Name Surname :________________________________ Group Nº ___________2025

Co**Enzymes** vitamin **B$_3$** NAD$^+$(Ox), NADH(Red). **A.** Task descriptions: for studies research of: **ADH**

Alcohol Dehydrogenase ChemScape MDLi **RasMol** MAGE FireFox application.

B. Task Lunch the molecular tutorial prepared by Aris Kaksis 2025 Riga Stradin's University look at: http://aris.gusc.lv/ChemFiles/AlhoDeHydrogenase/NadDehydrogenase.htm the **CPK** color scheme 1965

1. What N- and C-terminus amino acids have **ADH** IV? Menu Backbone, Termini Display option starting amino acid is SER1…..and finishing PHE374........? What total number (see 2nd page in 1AGN.pdb) ....386. and amino acids on **1JU9.pdb** polypeptide chain.........374.?

2. What Enzyme Class (of seven Classes 1.,2.,3.,4.,5.,6.,7.) present **ADH**? EC 1.1.1.1...

3. What particle in **ADH** transfer two reducing equivalents **(2e$^-$)** from alcohol to **NAD$^+$**? **H$^-$(2e$^-$+H$^+$)**............

4. Summary Red-Ox reaction studies (endoergic or exoergic) of **ADH** by reduced form ethanol and oxidised NAD$^+$ solutions **4.1 – 4.17** ! **Absolute** potential standard values **E˚** by David Harris, Kortly Shucha: at standard conditions of **absolute** scale temperature 298,15…... K degree according to the Celsius scale 25˚ C .

Alcohol dehydrogenase alcohol oxidation to aldehyde (aerobic).

4. Oks NAD$^+$+H$^-$(2e$^-$)=NADH ; **-E˚^{1}=0,4095 V absolute inverse** standard potential David Harris.

4. Red CH$_3$CH$_2$OH+H$_2$O=CH$_3$HC=O+H$_3$O$^+$+H$^-$(2e$^-$); **absolute** standard potential **E˚^{2}=-0.0550 V**

4.3 OksRed sum: **NAD$^+$+CH$_3$CH$_2$OH+H$_2$O=>NADH +CH$_3$HC=O +H$_3$O$^+$**.......................................

4.4 **ΔE˚=E˚$^2_{H2O}$+E˚^{1}=-0.0550+0,4095= 0,3545**.......... **V**, half reactions sum standard potencial ΔE˚.

4.5 ΔG$_{eqStandard}$=ΔE˚•F•n=0,3545*2*96485/1000=68,4......... $^{kJ}/_{mol}$ standard free energy change.

1> $K_{eqstandard} = \frac{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]} = e^{-\frac{\Delta G_{eqAerobi}}{R\bullet T}}$ =EXP(-68400/8,314/298,15)=**1,038•10^{-12}=10**$^{-12}$.............

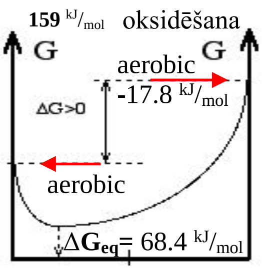
4.6 Is favored or unfavored aerobic reaction : Unfavored .... page 8; ;

ΔG$_{Hess}$=ΔG°$_{H3O}$-ΔG°$_{CH3CHO}$-ΔG°$_{NADH}$–(ΔG°$_{CH3CH2OH}$+ΔG°$_{H2O}$+ΔG°$_{NAD+}$)=

=**32,2824+1175,5732-151,549-(75,2864**+1059,11-237,191)=159........... $^{kJ}/_{mol}$ endoergic.............

Unfavored **equilibrium** constant **K$_{eq}$**$_{Aerobic}$**=10**$^{-12}$ value shows stability in mixture.

Endothermic and endoergic etanol **H$_3$CCH$_2$OH oxidation H$_3$CCH=O**



4.7 Hess free energy change positive **ΔG$_{Hess_oxidation}$**=159........$^{kJ}/_{mol}$, but

4.8 minimizes **ΔG$_{min}$=ΔG$_{eq}$=68,4**....... $^{kJ}/_{mol}$ reaching aerobic equilibrium mixture:

4.9 Aerobic oxidation with [**NAD$^+$**]/[**NADH**]=10^6 homeostasis pH=7,36 is favored .

ΔG$_{AerobicOx}$=**68,4**+8,3144*298,15*ln(1/10^6*1/1*10^($^{-7,36}$)/55,3457)/1000=**-17,8**.....$^{kJ}/_{mol}$;

