Name Surname :__________________________________ **Group Nº** ____________**2023**

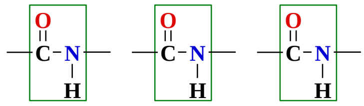
Carbonic Anhydrase 2015th Enzyme Proteins 1995th **A.**Task **human CA** studies for Molecule viewers: ChemScape MDLi **RasMol** (RasMac); MAGE FireFox application.

B. download: http://aris.gusc.lv/ChemFiles/CA/CarbonicAnhy.kin and start Mage file : CarbonicAnhy.kin and lunch **CA** Carbonic Anhydrase Elizabeth M. Boon '97, Aaron Downs '00, David Marcey: http://aris.gusc.lv/ChemFiles/CA/CAnhidrāzeII.htm

at Display conditions: **Stick** (on Menu Stripe) **Ball & Stick Spacefill**

Atom Name	Symbol	Color	Valence Number
Carbon	**C**	**Gray** lightly or **Black**	**4**
Hydrogen	H	White	**1**
Oxygen	**O**	**Red**	**2** (donor acceptor ligand up to 4)
Nitrogen	**N**	**Bluish**	**3** + 1 (donor acceptor ligand up to 4)
Sulfur	**S**	**Yellow**	**-2** , **+6**
Phosphor	**P**	**Yellow** Intensive dark	**5** (& 3)
Sodium ion	**Na^{+}**	**Blue**	**+1** (coordination up to 6)
Magnesium ion	**Mg^{2+}**	**Green**	**+2** (coordination up to 6)
Calcium ion	**Ca^{2+}**	**Gray** Dark	**+2** (coordination up to 6)
Iron ion	**Fe^{2+}**	**Yellow** Gray	**+2** (coordination up to 6)
Iron ion	**Fe^{3+}**	**Yellow** Gray	**+3** (coordination up to 6)

Corey, Pauling, Koltun the **CPK** color scheme 1965 **USA** patent for atomic modeling Protein **Backbone** is **Cα** carbon atoms of amino acids trace



Side chains: **Hydrophobic** *gray*
Polar *magenta and*
Polar *slightly bluish*
at Physiologic **pH=7.36** *conditions*
Acidic-COO^{-} negative *charge*
Basic–NH_3^{+} positive *charge*

1. N-terminus amino acid is His....... and C-terminus amino acid is Lys...... of **2VVA.pdb** chain. How many amino acids are on CA chain ... see 3rd page. Missing in Thr125-Lys127 sequence is ... and **2VVA.pdb** has (261-1 missing)=260; 260-3+1=....amino acids.

2. What 2° structures dose contains **CA**? ....**helixes** and.........**strands**

3. What number of **alpha helices** constitute **CA** polypeptide molecule?..........**Alpha-helices**

4. What type of **beta structure** and **sheets** and how many **beta strands** constitute Carbonic Anhydrase molecule?.............**stranded,** ....**beta-sheet**.

5. Describe the Carbonic Anhydrase active site geometry? active site is located the bottom .of a ......Å cone-shaped cavity that leads to the..............of the protein

6. To make seven measures of size? .....Å.... Å..... Å.... Å..... Å..... Å..... Å...

7. What three amino acids locate in active site Carbonic Anhydrase?

His.........,His.......... ,His..........

8.Which ion forms the coordination sphere?....Which three atoms in amino acids coordinated to central metal ion in metallo enzyme Carbonic Anhydrase? three Histidine ....atoms.

9. What water molecule and which atom of water make the coordinative donor-acceptor bond with central metal ion in metallo enzyme Carbonic Anhydrase? ....atom **HOH**............

10. What coordination number has central metal ion – complex maker?.....N =........................

11. What the water molecules ordered in active site of CA?.....,......,.....,........

12. To which water molecule is oriented carbon dioxide **O=C=O**? **HOH** Nr=............

13. Put in coordination sphere four ligand atoms! :N N: :O

14. What four amino acids lined at the bottom of the active site Carbonic Anhydrase form together with deep water **HOH**338

Leu..........,Trp..........,Val..........,Val....................

15. Write the collision **CO_2** with **E-Zn^{2+}-OH_2**+His64! (1a,1b)

(1a).water 263 HOH protolysis H^+_{His64} and OH^- collision OH^-+CO_{2aqua};

CA-**Zn^{2+}**-263H_2O+CO_{2aqua}+H_2O=>...

