mechanism of complex ion reactions.>> More from SciFinder ®
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Henehan, Gary T. M.; Oppenheimer, Norman J.Biochemistry (1993), 32 (3), 735-8CODEN: BICHAW; ISSN:0006-2960.The oxidn. of aldehydes by horse liver alc. dehydrogenase (HL-ADH) is more complex than previously recognized. At low enzyme concns. and/or high aldehyde concns., a pronounced lag in the assay progress curve is obsd. when the reaction is monitored for NADH prodn. at 340 nm. When the progress of the reaction is followed by 1H NMR spectroscopy, rapid dismutation of the aldehyde substrate into the corresponding acid and alc. is obsd. during the lag phase. Steady-state prodn. of NADH commences only after aldehyde concns. drop below 5% of their initial value; thereafter, NADH prodn. occurs with continuous adjustment of the equil. between aldehyde, alc., NADH, and NAD. The steady-state NADH prodn. exhibits normal Michaelis-Menten kinetics and is in accord with earlier studies using much higher enzyme concns. where no lag phase was reported. These results establish that the ability of HL-ADH to oxidize aldehydes is much greater than previously thought. The relationship between aldehyde dismutase and aldehyde dehydrogenase activities of HL-ADH is discussed.>> More from SciFinder ®
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Guy, Jodie E.; Isupov, Michail N.; Littlechild, Jennifer A.Journal of Molecular Biology (2003), 331 (5), 1041-1051CODEN: JMOBAK; ISSN:0022-2836. (Elsevier)
The structure of the recombinant medium chain alc. dehydrogenase (ADH) from the hyperthermophilic archaeon Aeropyrum pernix has been solved by the multiple anomalous dispersion technique using the signal from the naturally occurring zinc ions. The enzyme is a tetramer with 222 point group symmetry. The ADH monomer is formed from a catalytic and a cofactor-binding domain, with the overall fold similar to previously solved ADH structures. The 1.62 A resoln. A. pernix ADH structure is that of the holo form, with the cofactor NADH bound into the cleft between the two domains. The electron d. found in the active site has been interpreted to be octanoic acid, which has been shown to be an inhibitor of the enzyme. This inhibitor is positioned with its carbonyl oxygen atom forming the fourth ligand of the catalytic zinc ion. The structural zinc ion of each monomer is present at only partial occupancy and in its absence a disulfide bond is formed. The enhanced thermal stability of the A. pernix ADH is thought to arise primarily from increased ionic and hydrophobic interactions on the subunit interfaces.>> More from SciFinder ®
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Pauly, Thomas A.; Ekstrom, Jennifer L.; Beebe, David A.; Chrunyk, Boris; Cunningham, David; Griffor, Matthew; Kamath, Ajith; Lee, S. Edward; Madura, Rebecca; Mcguire, Dewitt; Subashi, Timothy; Wasilko, David; Watts, Paul; Mylari, Banavara L.; Oates, Peter J.; Adams, Paul D.; Rath, Virginia L.Structure (Cambridge, MA, United States) (2003), 11 (9), 1071-1085CODEN: STRUE6; ISSN:0969-2126. (Cell Press)
Sorbitol dehydrogenase (I) and aldose reductase form the polyol pathway that interconverts glucose and fructose. Redox changes from overprodn. of the coenzyme, NADH, by I may play a role in diabetes-induced dysfunction in sensitive tissues, making I a therapeutic target for diabetic complications. Here, the authors purified and detd. the crystal structures of human I alone, I with NAD, and I with NADH and an inhibitor, CP 166572, that is competitive with fructose. I was a tetramer of identical, catalytically active subunits. In the apoenzyme and NAD complex, the catalytic Zn was coordinated by His-69, Cys-44, Glu-70, and a water mol. Inhibitor CP 166572 coordinated the Zn through an O and a N atom with the concomitant dissocn. of Glu-70. The inhibitor formed hydrophobic interactions with NADH and likely sterically occluded substrate binding. The structure of the inhibitor complex provides a framework for developing more potent inhibitors of human I. The Km values of human I for NAD, NADH, and sorbitol were 225 μM, 210 μM, and 1.5 mM, resp. The Ki of CP 166572 for human I was 154 nM.>> More from SciFinder ®