A+B+C50%D+E+F

NAD$^+$+H$_3$CCH$_2$OH+H$_2$O

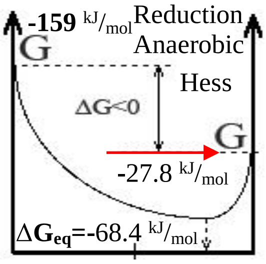
NADH+H$_3$CCHO+H$_3$O$^+$

Inverse symmetry: aerobic oxidation is **inverse** symmetric anaerobic reduction :

10^{-12}=$\frac{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]}$ =**K$_{eq}$**$_{Aerobic}$<1<**K$_{eq}$**$_{Anaerobic}$=$\frac{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]}{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}$ =10^{12}

Same number |**ΔG$_{Hess_oxidation}$**|=|159....... | $^{kJ}/_{mol}$ = |**ΔG**$_{Hesa}$|=|-159..... |.$^{kJ}/_{mol}$ of opposite sign

Inverse exothermic and exoergic ethanal **H$_3$CCH=O reduction H$_3$CCH$_2$OH**



4.10 hypoxic anaerobic ethanal reduction is **inverse** negative: **ΔG**$_{Hesa}$=-159......$^{kJ}/_{mol}$, but

4.11 minimized about **ΔG**$_{eq}$=**ΔE˚•F•n**=-0,3545 V•2 mol•96485 $^{C}/_{mol}$=**-68,4**....**$^{kJ}/_{mol}$.**

4.12 Ethanal reduction about **ethanol** with anaerobic ratio [**NAD$^+$**]/[**NADH**]=1/10 and pH=7,36 is favored, negative, exoergic free energy change $_l$:

D+E+F50%A+B+C

NADH+H$_3$CCHO+H$_3$O$^+$

NAD$^+$+H$_3$CCH$_2$OH+H$_2$O

$$\Delta G_{anaerobic} = \mathbf{-68{,}4} + 8{,}3144 * 298{,}15 * \mathbf{ln}\left(\frac{1}{10}\,\frac{1}{10}\,\frac{55{,}3}{10^{-7{,}36}}\right) = \mathbf{-27{,}8}......\ \mathbf{^{kJ}/_{mol}}\ ;$$

ΔG$_{AnaerobicRed}$=-68,4+8,3144*298,15*ln(1/10*1/10*55,3457/10^($^{-7,36}$))/1000==**-27,8**......$^{kJ}/_{mol}$;

5.0 What **ADH IV** isoelectric point IEP=pH=$pK_{a\text{-vid}}$ at physiologic pH=7.36 ? To determine water solution pH with **ADH IV** concentration $C=10^{-7.05339}$ M ($^{mol}/_{Litre}$)!

<u>Alcohol dehydrogenase **ADH** E.1.1.1.1. oxidoreductase</u>

Sequence of 386 AA amino acids for human ADH IV molecule 1AGN.pdb:

MFAEIQIQDKDRMGTAGKVIKCKAAVLWEQKQPFSIEEIEVAPPKTKEVRIKILATGICRTDDHVIKGTMVSKFPVI
VGH
EATGIVESIGEGVTTVKPGDKVIPLFLPQCRECNACRNPDGNLCIRSDITGRGVLADGTTRFTCKGKPVHHFMNTS
TFTE
YTVVDESSVAKIDDAAPPEKVCLIGCGFSTGYGAAVKTGKVKPGSTCVVFGLGGVGLSVIMGCKSAGASRIIGID
LNKDK
FEKAMAVGATECISPKDSTKPISEVLSEMTGNNVGYTFEVIGHLETMIDALASCHMNYGTSVVVGVPPSAKMLT
YDPMLL
FTGRTWKGCVFGGLKSRDDVPKLVTEFLAKKFDLDQLITHVLPFKKISEGFELLNSGQSIRTVLTF

