

Biochemistry 5700
Fall, 2001
Final Exam
December 10, 2001

INSTRUCTIONS:

Do not begin until 9:30 AM. The exam must be turned in by 11:20 AM. This exam should have 9 questions and 12 pages. Please print your name at the top of each page of the exam. Answer questions completely, concisely, and legibly in the spaces provided. Good Luck!!

Equations: $\Delta G' = \Delta G^{\circ'} + RT \ln Q$ $\Delta E' = \Delta E^{\circ'} - \frac{RT}{nF} \ln Q$ $\Delta G' = -nFE'$

Constants: $F = 96.5 \text{ kJ/V} \cdot \text{mol}$ $R = 8.3144 \text{ J/mol} \cdot \text{K}$

$$v = \frac{V_{\max} [S]}{K_m + [S]}$$

table 5-1

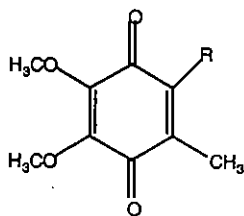
Properties and Conventions Associated with the Standard Amino Acids						
Amino acid	Abbreviated names		M_r	pK_a values		
				pK_1 ($-\text{COOH}$)	pK_2 ($-\text{NH}_3^+$)	pK_R (R group)
Nonpolar, aliphatic R groups						
Glycine	Gly	G	75	2.34	9.60	
Alanine	Ala	A	89	2.34	9.69	
Valine	Val	V	117	2.32	9.62	
Leucine	Leu	L	131	2.36	9.60	
Isoleucine	Ile	I	131	2.36	9.68	
Methionine	Met	M	149	2.28	9.21	
Aromatic R groups						
Phenylalanine	Phe	F	165	1.83	9.13	
Tyrosine	Tyr	Y	181	2.20	9.11	10.07
Tryptophan	Trp	W	204	2.38	9.39	
Polar, uncharged R groups						
Serine	Ser	S	105	2.21	9.15	
Proline	Pro	P	115	1.99	10.96	
Threonine	Thr	T	119	2.11	9.62	
Cysteine	Cys	C	121	1.96	10.28	8.18
Asparagine	Asn	N	132	2.02	8.80	
Glutamine	Gln	Q	146	2.17	9.13	
Positively charged R groups						
Lysine	Lys	K	146	2.18	8.95	10.53
Histidine	His	H	155	1.82	9.17	6.00
Arginine	Arg	R	174	2.17	9.04	12.48
Negatively charged R groups						
Aspartate	Asp	D	133	1.88	9.60	3.65
Glutamate	Glu	E	147	2.19	9.67	4.25

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

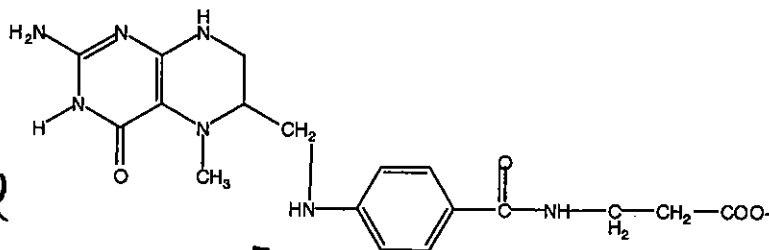
(1) (15 POINTS) You have an aqueous suspension of mitochondria to which you add succinate. Explain the effect(s) (e.g., increase, decrease, stop completely, no effect, and briefly why) of the following compounds on the (i) synthesis of ATP (ii) magnitude of the transmembrane proton-motive force and (iii) rate of consumption of oxygen when added to the mitochondrial suspension:

Inhibitor	(i) ATP synthesis	(ii) Protonmotive force	(iii) O ₂ consumption
a protonophore	will decrease, then cease as H ⁺ gradient is dissipated	will be dissipated due to free H ⁺ movement across membrane	no effect, or maybe increase?
a potassium ionophore	will decrease somewhat due to loss of elect potential portion of PMF	→	no effect
cyanide (inhibitor of complex IV)	will decrease to zero as PMF + e ⁻ transfer stops	will be dissipated as e ⁻ flow + H ⁺ pumping stop	will cease completely
rotenone (inhibitor of complex I)	no effect - succinate donates e ⁻ at complex II ←	→	→
oligomycin (inhibitor of F ₁)	will stop due to inhibition of ATP synthase	will reach max value then stop	will cease after H ⁺ gradient reached max. value

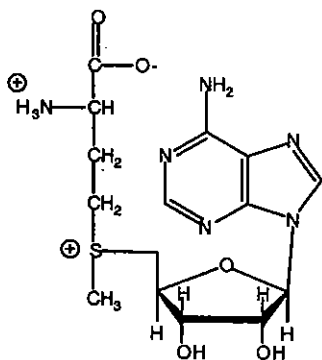
(2) (16 POINTS) Name the following compounds important in amino acid catabolism and electron transfer.