(1b) second water molecule protolytic protonation :

CA-**Zn^{2+}**<(-OH^-+CO_{2aqua})+H^+_{His64}+H_2O=>.......

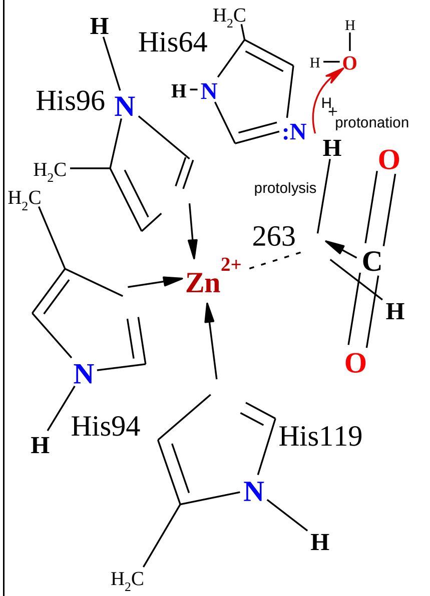
16. High rate CO_{2aqua} protolysis with $2H_2O$ overall reaction:

CO_{2aqua} + $2H_2O$<=>CA(**Zn^{2+}**)<=>.......

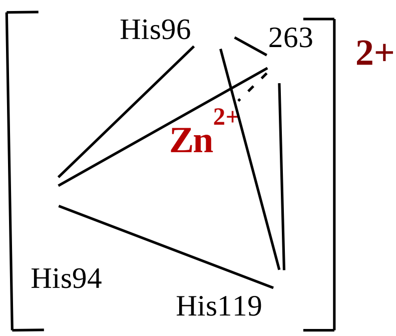
17. Write **H_2O_{236}** coordination by **Zn^{2+}** active site **CA-Zn^{2+}**!

His64+**E-Zn^{2+}+H_2O_{236}**⇔.......

High rate protolysis Biosphere attractor pH=7,36 stay at equilibrium state, while homeostasis irreversible continue generate concentration gradients H_3O^+ + HCO_3^- for transport and H_2O + O_{2aqua} osmosis, because is non-equilibrium state. Prigogine attractor Nobel prize in Chemistry 1977.



:N N: :O



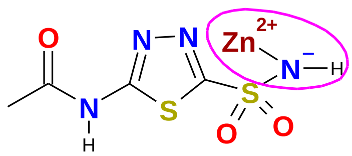
18. CO_{2aqua} slow exothermic reaction with hydroxide **OH^-** ions!

CO_{2aqua}+OH^-=>.......+Q=48.68 $^{kJ}/_{mol}$: $\mathbf{k_{1OH}}$=1.5*10^2 $M^{-1}s^{-1}$.

19. Calculate **CA** pKa=! $\mathbf{K_a}$=$\mathbf{K_{eq}}$ *[**H_2O**]^2=2.906*10^{-11}*55.3^2=10..........; pKa=............

20. Write Henderson Haselbalh buffer [**HCO_3^-**]/[**CO_2**] **pH** expression!

pH=pKa+log(n_{base} / n_{acid}**)=**..........**+log**...........



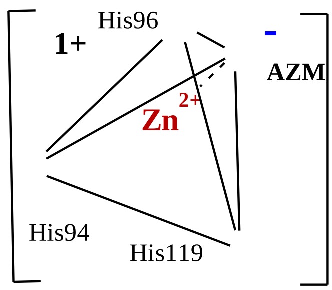
Acetazolamide **AZM**

21. How **AZM** inhibit Carbonic Anhydrase in tier solution and prevent glaucoma pressure on optic nerve fiber so prevent vision loss?.

tightly bound to ....

22. Put in coordination sphere four ligand atoms!

N: :N :N$^-$



23. Calculate the alkaline reserve ratio [**HCO_3^-**]/[**CO_2**] in blood

pH=7.36, **pK_a=7.0512**! [**HCO_3^-**]/[**CO_{2aqua}**]= **10$^{(7.36-7.0512)}$=10$^{0.3088}$**=.....

24. What the hazard for cells and life on pH=6.4188 in blood [**H_3O^+**]=**10^{-pH}=10$^{-6,4188}$** $^{mol}/_L$ at inhibition CA if concentration normal is [**H_3O^+**]$_{CA}$=**10$^{-pH_{CA}}$=10$^{-7,36}$** $^{mol}/_L$?