AA	pKa_{COO-}	pKa_{NH3+}	pK_{RR}	Nr	AA	pKa_{COO-}	pKa_{NH3+}	pK_{RR}	Nr
M	9.21		M	1	D		3.65	D	59
E		4,25	E	2	E		4,25	E	60
D		3,65	D	3	K		10,53	K	61
K		10,53	K	4	C		8,18	C	62
D		3,65	D	5	C		8,18	C	63
		12,48	R	6	Y		10,07	Y	64
K		10,53	K	7	K		10,53	K	65
K		10,53	K	8	K		10,53	K	66
C		8,18	C	9	K		10,53	K	67
K		10,53	K	10	C		8,18	C	68
E		4,25	E	11	C		8,18	C	69
K		10,53	K	12	K		10,53	K	70
E		4,25	E	13	R		12,48	R	71
E		4,25	E	14	D		3,65	D	72
E		4,25	E	15	K		10,53	K	73
K		10,53	K	16	D		3,65	D	74
K		10,53	K	17	K		10,53	K	75
E		4,25	E	18	E		4,25	E	76
R		12,48	R	19	K		10,53	K	77
K		10,53	K	20	E		4,25	E	78
C		8,18	C	21	C		8,18	C	79
R		12,48	R	22	K		10,53	K	80
D		3,65	D	23	D		3,65	D	81
D		3,65	D	24	K		10,53	K	82
H		6	H	25	E		4,25	E	83
K		10,53	K	26	E		4,25	E	84
K		10,53	K	27	Y		10,07	Y	85
H		6	H	28	E		4,25	E	86
E		4,25	E	29	H		6	H	87
E		4,25	E	30	E		4,25	E	88
E		4,25	E	31	D		3,65	D	89
K		10,53	K	32	C		8,18	C	90
D		3,65	D	33	H		6	H	91
K		10,53	K	34	Y		10,07	Y	92
C		8,18	C	35	K		10,53	K	93
R		12,48	R	36	Y		10,07	Y	94
E		4,25	E	37	D		3,65	D	95
C		8,18	C	38	R		12,48	R	96
C		8,18	C	39	K		10,53	K	97
R		12,48	R	40	C		8,18	C	98
D		3,65	D	41	K		10,53	K	99
C		8,18	C	42	R		12,48	R	100
R		12,48	R	43	D		3,65	D	101
D		3,65	D	44	D		3,65	D	102
R		12,48	R	45	K		10,53	K	103
D		3,65	D	46	E		4,25	E	104
R		12,48	R	47	K		10,53	K	105
C		8,18	C	48	K		10,53	K	106
K		10,53	K	49	D		3,65	D	107
K		10,53	K	50	D		3,65	D	108
H		6	H	51	H		6	H	109
H		6	H	52	K		10,53	K	110
E		4,25	E	53	K		10,53	K	111
Y		10,07	Y	54	E		4,25	E	112
D		3,65	D	55	E		4,25	E	113
E		4,25	E	56	R		12,48	R	114
K		10,53	K	57	F	1,83		F	115
D		3,65	D	58					

115 of 386 amino acids active values pKa

Sum = 881,66...............

$= \Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal} =$

$pKa_{mean} = (\Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal})/NpKa$

IEP= pKa_{mean} =881.66 / 115 =**7.6666**.............

Calculation tasks for human ADH IV molecule 1AGN.pdb

Protolytic constant, isoelectric point IEP= pKa_{mean} calculate of side chains $\Sigma pK_{aRside\ group}$.. $pK_{aNterminal}NH_3$

and $pK_{aCterminalCOO-}$ constants sum divide with number of acid groups NpK_a:

$$IEP = pKa_{mean} = (\Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal})/NpKa$$

1 Acid groups number in sum NpKa=113.......+2.........=115.....

386 amino acids of them protolytic constants pK_a for side groups 113+2 terminus N and C,

N-terminal metionine M $pK_{aNterminal}$=9.21 and C-terminąl phenilalanin F $pKa_{Cterminal}$=1,83

Sum are calculating as $\Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal}$ = 881,66.............

2 **1.** Average acid group constant pKa_{mean} = IEP **ISOELEKTRIC POINT**

IEP = pKa_{mean} =881,66 / 115 =**7.6666**.............

At pH value of amino acid and protein on isoelectric point pH**=IEP total charge** is zero „**0**"

0——— plus (+) acidic————zero charge „**0**" **IEP**=pH———— minus (-) basic———→ 14 pH scale

-COOH & **-NH$_3^+$** positive charge ....**-COO$^-$** & **-NH$_3^+$**..........................charge is negative **-COO$^-$** & **-NH$_2$**

Underline and determine existing: positive (+) or negative (-) or zero !

3 Determine ADH IV molecule charge sign (+). zero „**0**" or (-) at physiologic pH=7.36

Underline existing:

-COOH & **-NH$_3^+$** positive (+) charge ……. pH=7.36 < IEP=**7.67** .... charge negative(-) **-COO$^-$** & **-NH$_2$**.

4 Determine ADH IV molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH$_3^+$** positive (+) charge ……. IEP=**7.67**< pH=8.8 .... charge negative(-) **-COO$^-$** & **-NH$_2$**.