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Glu-60 of the zinc-dependent Thermoanaerobacter brockii alc. dehydrogenase (TbADH) is a strictly conserved residue in all members of the alc. dehydrogenase (ADH) family. Unlike most other ADHs, the crystal structures of TbADH and its analogs, ADH from Clostridium beijerinckii (CbADH), exhibit a unique zinc coordination environment in which this conserved residue is directly coordinated to the catalytic zinc ion in the native form of the enzymes. To explore the role of Glu-60 in TbADH catalysis, we have replaced it by alanine (E60A-TbADH) and aspartate (E60D-TbADH). Steady-state kinetic measurements show that the catalytic efficiency of these mutants is only four- and eightfold, resp., lower than that of wild-type TbADH. We applied X-ray absorption fine-structure (EXAFS) and near-UV CD to characterize the local environment around the catalytic zinc ion in the variant enzymes in their native, cofactor-bound, and inhibited forms. We show that the catalytic zinc site in the studied complexes of the variant enzymes exhibits minor changes relative to the analogous complexes of wild-type TbADH. These moderate changes in the kinetic parameters and in the zinc ion environment imply that the Glu-60 in TbADH does not remain bound to the catalytic zinc ion during catalysis. Furthermore, our results suggest that a water mol. replaces this residue during substrate turnover.>> More from SciFinder ®
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The coordination no. of the catalytic zinc ion in alc. dehydrogenase has been studied by integrated ab initio quantum-chem. and mol. mechanics geometry optimizations involving the whole enzyme. A four-coordinate active-site zinc ion is 100-200 kJ/mol more stable than a five-coordinate one, depending on the ligands. The only stable binding site for a fifth ligand at the zinc ion is opposite to the normal substrate site, in a small cavity buried behind the zinc ion. The zinc coordination sphere has to be strongly distorted to accommodate a ligand in this site, and the a ligand makes awkward contacts with surrounding atoms. Thus, the results do not support proposals attributing an important role to five-coordinate zinc complexes in the catalytic mechanism of alc. dehydrogenase. The present approach makes it possible also to quantify the strain induced by the enzyme on to the zinc ion and its ligands; it amts. to 42-87 kJ/mol for four-coordinate active-site zinc ion complexes and 131-172 kJ/mol for five-coordinate ones. The four-coordinate structure with a water mol. bound to the zinc ion is about 20 kJ/mol less strained than the corresponding structure with a hydroxide ion, indicating that the enzyme does not speed up the reaction by forcing the zinc coordination sphere into a structure similar to the reaction intermediates.>> More from SciFinder ®