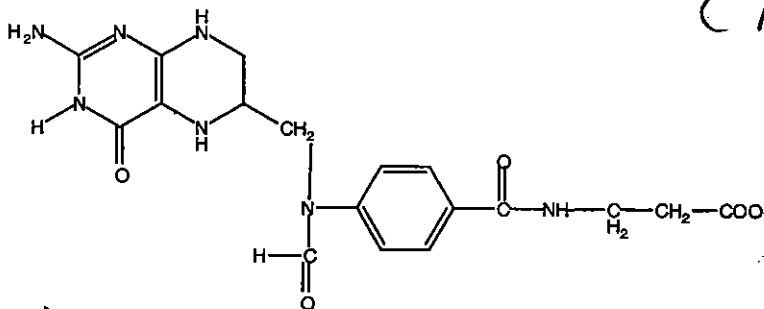
Ubiquinone; CoQ



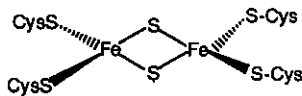
N⁵ methyl tetrahydrofolate (THF)



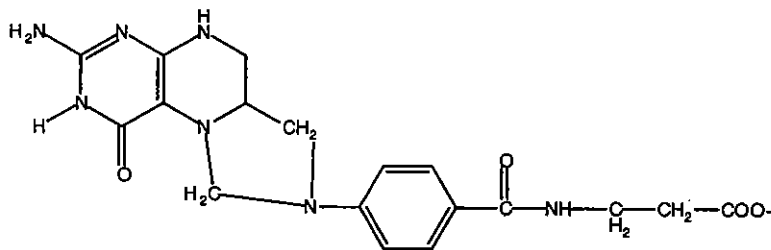
S-adenosylmethionine



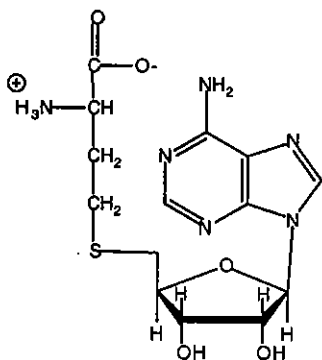
N¹⁰ formyl THF



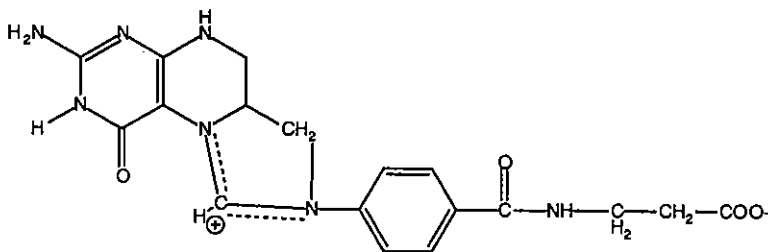
2Fe-2S cluster



N⁵, N¹⁰ methylene THF



S-adenosylhomocysteine



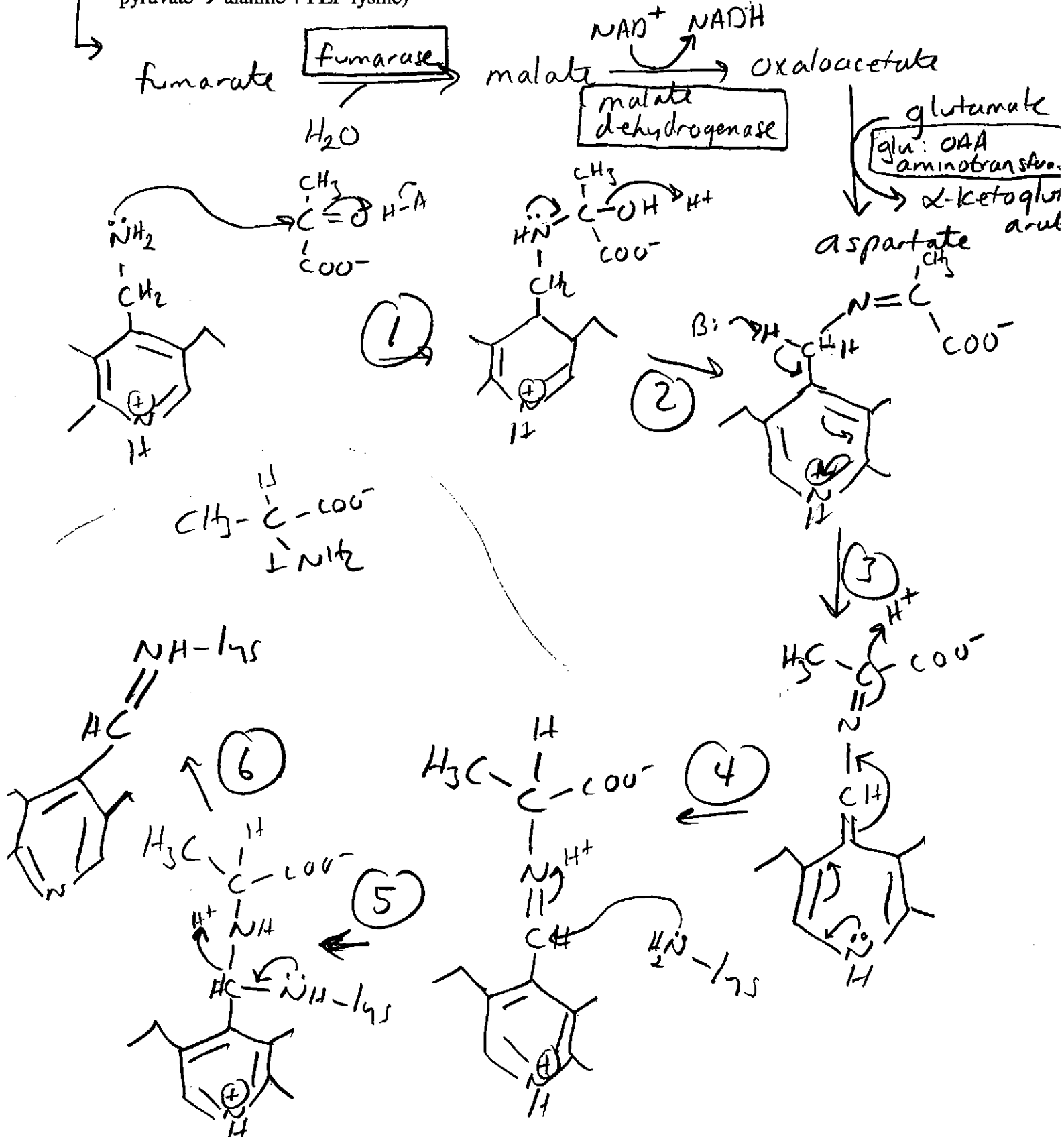
N⁵, N¹⁰ methenyl THF

(3) (14 points) **STUDENTS CHOICE QUESTION: ANSWER EITHER ONE OF THE FOLLOWING QUESTIONS-YOU CHOOSE WHICH ONE:**