5 Calculate ADH IV solution pH at concentration C=$10^{-6,8473}$ M (mol / Litre)

by *Ostwald dilution law* concentration M in logarithm:

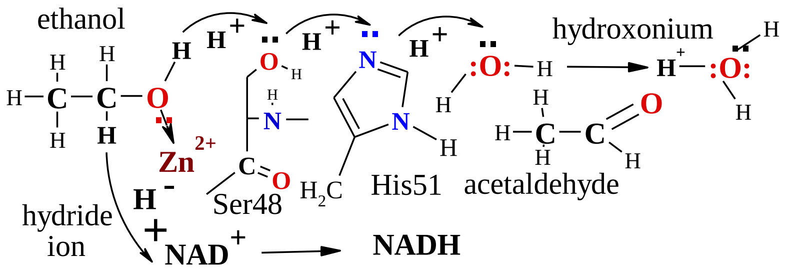
$$pH=\frac{pKa_{mean} - \log C}{2} = \frac{7.6666087 - \log 10^{-7,0533913}}{2} = \frac{7.6666087 + 7,0533913}{2} = \mathbf{14,72} / 2 = 7,36......$$

7,36 Attractor ADH IV concentration is C=$10^{-7,05339}$..............M .

5. . Place catalytic Zn^{2+}ion, ethanol oxygen atom **O** coordinate with donor acceptor bond and four jumping dissociated proton H^+ pathway from alcohol group **-CH_2-O-H** to Ser48 to His51 and resulting bound proton H^+ to water molecule **H_2O** forming hydronium ion **H_3O^+**.

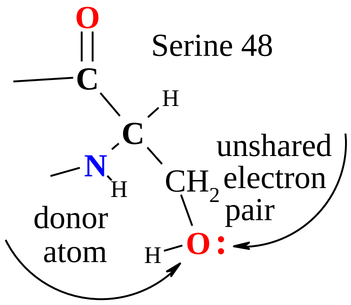
ADH during alcohol oxidation in water medium ,

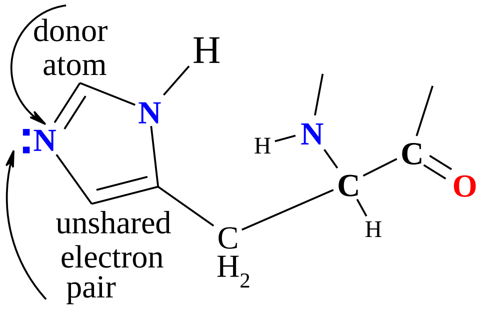
Place hydride ion H^- in to tunneling pathway from alcohol group carbon atom-CH_2- to NAD^+ cyclic carbon atom –HC^+– producing NADH.



that **H_2O** water molecule forming hydroxonium ion **H_3O^+** and aldehyde

6.Place in **Ser-48, His-51** structures **O**, **N** atoms and <u>electrons pair donor atoms</u> **O:**, **:N**!





Histidine 51

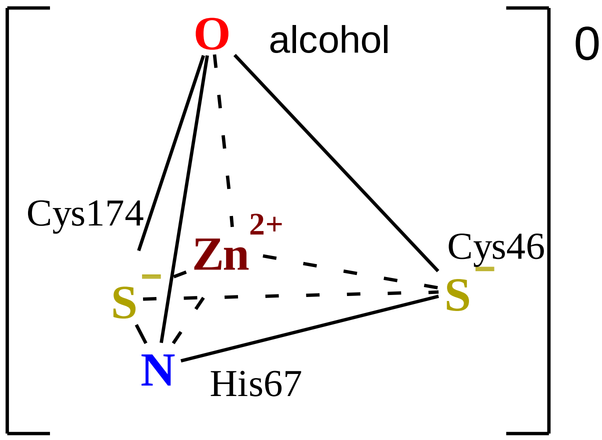
7. Place **O** , **Zn^{2+}**, **S**, **N** atoms and charge zero 0 of complex [Zn^{2+}(S^-Cys)$_2$(O-*spirts*)(NHis)]^0

for tetragonal geometry,

like trigonaāl pyramid!