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A detailed parameterization is presented of a zinc ion with one histidine and two cysteinate ligands, together with one or two water, hydroxide, aldehyde, alc., or alkoxide ligands. The parameterization is tailored for the active site of alc. dehydrogenase and is obtained entirely from quantum chem. computations. The force-field reproduces excellently the geometry of quantum chem. optimized zinc complexes as well as the crystallog. geometry of the active site of alc. dehydrogenase and small org. structures. The parameterization is used in mol. dynamics simulations and mol. mech. energy minimizations of alc. dehydrogenase with a four- or five-coordinate catalytic zinc ion. The active-site zinc ion seems to prefer four-coordination over five-coordination by at least 36 kJ/mol. The only stable binding site of a fifth ligand at the active-site zinc ion is opposite to the normal substrate site, in a narrow cavity behind the zinc ion. Only mols. of the size of water or smaller may occupy this site. There are large fluctuations in the geometry of the zinc coordination sphere. A four-coordinate water mol. alternates frequently (every 7 ps) between the substrate site and the fifth binding site and even two five-coordinate water mols. may interchange ligation sites without prior dissocn. Ligand exchange at the zinc ion probably proceeds by a dissociative mechanism. The results show that it is essential to allow for bond stretching degrees of freedom in mol. dynamics simulations to get a correct description of the dynamics of the metal coordination sphere; bond length constraints may restrict the accessible part of the phase space and therefore lead to qual. erroneous results.>> More from SciFinder ®
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1ADBX-ray2.40A/B2-375[»]1ADCX-ray2.70A/B2-375[»]1ADFX-ray2.90A2-375[»]1ADGX-ray2.70A2-375[»]
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3BTOX-ray1.66A/B/C/D2-375[»]**3OQ6**X-ray1.20A/B2-375[»]
4DWVX-ray1.14A/B2-375[»]**4DXH**X-ray1.12A/B2-375[»]4NFHX-ray1.20A/B2-375[»]
4NFSX-ray1.10A/B2-375[»]4NG5X-ray1.10A/B2-375[»]**4XD2**X-ray1.10A/B2-375[»]
5ADHX-ray2.90A2-375[»]
5CDGX-ray1.40A/B2-375[»]5CDSX-ray1.40A/B2-375[»]5CDTX-ray1.70A/B2-375[»]5CDUX-ray1.60A/B2-375[»]
5KCPX-ray1.10A/B2-375[»]5KCZX-ray1.14A/B2-375[»]
5KJ1X-ray1.20A/B2-375[»]5KJ6X-ray1.14A/B2-375[»]
5KJCX-ray1.20A/B2-375[»]5KJEX-ray1.26A/B2-375[»]5KJFX-ray1.20A/B2-375[»]
5VJ5X-ray1.90A/B2-375[»]**5VJG**X-ray1.90A2-375[»]5VKRX-ray1.80A2-375[»]**5VL0**X-ray1.20A/B/C/D2-375[»]
5VN1X-ray1.25A/B/C/D2-375[»]
6ADHX-ray2.90A/B2-375[»]6CXXX-ray1.26A/B2-375[»]6CY3X-ray2.30A2-375[»]
7ADHX-ray3.20A2-375[»]8ADHX-ray2.40A2-375[»] ADH1E HORSE Liver

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        10         20         30         40         50         60         70         80
MSTAGKVIKC KAAVLWEEKK PFSIEEVEVA PPKAHEVRIK MVATGICRSD DHVVSGTLVT PLPVIAGHEA AGIVESIGEG
        90        100        110        120        130        140        150        160
VTTVRPGDKV IPLFTPQCGK CRVCKHPEGN FCLKNDLSMP RGTMQDGTSR FTCRGKPIHH FLGTSTFSQY TVVDEISVAK
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       170        180        190        200        210        220        230        240
IDAASPLEKV CLIGCGFSTG YGSAVKVAKV TQGSTCAVFG LGGVGLSVIM GCKAAGAARI IGVDINKDKF AKAKEVGATE
       250        260        270        280        290        300        310        320
CVNPQDYKKP IQEVLTEMSN GGVDFSFEVI GRLDTMVTAL SCCQEAYGVS VIVGVPPDSQ NLSMNPMLLL SGRTWKGAIF
       330        340        350        360        370   375
GGFKSKDSVP KLVADFMAKK FALDPLITHV LPFEKINEGF DLLRSGESIR TILTF    ADH1E HORSE Liver

         10         20         30         40    4648 5051       60     67  70         80
STAGKVIKC KAAVLWEEKK PFSIEEVEVA PPKAHEVRIK MVATGICRSD DHVVSGTLVT PLPVIAGHEA AGIVESIGEG V
         90        100        110        120        130        140        150        160
TTVRPGDKV IPLFTPQCGK CRVCKHPEGN FCLKNDLSMP RGTMQDGTSR FTCRGKPIHH FLGTSTFSQY TVVDEISVAK I
        170 174    180        190        200        210        220 224 228230        240
DAASPLEKV CLIGCGFSTG YGSAVKVAKV TQGSTCAVFG LGGVGLSVIM GCKAAGAARI IGVDINKDKF AKAKEVGATE C
        250        260     269270271     280        290        300        310        320