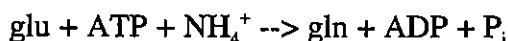
Aspartate feeds into the Urea cycle while fumarate is later expelled. Diagram the reactions necessary for converting fumarate back to aspartate and name the enzymes that catalyze the necessary reactions.

OR

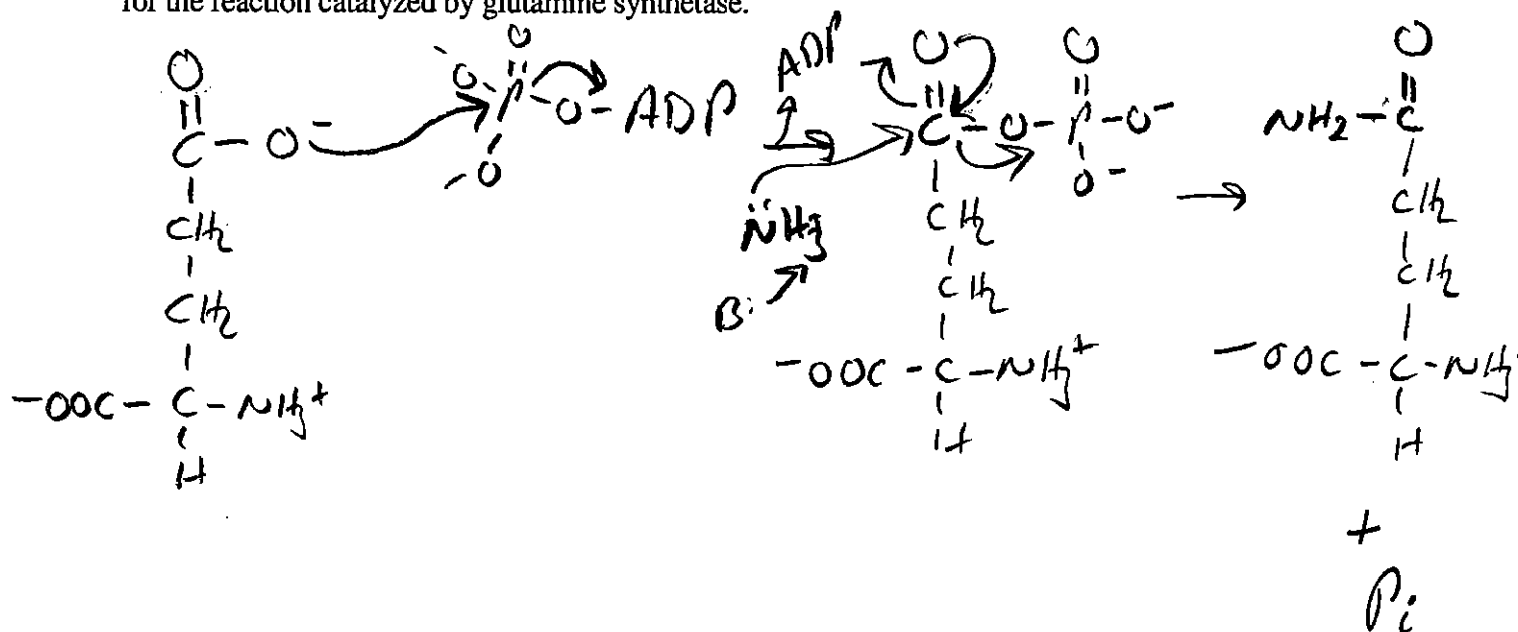
Starting with the pyridoxamine form of PLP, diagram the reactions whereby pyruvate is converted to alanine while pyridoxamine is converted back to the lysine-bound form of PLP. (pyridoxamine + pyruvate $\rightarrow$ alanine + PLP-lysine)