[**H_3O^+**]/[**H_3O^+**]$_{CA}$=**10^{-pH}/10$^{-pH_{CA}}$=10$^{-6.4188}$/10$^{-7.36}$$_{CA}$**=......................... times acidification,

bubbling CO_2↑$_{gas}$ ...., ..., ... stress..

27. What **CA2** isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 ? To determine friendly water solution pH=7,36 with **CA2** concentration C=$10^{-7,3502}$ M ($^{mol}/_{Litre}$)!

http://aris.gusc.lv/ChemFiles/CA/2VVApIStud.doc ; http://aris.gusc.lv/ChemFiles/CA/2VVApI.xls

Sequence of 260 AA Amino Acids in CA2 2VVA,2VVB.4G0C.pdb molecule

```
        10         20         30         40         50         60         70         80
MSHHWGYGKH NGPEHWHKDF PIAKGERQSP VDIDTHTAKY DPSLKPLSVS YDQATSLRIL NNGHAFNVEF DDSQDKAVLK
        90        100        110        120        130        140        150        160
GGPLDGTYRL IQFHFHWGSL DGQGSEHTVD KKKYAAELHL VHWNTKYGDF GKAVQQPDGL AVLGIFLKVG SAKPGLQKVV
       170        180        190        200        210        220        230        240
DVLDSIKTKG KSADFTNFDP RGLLPESLDY WTYPGSLTTP PLLECVTWIV LKEPISVSSE QVLKFRKLNF NGEGEPEELM
       250        260
VDNWRPAQPL KNRQIKASFK     CAH2  Human
```

AA	pKa_{COO-} - pKa_{NH3+} - pK_{RR}		Nr	AA	pKa_{COO-} - pKa_{NH3+} - pK_{RR}		Nr
M	9,21	1	1	Y	10,07	114	44
H	6	3	2	E	4,25	117	45
H	6	4	3	H	6	119	46
Y	10,07	7	4	H	6	122	47
K	10,53	9	5	K	10,53	126	48
H	6	10	6	Y	10,07	127	49
E	4,25	14	7	D	3,65	129	50
H	6	15	8	K	10,53	132	51
H	6	17	9	D	3,65	138	52
K	10,53	18	10	K	10,53	148	53
D	3,65	19	11	K	10,53	153	54
K	10,53	24	12	K	10,53	158	55
E	4,25	26	13	D	3,65	161	56
R	12,48	27	14	D	3,65	164	57
D	3,65	32	15	K	10,53	167	58
D	3,65	34	16	K	10,53	169	59
H	6	36	17	K	10,53	171	60
K	10,53	39	18	D	3,65	174	61
Y	10,07	40	19	D	3,65	179	62
D	3,65	41	20	R	12,48	181	63
K	10,53	45	21	E	4,25	186	64
Y	10,07	51	22	D	3,65	189	65
D	3,65	52	23	Y	10,07	190	66
R	12,48	58	24	Y	10,07	193	67
H	6	64	25	E	4,25	204	68
E	4,25	69	26	C	8,18	205	69
D	3,65	71	27	K	10,53	212	70
D	3,65	72	28	E	4,25	213	71
D	3,65	75	29	E	4,25	220	72
K	10,53	76	30	K	10,53	224	73
K	10,53	80	31	R	12,48	226	74
D	3,65	85	32	K	10,53	227	75
Y	10,07	88	33	E	4,25	233	76
R	12,48	89	34	E	4,25	235	77
H	6	94	35	E	4,25	237	78
H	6	96	36	E	4,25	238	79
D	3,65	101	37	D	3,65	242	80
E	4,25	106	38	R	12,48	245	81
H	6	107	39	K	10,53	251	82
D	3,65	110	40	R	12,48	253	83
K	10,53	111	41	K	10,53	256	84
K	10,53	112	42	G	2,34	260	85
K	10,53	113	43				

CA2 7,36988 ; 85 ; sum 624,1

In account are present Cysteine 204 residue pK_{RR} =8.18;

Sum of 85 pKa values in table sum 624,1.

Tasks for carbonic anhydrase molecule CA2

Protolytic constant pK_a isoelectric point IEP=pK_a 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=pK_a=($\Sigma pKa_{Rside\ group}$+ $pKa_{Nterminal}$+ $pKa_{Cterminal}$)/NpKa

1. Acid groups number in sum NpKa=83.......+2.........=.....

260 amino acids of them for pK_a side groups number 83+2. N-terminal Methionine M $pK_{aNterminal}$=9.21 and C-terminal Lysine K $pKa_{Cterminal}$=2.34

Sum are calculate as

$\Sigma pKa_{Rside\ group}$+$pK_{aNterminal}$+$pKa_{Cterminala}$=..........