VNPQDYKKP IQEVLTEMSN GGVDFSFEVI GRLDTMVTAL SCCQEAYGVS VIVGVPPDSQ NLSMNPMLLL SGRTWKGAIF G
        330        340        350        360     369370  375    4DXH, 4XD2, 5VJ5
GFKSKDSVP KLVADFMAKK FALDPLITHV LPFEKINEGF DLLRSGESIR TILTF    ADH1E HORSE Liver
5VJ5(1-375,1-374,2-375)      ADH1E  HORSE Liver
ZN ZINC ION 4(ZN 2+) *228(H2 O)
PHN 1,10-PHENANTHROLINE 2(C12 H8 N2)
HELIX    1 AA1 CYS A   46  SER A   54  1  9
HELIX    2 AA2 CYS A  100  HIS A  105  1  6
HELIX    3 AA3 PRO A  165  CYS A  170  1  6
HELIX    4 AA4 LEU A  171  GLY A  173  5  3
HELIX    5 AA5 CYS A  174  LYS A  185  1 12
HELIX    6 AA6 GLY A  201  GLY A  215  1 15
HELIX    7 AA7 ASN A  225  ASP A  227  5  3
HELIX    8 AA8 LYS A  228  GLY A  236  1  9
HELIX    9 AA9 ASN A  242  TYR A  246  5  5
HELIX   10 AB1 PRO A  249  SER A  258  1 10
HELIX   11 AB2 ARG A  271  CYS A  281  1 11
HELIX   12 AB3 PRO A  305  SER A  310  1  6
HELIX   13 AB4 ILE A  318  PHE A  322  5  5
HELIX   14 AB5 LYS A  323  ALA A  337  1 15
HELIX   15 AB6 LEU A  342  ILE A  346  5  5
HELIX   16 AB7 LYS A  354  SER A  364  1 11
SHEET 1 AA1 5 VAL A  63  ILE A  64  0
LINK   SG  CYS A  46       ZN    ZN A 401  1555 2.48
LINK   NE2 HIS A  67       ZN    ZN A 401  1555 2.30
LINK   SG  CYS A  97       ZN    ZN A 402  1555 2.34
LINK   SG  CYS A 100       ZN    ZN A 402  1555 2.38
LINK   SG  CYS A 103       ZN    ZN A 402  1555 2.14
LINK   SG  CYS A 111       ZN    ZN A 402  1555 2.45
LINK   SG  CYS A 174       ZN    ZN A 401  1555 2.19
LINK ZN    ZN A 401        N10 PHN A 403  1555 2.40
LINK ZN    ZN A 401        N1  PHN A 403  1555 2.31
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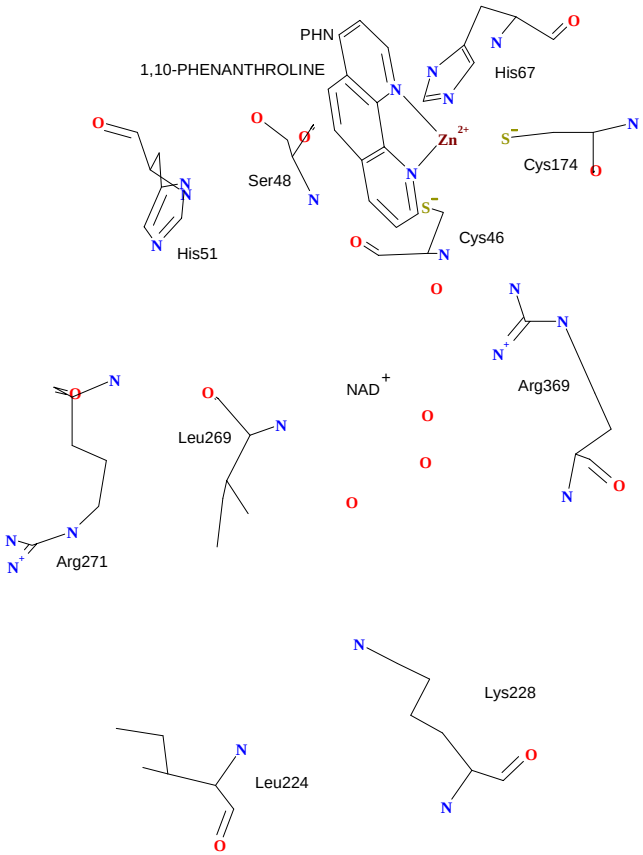


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SHEET 2 AA1 5 ILE A   7  LEU A  14 -1  N  LEU A  14   O  VAL A  63
SHEET 3 AA1 5 SER A  22  VAL A  28 -1  O  VAL A  26   N  CYS A   9
SHEET 4 AA1 5 PHE A 130  CYS A 132 -1  O  THR A 131   N  GLU A  27
SHEET 5 AA1 5 LYS A 135  ILE A 137 -1  O  LYS A 135   N  CYS A 132
SHEET 1 AA2 5 TYR A 149  ASP A 153  0
SHEET 2 AA2 5 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3 AA2 5 GLU A  68  ILE A  76 -1  O  ILE A  72   N  LYS A  39
SHEET 4 AA2 5 LYS A  88  PRO A  91 -1  O  VAL A  89   N  GLY A  71
SHEET 5 AA2 5 VAL A 157  LYS A 159 -1  O  ALA A 158   N  ILE A  90
SHEET 1 AA3 4 TYR A 149  ASP A 153  0
SHEET 2 AA3 4 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3 AA3 4 THR A 370  THR A 373 -1  O  LEU A 372   N  THR A  43
SHEET 4 AA3 4 HIS A 348  PRO A 351  1  N  LEU A 350   O  ILE A 371
SHEET 1 AA412 GLU A 239  VAL A 241  0
SHEET 2 AA412 ARG A 218  VAL A 222  1  N  GLY A 221   O  VAL A 241
SHEET 3 AA412 THR A 194  PHE A 198  1  N  CYS A 195   O  ILE A 220
SHEET 4 AA412 PHE A 264  GLU A 267  1  O  PHE A 266   N  PHE A 198
SHEET 5 AA412 VAL A 288  ILE A 291  1  O  VAL A 290   N  SER A 265
SHEET 6 AA412 THR A 313  GLY A 316  1  O  LYS A 315   N  ILE A 291
SHEET 1 AA5 2 LEU A 301  MET A 303  0
CISPEP 1 LEU A 61 PRO A 62          0        -7.74
SITE     1 AC1  4 CYS A  46  HIS A  67  CYS A 174  PHN A 403
SITE     1 AC2  4 CYS A  97  CYS A 100  CYS A 103  CYS A 111
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SITE     1 AC3  8 CYS A  46  SER A  48  HIS A  67  PHE A  93
SITE     2 AC3  8 CYS A 174  VAL A 292  VAL A 294   ZN A 401
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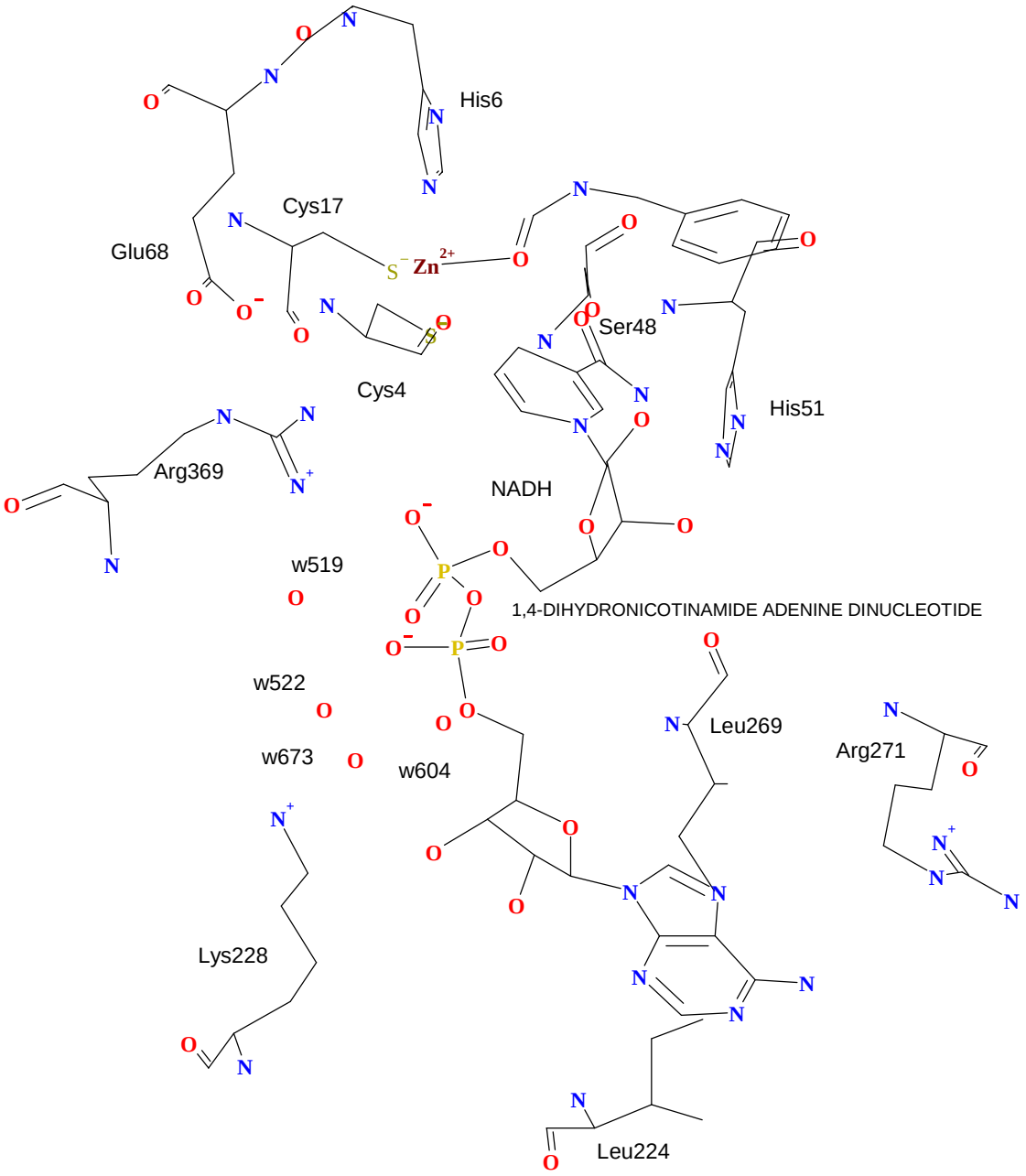

5VL0X-ray1.20A/B/C/D(1-375,1-374,2-375) NADH ADH1E HORSE Liver
ZN ION 8(ZN 2+) *1347(H2 O)
NAI 1,4-DIHYDRONICOTINAMIDE ADENINE DINUCLEOTIDE NADH 4(C21 H29 N7 O14 P2)
BNF N-BENZYLFORMAMIDE 4(C8 H9 N O)
MRD (4R)-2-METHYLPENTANE-2,4-DIOL 2(C6 H14 O2)

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HELIX    1 AA1 CYS A   46  SER A   54  1                                   9