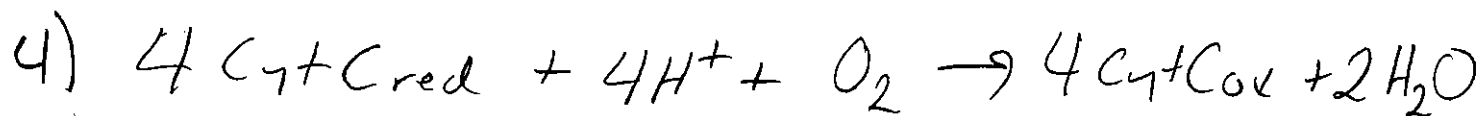
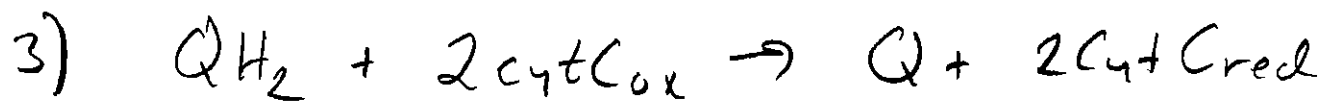
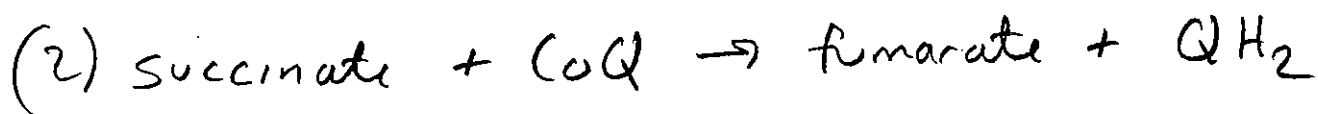
(4) (10 points) Glutamine synthetase catalyzes the ATP-dependent addition of ammonia to glutamate to produce glutamine according to the equation:



Based on your knowledge of how ATP hydrolysis drives biochemical reactions, draw the mechanism for the reaction catalyzed by glutamine synthetase.

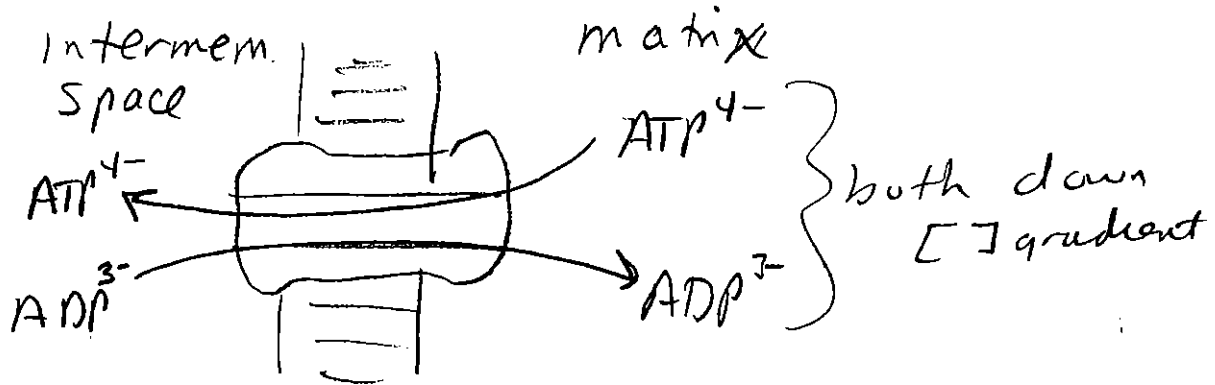


(5) (10 POINTS) Write the net reactions catalyzed by each of the four electron transfer chain complexes (comp. I-IV).