2. Average constant pK_{mean}=pK_a=IEP **ISOELEKTRIC POINT** NpKa=83+2=....... ; IEP= =624,1 / 85 =.............

At pH value on isoelectric point pH=**IEP total charge** is zero „**0**"

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

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

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

3. CA2 molecule charge sign (+). zero „**0**" or (-) at pH=7.36

Underline existing:

COOH,**NH$_3^+$** positive + pH=7.36<IEP=**7,64** negative-**COO$^-$**,**NH$_2$**.

4. CA2 molecule charge **+**. zero „**0**" or **-** at **electrophoresis** pH **8.8**

Underline existing:

COOH,**NH$_3^+$** positive +IEP=**7,64**<pH = **8,8** negative - **COO$^-$**,**NH$_2$**

5. Calculate C=$10^{-7,3502}$ M CA2 solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7{,}36988-\log 10^{-7{,}3502}}{2}=\frac{7{,}36988+7{,}3502}{2}=\mathbf{14{,}7201}/2=......$$

Attractor 7,36 CA2 concentration is ....M.

Carbonic Anhydrase CA synthesis indispensible solubility attractor CO_{2gas} for $CO_{2aqua}+2H_2O$ activation.

CO_{2gas} endoergic solubility $G_{šķCO2}$=8.38 kJ/mol activation $CO_{2aqua}+2H_2O$ indispensible carbonic anhydrase (**CA**) reactivity with high rate protolysis in products $H_3O^++HCO_3^-$ create multi functional global attractor value pH=7.36. Biosphere Self-Organization attractors **CA** and pH=7.36 generate $H_3O^++HCO_3^-$ concentration gradients accumulate free energy $G_{H3O++HCO3-}=G_{šķCO2}+G_{CA}$=8.38 kJ/mol+60 kJ/mol , what as Brawnian molecular engines drive irreversible homeostasis for evolution and for survival.

No reaction CO_2 with water H_2O at absence of CA. CO_2 is slightly soluble and slow reacts with OH^-.

Solubility product: $K_{sp}=\frac{[CO_2\ aqua]}{[CO_2\ gas]\cdot[H_2O]}$ =EXP(-ΔGsp/R/T)=EXP(-8379/8.3144/298.15)=............ .

Substance	ΔH°Hess, kJ/mol	ΔS°Hess, J/mol/K	ΔG°Hess, kJ/mol
H_3O^+	-285.81	-3.854	-213.274599
HCO_3^-	-689.93	98.324	-586.93988
HCO_3^-	**-692.4948**	**-494.768**	**-544.9688**
H_2O	-285.85	69.9565	-237.191
H_2O	**-286.65**	**-453.188**	**-151.549**
CO_{2aqua}	-413.7976	117.5704	-385.98
$CO_2\uparrow_{gas}$	-393.509	213.74	-394.359

Solubility $CO_2\uparrow_{gas}+\Delta G \Leftrightarrow CO_{2aqua}+Q$=20.3 kJ/mol;

$\Delta H_{sp}=\Delta H°_{CO2aq}-\Delta H°_{CO2gas}$=-413.7976-(-393.509)=-20.3 kJ/mol;

$G_{spCO2}=\Delta G°_{CO2aq}-\Delta G°_{CO2gas}$=-385.98+394.359=8.379 kJ/mol;

Pure gas 100% $[CO_{2gas}]$]=X_{CO2gas}=1 mol fraction solubility is $[CO_{2aqua}]=K_{sp}*1*[H_2O]$=0.034045*55.3457339=1.884 M.

Atmospheric 0.04%=$[CO_{2gas}]=X_{CO2gas}$=0.0004 mol fraction solubility $[CO_{2aqua}]=K_{sp}*[CO_2\uparrow_{gas}]*[H_2O]$ is

$[CO_{2aqua}]=K_{sp}*[CO_2\uparrow_{gas}]*[H_2O]$=0.034045*0.0004*55.3=0.000754 M; 4th, 45th, 46th pages .

$\Delta G_{hydrationHess}=\Delta H_{hydrationHess}-T*\Delta S_{hydrationHess}$ = -17.9-298.15*-0.09617=............. kJ/mol ....