HELIX    2 AA2 CYS A  100  HIS A  105  1                                   6
HELIX    3 AA3 PRO A  165  CYS A  170  1                                   6
HELIX    4 AA4 LEU A  171  GLY A  173  5                                   3
HELIX    5 AA5 CYS A  174  LYS A  185  1                                  12
HELIX    6 AA6 GLY A  201  ALA A  214  1                                  14
HELIX    7 AA7 ASN A  225  ASP A  227  5                                   3
HELIX    8 AA8 LYS A  228  GLY A  236  1                                   9
HELIX    9 AA9 ASN A  242  TYR A  246  5                                   5
HELIX   10 AB1 PRO A  249  SER A  258  1                                  10
HELIX   11 AB2 ARG A  271  CYS A  282  1                                  12
HELIX   12 AB3 PRO A  305  SER A  310  1                                   6
HELIX   13 AB4 ILE A  318  PHE A  322  5                                   5
HELIX   14 AB5 LYS A  323  ALA A  337  1                                  15
HELIX   15 AB6 LEU A  342  PRO A  344  5                                   3
HELIX   16 AB7 LYS A  354  SER A  364  1                                  11
SHEET    1 AA1 5 VAL A  63  ILE A  64  0
SHEET    2 AA1 5 ILE A   7  LEU A  14 -1  N  LEU A  14   O  VAL A  63
SHEET    3 AA1 5 SER A  22  VAL A  28 -1  O  VAL A  28   N  ILE A   7
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SHEET    4 AA1 5 PHE A 130  CYS A 132 -1  O  THR A 131   N  GLU A  27
SHEET    5 AA1 5 LYS A 135  ILE A 137 -1  O  LYS A 135   N  CYS A 132
SHEET    1 AA2 5 TYR A 149  ASP A 153  0
SHEET    2 AA2 5 GLU A  35  GLY A  44 -1  N  VAL A  36   O  VAL A 152
SHEET    3 AA2 5 ALA A  69  ILE A  76 -1  O  ILE A  72   N  LYS A  39
SHEET    4 AA2 5 LYS A  88  PRO A  91 -1  O  VAL A  89   N  GLY A  71
SHEET    5 AA2 5 VAL A 157  LYS A 159 -1  O  ALA A 158   N  ILE A  90
SHEET    1 AA3 4 TYR A 149  ASP A 153  0
SHEET    2 AA3 4 GLU A  35  GLY A  44 -1  N  VAL A  36   O  VAL A 152
SHEET    3 AA3 4 ARG A 369  THR A 373 -1  O  LEU A 372   N  THR A  43
SHEET    4 AA3 4 ILE A 346  PRO A 351  1  N  LEU A 350   O  ILE A 371
SHEET    1 AA412 GLU A 239  VAL A 241  0
SHEET    2 AA412 ARG A 218  VAL A 222  1  N  GLY A 221   O  VAL A 241
SHEET    3 AA412 THR A 194  PHE A 198  1  N  CYS A 195   O  ILE A 220
SHEET    4 AA412 PHE A 264  GLU A 267  1  O  PHE A 266   N  PHE A 198
SHEET    5 AA412 VAL A 288  ILE A 291  1  O  VAL A 290   N  SER A 265
SHEET    6 AA412 THR A 313  GLY A 316  1  O  LYS A 315   N  ILE A 291
LINK         SG  CYS A  46                ZN    ZN A 401     1555   1555  2.32
LINK         NE2 HIS A  67                ZN    ZN A 401     1555   1555  2.01
LINK         SG  CYS A  97                ZN    ZN A 402     1555   1555  2.34
LINK         SG  CYS A 100                ZN    ZN A 402     1555   1555  2.35
LINK         SG  CYS A 103                ZN    ZN A 402     1555   1555  2.32
LINK         SG  CYS A 111                ZN    ZN A 402     1555   1555  2.32
LINK         SG  CYS A 174                ZN    ZN A 401     1555   1555  2.25
LINK        ZN    ZN A 401                O16 BNF A 404     1555   1555  2.10
CISPEP   1 LEU A   61    PRO A   62          0        -3.76
SITE     1 AC1  5 CYS A  46  HIS A  67  CYS A 174  NAI A 403
SITE     2 AC1  5 BNF A 404
SITE     1 AC2  4 CYS A  97  CYS A 100  CYS A 103  CYS A 111
SITE     1 AC3 31 ARG A  47  SER A  48  HIS A  51  CYS A 174
SITE     2 AC3 31 THR A 178  GLY A 199  GLY A 201  GLY A 202
SITE     3 AC3 31 VAL A 203  ASP A 223  ILE A 224  LYS A 228
SITE     4 AC3 31 VAL A 268  ILE A 269  ARG A 271  VAL A 292
SITE     5 AC3 31 GLY A 293  VAL A 294  ALA A 317  ILE A 318
SITE     6 AC3 31 PHE A 319  ARG A 369   ZN A 401  BNF A 404
SITE     7 AC3 31 HOH A 503  HOH A 519  HOH A 590  HOH A 604
SITE     8 AC3 31 HOH A 673  HOH A 688  HOH A 698