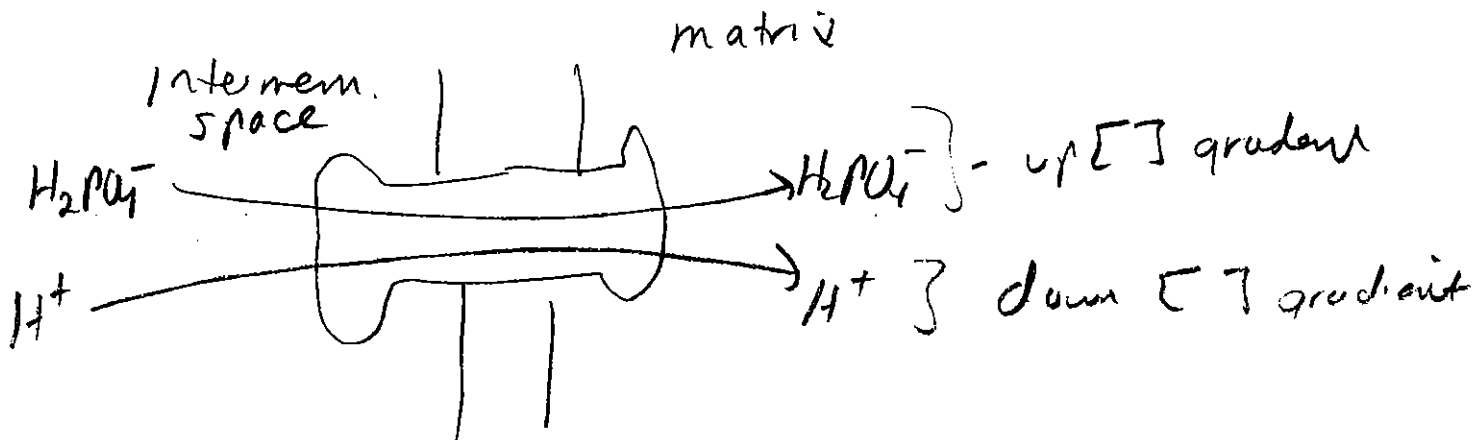


(6) (10 POINTS) Outline the transport mechanisms used to transport ATP, ADP, and P_i across the inner mitochondrial membrane. Name the transporters. What type of transporter (e.g. primary or secondary active, passive, antiport, uniport, synport, etc.) is each?

adenine nucleotide translocase - passive antiport



phosphate translocase - uniport driven by 2° active transport



Comprehensive section: 75 points.

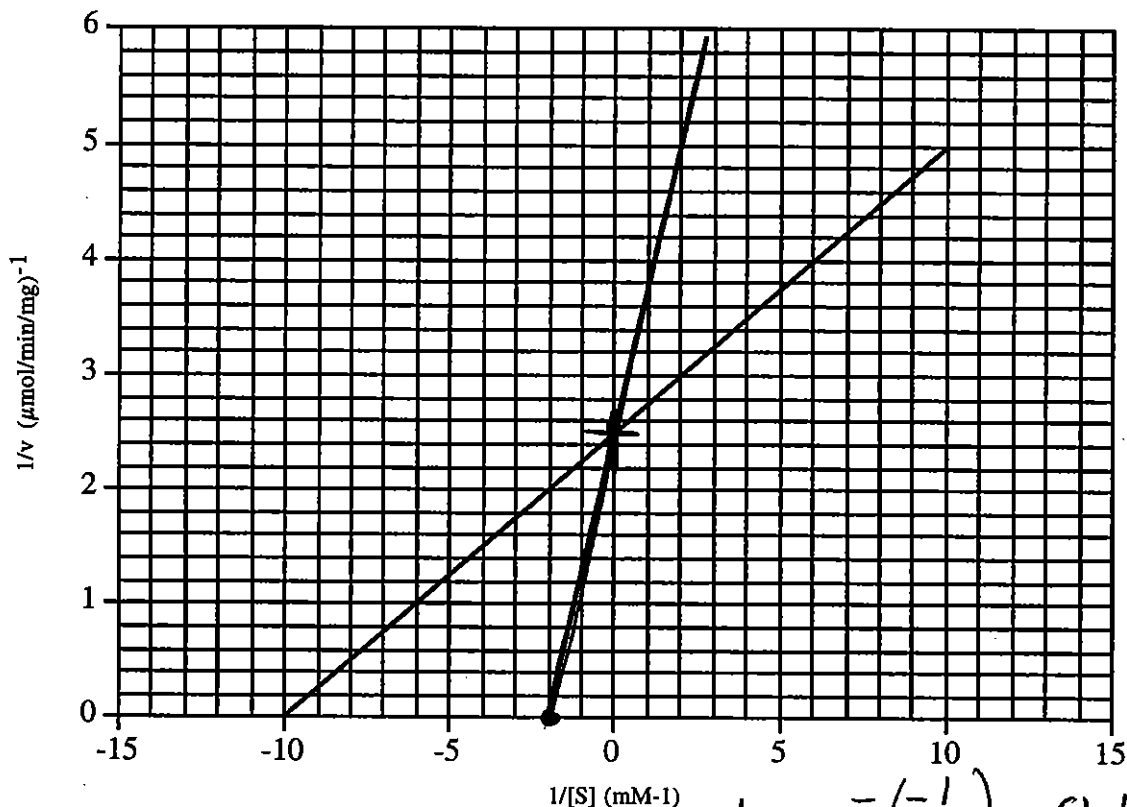
(7) (25 points) Pot pourri of definitions.