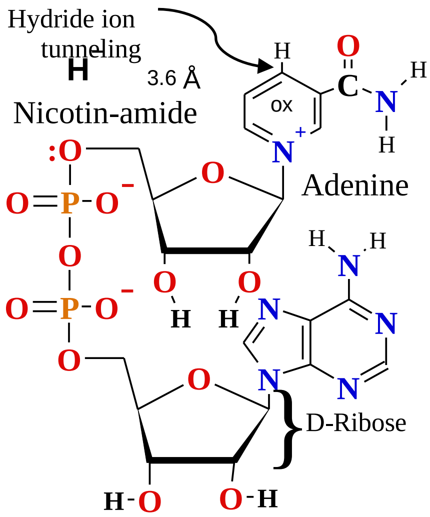
1HLD.pdb **Zn^{2+}** coordinates:

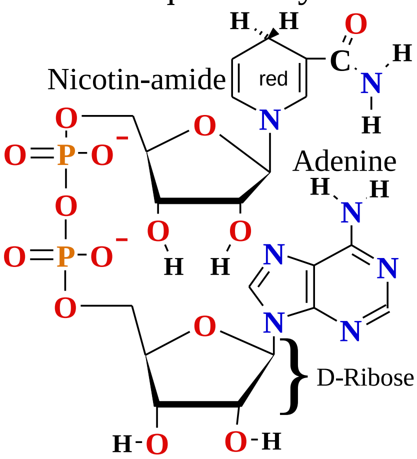
Cys46-Cys174-His67-**O** alcohol



8. What vitamin-cofactor oxidizes alcohols in **ADH dimer** quaternary structure?.....B_3 vitamin..

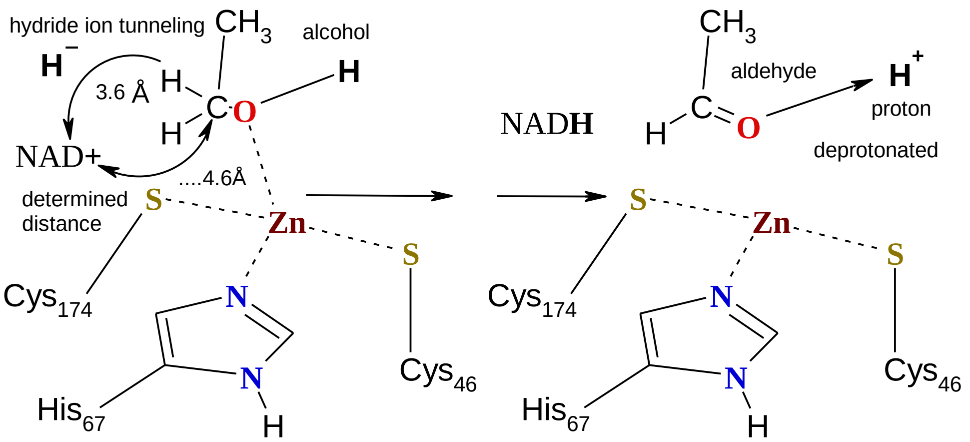
9. Oxidised **NAD^+** hydride tunneling **nicotine adenine dinucleotide**: Nicotin-amide, Adenine, two riboses, two phosphates with anhydride bond between phosphates





10. NADH hydride **nicotine adenine dinucleotide** reduced form: Nicotin-amide, Adenine, two riboses, two phosphates with anhydride bond between phosphates

ADH 1HLD.pdb **Zn^{2+}** coordinates Cys46-Cys174-His67-**O** : Tunnel distance 3,6 Å for hydride



ion **H**$^-$ to NAD$^+$ nicotine amide positive charged cycle carbon atom –CH–. Measure distance ......4,6 Å from alcohol –CH_2- carbon atom to NAD+ aromatic cycle –CH–in 1HLD.pdb molecule. With right button click in menu choose „Distance"

from „Select Mouse Click Action" measure distance from alcohol carbon atom –CH_2- ......4.6 Å to NAD$^+$ nicotine amide cyclic carbon atom –CH–!

11. Place amino acid numbers for coordination sphere and measure distance in angstroms units.

12. Secondary structures in **ADH** are............alpha helixes and................beta sheets.

13. Count **alpha-helixes** on **ADH** polypeptide molecule? ....**16 alpha-helixes**........................

14. Count **beta strands - sheets** in **ADH** molecule? **4**.......**beta strands in-sheet** ....

..................**6 beta strands in-sheet**.........and single alone ....**beta strand**

15. Count quaternary 4° structure components of 3° subunits in **ADH** molecule **1JU9zn.pdb** and **1HLDznNAD.pdb**? identic **ADH** molecules two........, each bind co**enzime** $\mathbf{B_3}$..............

each in domain binds **substrate alcohol group** like ethanol..............., retinol..............