SITE     1 AC4 10 CYS A  46  SER A  48  HIS A  67  PHE A  93
SITE     2 AC4 10 LEU A 116  CYS A 174  ILE A 318   ZN A 401
SITE     3 AC4 10 NAI A 403  LEU B 309
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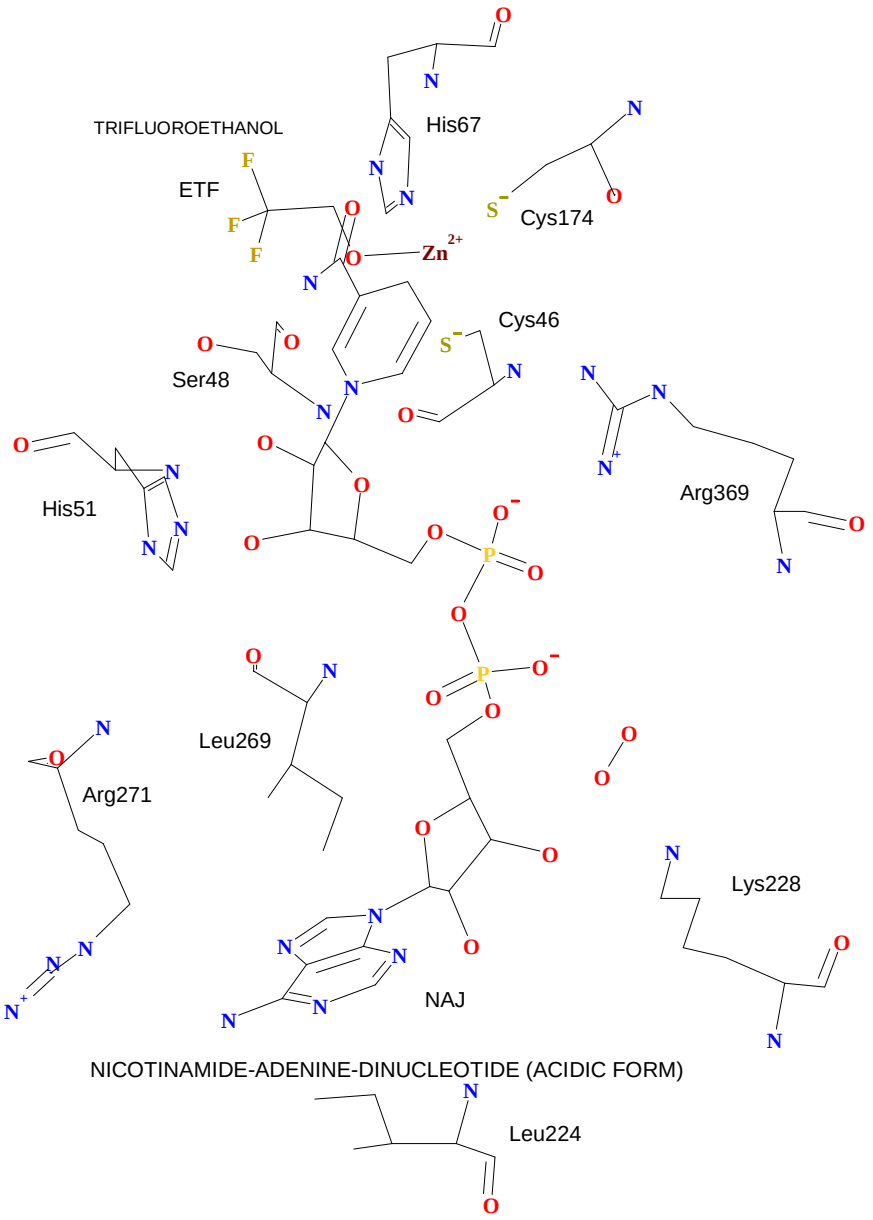
5VJGX-ray1.90A2-375[»] NADH ADH1E HORSE Liver

5VN1X-ray1.25A/B/C/D2-375[»] NADH ADH1E HORSE Liver



4DXH(1-375,1-374,2-375) ADH1E HORSE Liver
ZN ZINC ION **NAJ NICOTINAMIDE**-ADENINE-DINUCLEOTIDE (ACIDIC FORM) 15 HOH *1025(H2 O)
ETF TRIFLUORO**ETHANOL** MRD (4R)-2-**METHYL**PENTANE-2,4-DIOL
ZN 4(**ZN** 2+) 5 **NAJ** 2(C21 H27 N7 O14 P2) 6 ETF 2(C2 H3 F3 O) 4(C6 H14 O2)

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HELIX    1   1 CYS A   46  SER A   54  1  9    CYS46  SER54
HELIX    2   2 CYS A  100  HIS A  105  1  6    CYS100  HIS105
HELIX    3   3 PRO A  165  CYS A  170  1  6    PRO165  CYS170
HELIX    4   4 LEU A  171  GLY A  173  5  3    LEU171  GLY173
HELIX    5   5 CYS A  174  LYS A  185  1 12    CYS174  LYS185
HELIX    6   6 GLY A  201  ALA A  214  1 14    GLY201  ALA214
HELIX    7   7 ASN A  225  ASP A  227  5  3    ASN225  ASP227
HELIX    8   8 LYS A  228  GLY A  236  1  9    LYS228  GLY236
HELIX    9   9 ASN A  242  TYR A  246  5  5    ASN242  TYR246
HELIX   10  10 PRO A  249  SER A  258  1 10    PRO249  SER258
HELIX   11  11 ARG A  271  CYS A  282  1 12    ARG271  CYS282
HELIX   12  12 PRO A  305  SER A  310  1  6    PRO305  SER310
HELIX   13  13 ILE A  318  PHE A  322  5  5    ILE318  PHE322
HELIX   14  14 LYS A  323  ALA A  337  1 15    LYS323  ALA337
HELIX   15  15 LEU A  342  PRO A  344  5  3    LEU342  PRO344
HELIX   16  16 LYS A  354  SER A  364  1 11    LYS354  SER364
SHEET 1   A 5 VAL A  63  ILE A  64  0
SHEET 2   A 5 ILE A   7  LEU A  14 -1  N  LEU A  14   O  VAL A  63
SHEET 3   A 5 SER A  22  VAL A  28 -1  O  VAL A  28   N  ILE A   7
SHEET 4   A 5 PHE A 130  CYS A 132 -1  O  THR A 131   N  GLU A  27
SHEET 5   A 5 LYS A 135  ILE A 137 -1  O  LYS A 135   N  CYS A 132
SHEET 1   B 5 TYR A 149  ASP A 153  0
SHEET 2   B 5 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3   B 5 ALA A  69  ILE A  76 -1  O  ILE A  72   N  LYS A  39
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SHEET 4   B 5 LYS A  88  PRO A  91 -1  O  VAL A  89   N  GLY A  71
SHEET 5   B 5 VAL A 157  LYS A 159 -1  O  ALA A 158   N  ILE A  90
SHEET 1   C 4 TYR A 149  ASP A 153  0
SHEET 2   C 4 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3   C 4 ARG A 369  THR A 373 -1  O  LEU A 372   N  THR A  43
SHEET 4   C 4 ILE A 346  PRO A 351  1  N  LEU A 350   O  ILE A 371
SHEET 1   D12 GLU A 239  VAL A 241  0
SHEET 2   D12 ARG A 218  VAL A 222  1  N  GLY A 221   O  VAL A 241
SHEET 3   D12 THR A 194  PHE A 198  1  N  CYS A 195   O  ILE A 220
SHEET 4   D12 PHE A 264  GLU A 267  1  O  PHE A 266   N  PHE A 198
SHEET 5   D12 VAL A 288  ILE A 291  1  O  VAL A 290   N  SER A 265
SHEET 6   D12 THR A 313  GLY A 316  1  O  LYS A 315   N  ILE A 291
SHEET 7   D12 N  TRP A 314
SHEET 1   E 2 LEU A 301  MET A 303  0
SHEET 2   E 2 N  LEU A 301
LINK ZN    ZN A 375  O   ETF A 378 1555 1.95
LINK  NE2 HIS A  67  ZN    ZN A 375 1555 2.04
LINK  SG  CYS A 174  ZN    ZN A 375 1555 2.29
LINK  SG  CYS A 111  ZN    ZN A 376 1555 2.33
LINK  SG  CYS A  46  ZN    ZN A 375 1555 2.35
LINK  SG  CYS A  97  ZN    ZN A 376 1555 2.35
LINK  SG  CYS A 100  ZN    ZN A 376 1555 2.35
LINK  SG  CYS A 103  ZN    ZN A 376 1555 2.35
CISPEP   1 LEU A   61    PRO A   62          0        -6.26
SITE     1 AC1  5 CYS A  46  HIS A  67  CYS A 174  NAJ A 377
SITE     2 AC1  5 ETF A 378
SITE     1 AC2  4 CYS A  97  CYS A 100  CYS A 103  CYS A 111
SITE     1 AC3 33 ARG A  47  SER A  48  HIS A  51  CYS A 174
SITE     2 AC3 33 THR A 178  GLY A 199  GLY A 201  GLY A 202
SITE     3 AC3 33 VAL A 203  ASP A 223  ILE A 224  LYS A 228