Name the protein that maintains the transmembrane potential within cells	$\text{Na}^+ \text{K}^+ \text{ATPase}$
Name a fibrous protein that forms a two-chain coiled coil	α -Keratin
Which of the 20 amino acids is an imino acid?	proline
Name a cofactor used for the transfer of CO_2 to an organic acceptor molecule	biotin
Name a cofactor involved in nonoxidative decarboxylation of alpha-keto acids	thiamine pyrophosphate
Name an enzyme from glycolysis catalyzing substrate level phosphorylation.	pyruvate kinase or phosphoglycerate kinase
Name a cofactor that is both involved in oxidation/reduction and acyl group transfer	lipoic acid (lipoamide)
What type of amino acid residue in hemoglobin is reversibly carbamylated?	lysine
Name the unbranched form of starch	α -amylose
Name a nucleotide base found in RNA but not DNA	uracil
Name a homopolymeric biomolecule containing N-acetylglucosamine residues linked in $\beta(1,4)$ configuration	chitin
Name the lipid molecule that serves as a "membrane plasticizer"	cholesterol
Name the organic phosphate molecule that regulates oxygen binding to hemoglobin	2,3-bisphosphoglycerate
Name the protein that transports fatty acids in the bloodstream	albumin
Name the principle component of bacterial cell walls	peptidoglycan

(7) (definitions (continued))

Name an enzyme catalyzing a claisen condensation reaction	Citrate synthase
Name the cofactor used by methylmalonyl-CoA mutase	Cobalamin (B ₁₂)
What type of inhibitor binds to both free E and the ES complex?	NON-comp. (or mixed)
Name the type of cell found within fat tissue	adipocyte
Name an amino acid that can serve as an effective nucleophile at physiological pH	glu, his, asp
What class of lipids are built from a long-chain amino alcohol?	Sphingolipids
Name a cofactor that is both involved in oxidation/reduction and acyl group transfer <i>what is the product of odd chain FA ox?</i>	propionyl-CoA
Name an enzyme subject to reversible covalent modification.	glycogen phosphorylase
What type of sugar linkage(s) are found in cellulose?	B1,4
Name a nucleotide base found in DNA but not RNA	thymine

(8) (12 points) The following graph shows a Lineweaver-Burke ($1/v$ vs. $1/[S]$) plot for an enzymatic reaction transforming substrate S to product P.