Carbonic anhydrase CA protolysis reactivity create functional active bicarbonate buffer. [9,14]

Carbonic anhydrase CA drive high rate protolysis CO_{2aqua} with two water molecules:

$CO_{2aqua}+2H_2O+\Delta G+Q$=v1 =CA> $H_3O^++HCO_3^-$, which velocity constant is: $k_{1CO2aqua}=1.5\times10^8\ M^{-1}s^{-1}$. [9]

.... ΔH_{Hess}=9.7576 kJ/mol ; .... ΔG_{Hess}=**102** kJ/mol;. [9]; Hess expressions:

$\Delta H_{Hess}=\Delta H°_{H3O}+\Delta H°_{HCO3}-2\Delta H°_{H2O}-\Delta H°_{CO2}$=-285.81-689.93-(2*-285.85-413.7976) =9.7576..............kJ/mol;

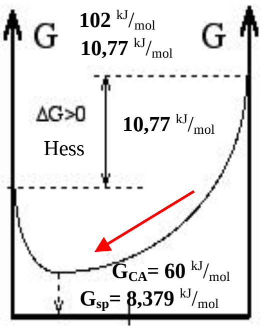
$\Delta G_{protolysisHess}=\Delta G°_{H3O}+\Delta G°_{HCO3}-2\Delta G°_{H2O}-\Delta G°_{CO2}$=-213.2746-**544.9688**-(2*-237.191-385.98)=**102**.........kJ/mol;

$\Delta G_{Absolute}=G_{H3O}+G_{HCO3}-(2G_{H2O}+G_{CO2qua})$=22.44+46.08-(2*0+8.379)=**60.14** kJ/mol ;

CA weak acid equilibrium $K_{eqCA}=\frac{[HCO_3^-]_{aqua}\cdot[H_3O^+]}{[CO_2]_{aqua}\cdot[H_2O]^2}$ =$K_a/[H_2O]^2$=10^(-7.0512)/55.3457339^2=2.906*10^-11

Exothermic ΔH_{spHess}=-....... kJ/mol and endoergic **solubility** $[CO_{2aqua}]$=0.000754 M for dissolution is ΔG_{spHess}=.......kJ/mol and protolysis constant is K_{eqCA}=2.906*10^-11 <1: therefore positive endoergic free energy change minimum:

$G_{CA}=-R\cdot T\cdot \ln(K_{eqCA})$=-8.3144*298.15*ln(2.906*10^(-11))= .... kJ/mol.



A+2B 50% C+D

$CO_{2aq}+2H_2O$ reactants

products $HCO_3^-+H_3O^+$

A 50% B

$CO_2\uparrow_{gas}$ reactant

product CO_{2aqua}

Endoergic CO_{2gas} solubility and CO_{2aq} protolysis Hess free energy change positive ΔG_{spHess} 10.77 kJ/mol and $\Delta G_{protolysisHess}$ 102 kJ/mol , but minimizes reaching equilibrium mixture of solubility G_{sp}=8.38 kJ/mol and of protolysis $\Delta G_{min}=G_{CA}$ 60 kJ/mol :

CO_2+2H_2O protolysis generate indispensable concentrations $H_3O^++HCO_3^-$ gradients of free energy accumulation $G_{spCO2}+G_{CA}$=8.38 kJ/mol+60 kJ/mol . Using the gradients energy Brownian molecular engines drive irreversible homeostasis of $H_3O^++HCO_3$ for transport down the gradient through membrane cannels exhaling $CO_{2gas}+H_2O$ and of $O_{2aqua}+H_2O$ for osmosis against the gradients through aquaporins inhaling oxygen O_2. Photosynthesis with CA inhale $CO_{2gas}+H_2O$ through proton H^++HCO_3 bicarbonate cannels and exhale $O_{2aqua}+H_2O$ through aquaporins cannels in osmosis manner.

Prigogine attractor free energy change minimum ΔG_{min} reaching is Le Chatelier principle of equilibrium mixture. High rate protolysis attractor stay at equilibrium, while homeostasis continues, because is non-equilibrium state. Prigogine: "This equilibrium state is an “attractor” for non-equilibrium states." 1977. [4]

CA Carbonic Anhydrase drive irreversible dissolute carbon dioxide protolysis with two water molecules cooling Earth biosphere in photosynthesis : $CO_{2aqua}+2H_2O+\Delta G+Q$ =CA> $H_3O^++HCO_3^-$ high solubility ratio $K_{CO2aqua+HCO3-}=[CO_{2aqua}+HCO_3^-]/[CO_2\uparrow_{air}]$=0.023 M/0.000754 M=...... times for inhale . $CO_{2gas}+H_2O$. [14]

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