SITE     4 AC3 33 VAL A 268  ILE A 269  ARG A 271  VAL A 292
SITE     5 AC3 33 GLY A 293  VAL A 294  ALA A 317  ILE A 318
SITE     6 AC3 33 PHE A 319  ARG A 369   ZN A 375  ETF A 378
SITE     7 AC3 33 HOH A 406  HOH A 430  HOH A 439  HOH A 517
SITE     8 AC3 33 HOH A 588  HOH A 647  HOH A 664  HOH A 686
SITE     9 AC3 33 HOH A 711
SITE     1 AC4 10 CYS A  46  SER A  48  LEU A  57  HIS A  67
SITE     2 AC4 10 PHE A  93  LEU A 116  CYS A 174  VAL A 294
SITE     3 AC4 10  ZN A 375  NAJ A 377
SITE     1 AC5  8 ASP A 297  GLN A 299  LYS A 338  LYS A 339
SITE     2 AC5  8 PHE A 340  HOH A 735  HOH A 752  HOH A 814
SITE     1 AC6  6 ARG A 218  THR A 238  GLU A 239  HOH A 998
SITE     1 AC7  6 LYS A 168  ASP A 343  ILE A 346  THR A 347
SITE     2 AC7  6 HOH A 840  HOH A 852
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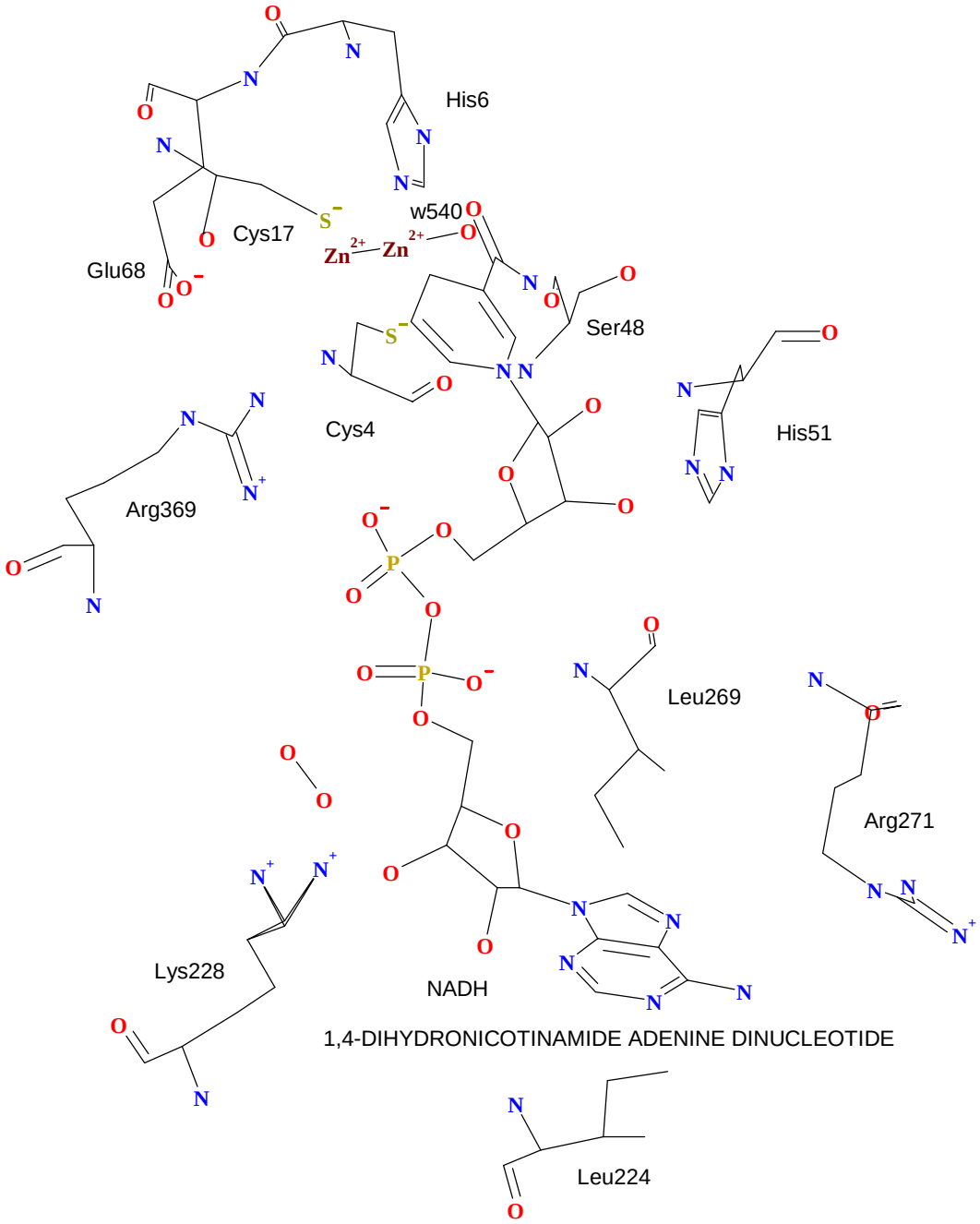

4XD2(1-375,1-374,2-375) NADH ADH1E HORSE Liver
ZN ZINC ION 4(ZN 2+) *804(H2 O)
NAI NADH 1,4-DIHYDRONICOTINAMIDE ADENINE DINUCLEOTIDE 2(C21 H29 N7 O14 P2)
MRD (4R)-2-METHYLPENTANE-2,4-DIOL 3(C6 H14 O2

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HELIX    1 AA1 CYS A   46  SER A   54  1  9
HELIX    2 AA2 CYS A  100  HIS A  105  1  6
HELIX    3 AA3 PRO A  165  CYS A  170  1  6
HELIX    4 AA4 LEU A  171  GLY A  173  5  3
HELIX    5 AA5 CYS A  174  LYS A  185  1 12
HELIX    6 AA6 GLY A  201  ALA A  214  1 14
HELIX    7 AA7 ASN A  225  ASP A  227  5  3
HELIX    8 AA8 LYS A  228  GLY A  236  1  9
HELIX    9 AA9 ASN A  242  TYR A  246  5  5
HELIX   10 AB1 PRO A  249  SER A  258  1 10
HELIX   11 AB2 ARG A  271  CYS A  282  1 12
HELIX   12 AB3 PRO A  305  SER A  310  1  6
HELIX   13 AB4 ILE A  318  PHE A  322  5  5
HELIX   14 AB5 LYS A  323  ALA A  337  1 15
HELIX   15 AB6 LEU A  342  PRO A  344  5  3
HELIX   16 AB7 LYS A  354  SER A  364  1 11
SHEET 1 AA1 4 ILE A   7  VAL A  13  0
SHEET 2 AA1 4 SER A  22  VAL A  28 -1  O  VAL A  28   N  ILE A   7
SHEET 3 AA1 4 PHE A 130  CYS A 132 -1  O  THR A 131   N  GLU A  27
SHEET 4 AA1 4 LYS A 135  ILE A 137 -1  O  LYS A 135   N  CYS A 132
SHEET 1 AA2 5 TYR A 149  ASP A 153  0
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SHEET 2 AA2 5 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3 AA2 5 ALA A  69  ILE A  76 -1  O  ILE A  72   N  LYS A  39
SHEET 4 AA2 5 LYS A  88  PRO A  91 -1  O  VAL A  89   N  GLY A  71
SHEET 5 AA2 5 VAL A 157  LYS A 159 -1  O  ALA A 158   N  ILE A  90
SHEET 1 AA3 4 TYR A 149  ASP A 153  0
SHEET 2 AA3 4 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET 3 AA3 4 ARG A 369  THR A 373 -1  O  LEU A 372   N  THR A  43
SHEET 4 AA3 4 ILE A 346  PRO A 351  1  N  LEU A 350   O  ILE A 371
SHEET 1 AA412 GLU A 239  VAL A 241  0
SHEET 2 AA412 ARG A 218  VAL A 222  1  N  GLY A 221   O  GLU A 239
SHEET 3 AA412 THR A 194  PHE A 198  1  N  CYS A 195   O  ILE A 220
SHEET 4 AA412 PHE A 264  GLU A 267  1  O  PHE A 266   N  PHE A 198
SHEET 5 AA412 VAL A 288  ILE A 291  1  O  VAL A 290   N  SER A 265
SHEET 6 AA412 THR A 313  GLY A 316  1  O  THR A 313   N  SER A 289
LINK   SG  CYS A  46       ZN   A ZN A 401 1555 2.31
LINK   NE2 HIS A  67       ZN   A ZN A 401 1555 2.07
LINK   SG  CYS A  97       ZN     ZN A 402 1555 2.33
LINK   SG  CYS A 100       ZN     ZN A 402 1555 2.31
LINK   SG  CYS A 103       ZN     ZN A 402 1555 2.33
LINK   SG  CYS A 111       ZN     ZN A 402 1555 2.33
LINK   SG  CYS A 174       ZN   A ZN A 401 1555 2.28
LINK ZN   A ZN A 401        O   HOH A 540 1555 2.04
CISPEP   1 LEU A   61    PRO A   62          0        -4.36
CISPEP   2 LEU B   61    PRO B   62          0        -1.43
SITE     1 AC1  5 CYS A  46  HIS A  67  CYS A 174  NAI A 403
SITE     2 AC1  5 HOH A 540
SITE     1 AC2  4 CYS A  97  CYS A 100  CYS A 103  CYS A 111
SITE     1 AC3 31 ARG A  47  SER A  48  HIS A  51  CYS A 174