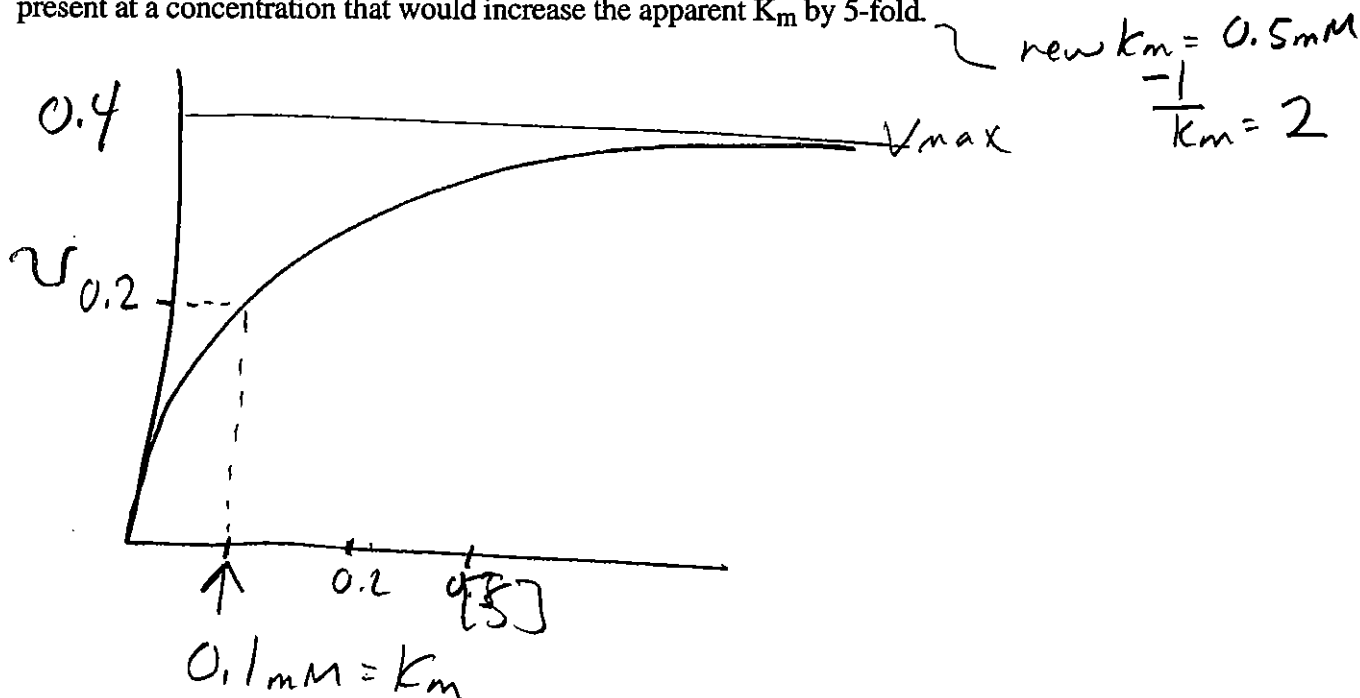
$$K_m = -\left(-\frac{1}{10}\right) = 0.1 \text{ mM}$$

(a) Determine the values of K_m and $V_{\max}$ for this enzyme (remember to include proper units in your answer).

$$V_{\max} = \frac{1}{2.5} = 0.4 \mu\text{mol/min/mg}$$

(b) At the bottom of this page, draw a new graph replotting the data as v vs. $[S]$. Label $V_{\max}$ and K_m on your new graph.

(c) Draw and label a line on the above graph representing the effect of a competitive inhibitor present at a concentration that would increase the apparent K_m by 5-fold.



(9) (38 points) **rehashed quiz questions**

3 (4 POINTS) The aggregation of nonpolar molecules or groups in water is thermodynamically favorable due to:

1. increased entropy of the nonpolar molecules when they associate
2. decreased enthalpy of the system
3. increased entropy of the water molecules
4. very strong van der Waals forces among the nonpolar molecules or groups

(3 POINTS) Acetic acid has a pKa of 4.8. How many milliliters of 0.2 M acetic acid and 0.1 M sodium acetate are required to prepare 1 liter of 0.1 M buffer solution having a pH of 4.8?

1. 500 ml acetic acid and 500 ml sodium acetate
2. 250 ml acetic acid and 250 ml sodium acetate, then 500 ml water
3. 250 ml acetic acid and 500 ml sodium acetate, then 250 ml water
4. 500 ml acetic acid and 250 ml sodium acetate, then 250 ml water

$$\text{need } [HA] = [A^-] = 0.05M$$

$$1L(0.05M HA) = xL(0.2M)$$

$$x = 0.25L$$

$$1L(0.05M A^-) = yL(0.1M A^-)$$

$$y = 0.5L$$

(3 POINTS) In a mixture of the five proteins listed below, which should elute fourth in size-exclusion (gel filtration) chromatography?