16. What physiological functions in human body have ADH against ethanol?......................

.... remove two hydrogen atoms and so oxidize ethanol and form the acetaldehyde.....................

18. What toxic physiological functions in human body have ethanol molecules at long time abused? CSDD Auto transport certification limited concentration in blood 0.5 promilles and more? a) slow down the transport through membrane aquaporin-channels of water + oxygen .....

b).... long time abused in body leads to ....and hypoxia..............

c).... ethanol compete with retinol oxidation to prevent A vitamin formation ....

19. What toxic functions in human body have **ADH** against methanol?

.... to poison human body with formaldehyde...................

remove two hydrogen atoms of alcohol so to oxidize methanol and form the formaldehyde ....

20. Complete the oxidation reaction for methanol: $\mathbf{H_3C\text{-}OH + NAD^+}$ in water.

$\mathbf{H_3C\text{-}OH + NAD^+ + H_2O}$(His51)<=>$\mathbf{H_2C{=}O}$ + **NADH** $\mathbf{+H_3O^+}$ + His51 ......

Methanol B_3 vitamin Formaldehyde B_3 vitamin reduced

21. How compete ethanol with methanol? What ist the anti dot against methanol misuse in human body? high ethanol concentration oppress methanol to oxidation ....

methanol oxidation silencing lets throw aquaporins eliminate from human body methanol ......

22. To call six crystalline shapes for ADH subunits designation by Greek alphabet letters!

1. alpha α......, 2. beta β......,. 3. gamma γ........., 4. pee π...., 5. chi χ....., 6. sigma σ........

23. What kind human alcohol dehydrogenase crystallization failed? ADH6_HUMAN.........

To depict what kind of human alcohol dehydrogenases seven types - proteins identified in organism from data bank of Uni-Prot KB files:

1. ....ADH1A_HUMAN, subunits designated alpha α ....
2. ....ADH1B_HUMAN, subunits designated beta β ....
3. ....ADH1G_HUMAN, subunits designated gamma γ ....
4. ....ADH4_HUMAN, subunits designated pi π ....
5. ....ADHX_HUMAN, subunits designated chi χ ....
6. ....ADH7_HUMAN, subunits designated sigma σ ....
7. ....ADH6_HUMAN, absent crystalline filed ....

http://aris.gusc.lv/ChemFiles/AlhoDeHydrogenase/4DXH5VJ5hOhBioChem1718/5VJ5hOhBioChem17.pdf

Table 1: Nomenclature for Human Alcohol Dehydrogenase

The Class	System	Protein gene	Uni-Prot KB	Gene New	Gene Old
Class I 1HSO	α-subunit	ADH1A	ADH1A_HUMAN	ADH1	ADH1A
Class I 1DEH	β-subunit	ADH1B	ADH1B_HUMAN	ADH2	ADH1B
Class I 1HT0	γ-subunit	ADH1C	ADH1G_HUMAN	ADH3	ADH1C
Class II	π-subunit	ADH2	ADH4_HUMAN	ADH4	ADH4
Class III 1MP0	χ-subunit	ADH3	ADHX_HUMAN	ADH5	ADH5
Class IV 1AGN	σ-subunit	ADH4	ADH7_HUMAN	ADH7	ADH7
Class V		ADH5	ADH6_HUMAN	ADH6	ADH6

Abstract Background

All known attempts to isolate and characterize mammalian class V alcohol dehydrogenase (class V ADH), a member of the large ADH protein family, at the protein level have failed. This indicates that the class V ADH according Uni-Prot KB ADH6_HUMAN protein is not stable in a non-cellular environment, which is in contrast to all other human ADH enzymes. In this report we present evidence, supported with results from computational analyses performed in combination with earlier in vitro studies, why this ADH behaves in an atypical way.

Arch Biochem Biophys. 2018;653:97-106.4**DXHa**

Biochemistry, 2017, 56 (28), pp 3632-3646.

5ENV,8ADH,1QLH,4DWV,1N92,1N8K,1P1R,4DXH,1N92,1N8K,1LDE,1LDY,1MGO,5VKR,
1HEU,2JHF,1HET,2JHG,1H2B,1MAO,1PL6,1PL6,1YKF,**1YE3**,**4XD2**,**5VJ5**,**5VJG**,**5VKR**,**5VL0**,**5VN1**,,, 6

http://aris.gusc.lv/ChemFiles/AlhoDeHydrogenase/4DXH5VJ5hOhBioChem1718/5VJ5hOhBioChem17.pdf