SITE     2 AC3 31 THR A 178  GLY A 199  GLY A 201  GLY A 202
SITE     3 AC3 31 VAL A 203  ASP A 223  ILE A 224  LYS A 228
SITE     4 AC3 31 VAL A 268  ILE A 269  ARG A 271  VAL A 292
SITE     5 AC3 31 GLY A 293  VAL A 294  ALA A 317  ILE A 318
SITE     6 AC3 31 PHE A 319  ARG A 369   ZN A 401  HOH A 561
SITE     7 AC3 31 HOH A 625  HOH A 658  HOH A 660  HOH A 670
SITE     8 AC3 31 HOH A 698  HOH A 734  HOH A 774
SITE     1 AC4  7 ASP A 297  GLN A 299  LYS A 338  PHE A 340
SITE     2 AC4  7 ALA A 341  HOH A 616  HOH A 863
SITE     1 AC5  7 ARG A 218  THR A 238  GLU A 239  HOH A 515
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1YE3(1-375,1-374,2-375) NADH ADH1E HORSE Liver

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ZN ZINC ION 2(ZN 2+) *200(H2 O)
MPD (4S)-2-METHYL-2,4-PENTANEDIOL C6 H14 O2
HELIX    1   1 CYS A   46  SER A   54  1  9
HELIX    2   2 PRO A  165  CYS A  170  1  6
HELIX    3   3 LEU A  171  GLY A  173  5  3
HELIX    4   4 CYS A  174  LYS A  185  1 12
HELIX    5   5 GLY A  201  ALA A  214  1 14
HELIX    6   6 ASN A  225  ASP A  227  5  3
HELIX    7   7 LYS A  228  GLY A  236  1  9
HELIX    8   8 ASN A  242  TYR A  246  5  5
HELIX    9   9 PRO A  249  SER A  258  1 10
HELIX   10  10 ARG A  271  CYS A  282  1 12
HELIX   11  11 PRO A  305  SER A  310  1  6
HELIX   12  12 ILE A  318  PHE A  322  5  5
HELIX   13  13 LYS A  323  ALA A  337  1 15
HELIX   14  14 LEU A  342  ILE A  346  5  5
HELIX   15  15 LYS A  354  SER A  364  1 11
SHEET    1   A 4 ILE A   7  VAL A  13  0
SHEET    2   A 4 SER A  22  VAL A  28 -1  O  GLU A  24   N  ALA A  11
SHEET    3   A 4 PHE A 130  CYS A 132 -1  O  THR A 131   N  GLU A  27
SHEET    4   A 4 LYS A 135  ILE A 137 -1  O  LYS A 135   N  CYS A 132
SHEET    1   B 5 TYR A 149  ASP A 153  0
SHEET    2   B 5 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET    3   B 5 ALA A  69  ILE A  76 -1  O  ILE A  72   N  LYS A  39
SHEET    4   B 5 LYS A  88  PRO A  91 -1  O  VAL A  89   N  GLY A  71
SHEET    5   B 5 VAL A 157  ILE A 160 -1  O  ILE A 160   N  LYS A  88
SHEET    1   C 4 TYR A 149  ASP A 153  0
SHEET    2   C 4 GLU A  35  GLY A  44 -1  N  ILE A  38   O  THR A 150
SHEET    3   C 4 THR A 370  THR A 373 -1  O  LEU A 372   N  THR A  43
SHEET    4   C 4 HIS A 348  PRO A 351  1  N  LEU A 350   O  ILE A 371
SHEET    1   D 6 GLU A 239  VAL A 241  0
SHEET    2   D 6 ARG A 218  VAL A 222  1  N  GLY A 221   O  VAL A 241
SHEET    3   D 6 THR A 194  PHE A 198  1  N  CYS A 195   O  ILE A 220
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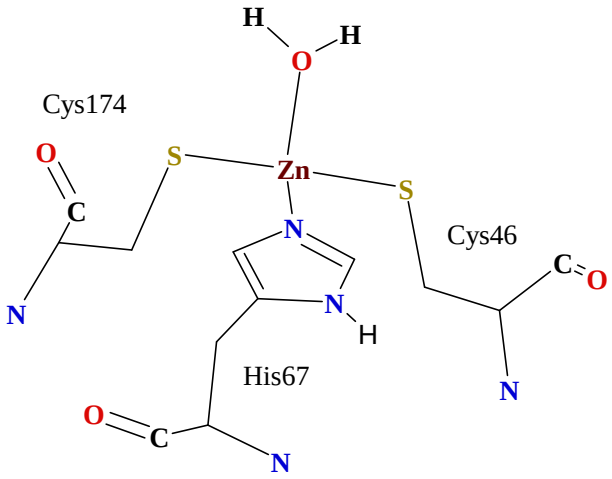

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SHEET    4   D 6 PHE A 264  GLU A 267  1  O  PHE A 266   N  PHE A 198
SHEET    5   D 6 VAL A 288  ILE A 291  1  O  VAL A 290   N  SER A 265
SHEET    6   D 6 THR A 313  GLY A 316  1  O  LYS A 315   N  ILE A 291
LINK         ZN    ZN A 376                 SG  CYS A  97     1555   1555  2.30
LINK         ZN    ZN A 376                 SG  CYS A 100     1555   1555  2.37
LINK         ZN    ZN A 376                 SG  CYS A 103     1555   1555  2.35
LINK         ZN    ZN A 376                 SG  CYS A 111     1555   1555  2.33
LINK         ZN    ZN A 375                 SG  CYS A  46     1555   1555  2.42
LINK         ZN    ZN A 375                 SG  CYS A 174     1555   1555  2.21
LINK         ZN    ZN A 375                 NE2 HIS A  67     1555   1555  2.05
LINK         ZN    ZN A 375                 O   HOH A 463     1555   1555  2.11
CISPEP   1 LEU A   61    PRO A   62          0        -0.99
SITE     1 AC1  4 CYS A  46  HIS A  67  CYS A 174  HOH A 463
SITE     1 AC2  4 CYS A  97  CYS A 100  CYS A 103  CYS A 111
SITE     1 AC3  5 LEU A 116  VAL A 294  PRO A 296  MET A 306
SITE     2 AC3  5 HOH A 464
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