- a. cytochrome c, MW = 13,000 5
- b. immunoglobulin, MW = 145,000 2
- c. ribonuclease, MW = 13,700 4
- d. RNA polymerase, MW = 450,000 1
- e. serum albumin, MW = 68,500 3

biggest 1st smallest last

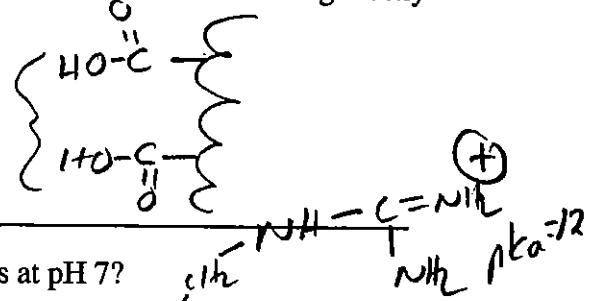
(4 POINTS) An enzyme has a specific activity of 30 U/mg in a crude lysate. The purified enzyme has a specific activity of 20,000 U/mg. About what percentage of total proteins in the original cell lysate was made up by this enzyme?

answer: 0.15%

$$\frac{30 \text{ U/mg}}{20,000 \text{ U/mg}} \times 100 = 0.15\%$$

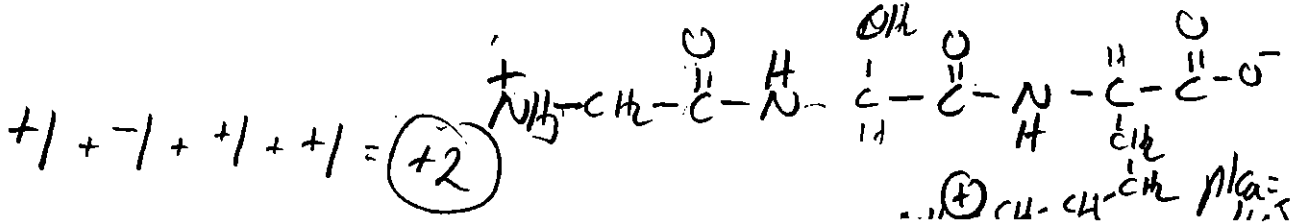
(2 POINTS) What would you expect about the formation of an alpha-helix for a segment of a protein chain that contains aspartate approximately every fourth residue with all other residues being mostly hydrophobic?

- a. helix formation would be favored at low pH
- b. helix formation would be favored at high pH
- c. helix formation would be favored at neutral pH
- d. helix formation would never occur regardless of pH



(4 POINTS) What is the net charge on the tripeptide Gly-Arg-Lys at pH 7?

answer: +2



Key

(2 POINTS) The conformation of the backbone of a polypeptide is described completely by the angle(s) of rotation about which bond(s)?

- a. the peptide bond only
- b. N-C α only
- c. N-C α , C α -C, and peptide bonds
- ☒ d. N-C α and C α -C bonds only
- e. C α -C bond only

(2 POINTS) Which of the following is least likely to result in the denaturation of a typical protein?

- a. boiling
- b. addition of an organic solvent
- ☒ c. changing the concentration of salt
- d. changing the pH
- e. exposure to detergents

(2 POINTS) Which would you expect for the fatty acyl chains of the membrane phospholipids of bacteria grown at high temperature?

- a. proportion of unsaturated acyl groups increases
- b. proportion of unsaturated acyl groups decreases
- c. the length of the fatty acyl side chains will increase
- d. more cholesterol is produced and inserted in the membrane
- ☒ e. both B and C are correct

$\leftarrow$ to $>$ VDW forces

(2 POINTS) How will the presence of a positive heterotropic modulator affect the shape of the v vs. S plot for an allosteric enzyme?

- a. the curve will shift to the right
- ☒ b. the curve will shift to the left
- c. the curve will not be affected
- d. it may shift left or right depending on where it binds



(2 POINTS) Pyranose rings are usually most stable when the ring adopts a _____ conformation with the bulkiest ring substituents in _____ positions

- ☒ a. chair; equatorial
- b. chair; axial
- c. boat; equatorial
- d. boat; axial

(2 POINTS) The hydrolysis of phosphoenolpyruvate proceeds with a $\Delta G'^{\circ}$ of about -62 kJ/mol. The greatest contributing factors to this reaction are the destabilization of the reactants by electrostatic repulsion and stabilization of the product pyruvate by:

- 1. ionization.
- 2. electrostatic attraction.
- 3. resonance.
- ☒ 4. tautomerization.

(5 POINTS) Match the indicated glycolytic enzyme with an appropriate answer that relates to the mechanism of the transformation.

Enzymealcohol dehydrogenase eglyceraldehyde-3-phosphate dehydrogenase ctriose phosphate isomerase dtype I aldolase aphosphoglycerate mutase bPossible matches

- (a) schiff base intermediate
- (b) phosphoenzyme intermediate
- (c) acyl enzyme intermediate
- (d) cis-enediolate intermediate
- (e) zinc cofactor

(2 POINTS) All of the oxidative steps of the citric acid cycle are linked to the reduction of NAD^+ except the reaction catalyzed by:

- a. isocitrate dehydrogenase.
- b. malate dehydrogenase.
- c. the α -ketoglutarate dehydrogenase complex.
- d. succinate dehydrogenase.