Name Key

Biochemistry 5700
Fall, 2003
Final Exam
December 10, 2003

INSTRUCTIONS:

Do not begin until 7:30 AM. The exam must be turned in by 9:20 AM. This exam contains two sections: the "new material" section and the "comprehensive" section. Answer the "new material" questions in the spaces provided. Answer the "comprehensive" questions by marking on the scantron sheet using a #2 pencil. Turn in both the scantron and the entire exam when you are finished. Good Luck!!

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

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

Constants: F = 96.5 kJ/V•mol R = 8.3144 J/mol•K

Table 5-1

Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	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

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (values) or a hydrophobic environment (values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol* **157**, 105 – 132.

†Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

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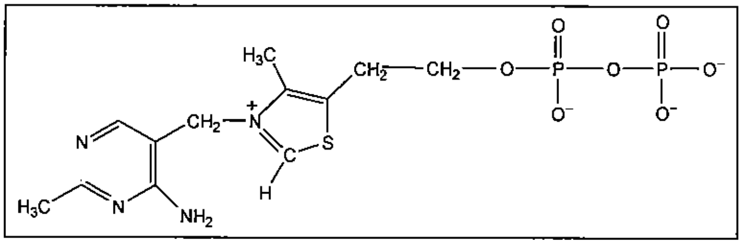

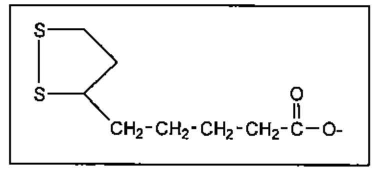

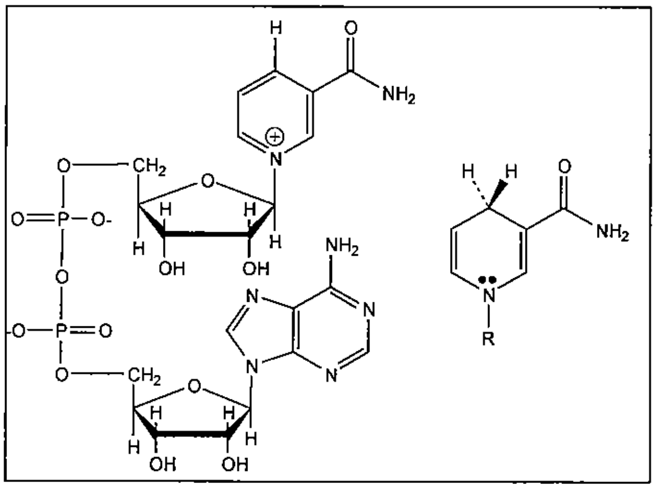

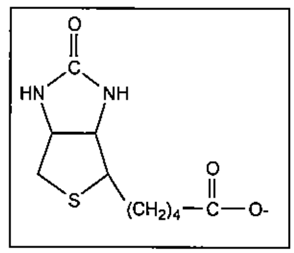

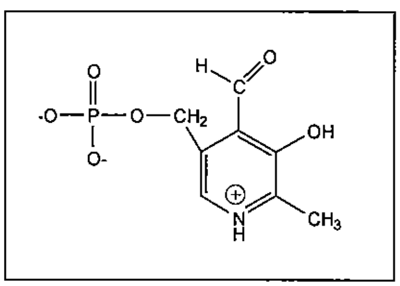

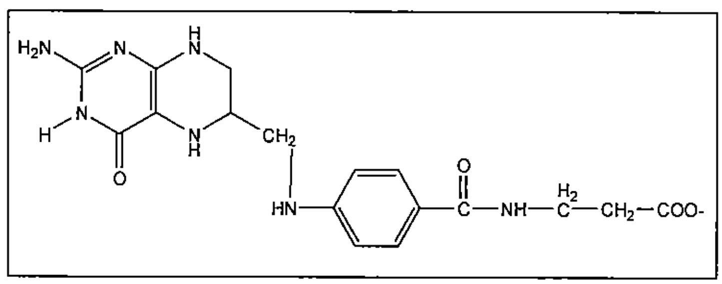

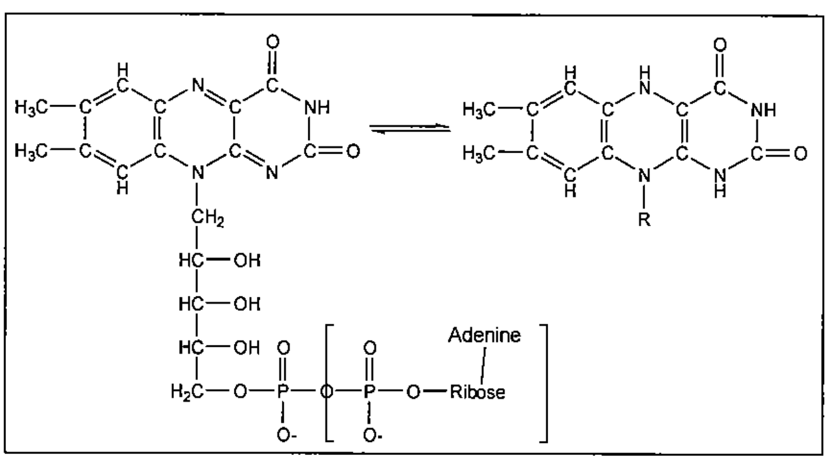

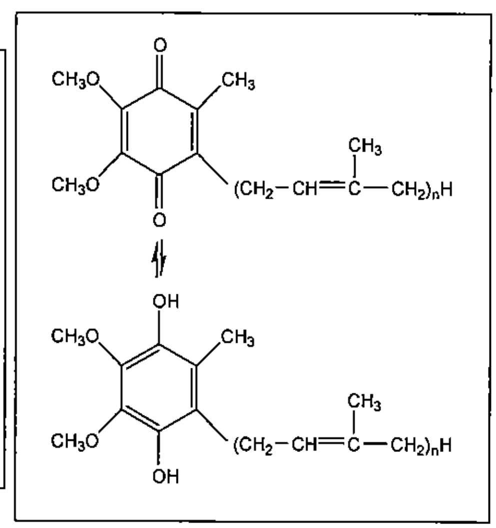

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(1) (8 points) Write the net reactions catalyzed by each of the four electron transfer chain complexes (comp. I-IV). (Please balance protons in the reactions.)

I. $NADH + H^+ + CoQ \rightarrow NAD^+ + QH_2$

II $\text{succinate} + CoQ \rightarrow \text{fumarate} + QH_2$

III $QH_2 + 2\,CytC_{ox} \rightarrow Q + 2\,CytC_{red} + 2H^+$

IV $4H^+ + 4\,CytC_{red} + O_2 \rightarrow 2H_2O + 4\,CytC_{ox}$

(2) (10 points) Write the balanced chemical equations (NO MECHANISMS) and name the enzymes used for each of the following reactions of amino acid catabolism:

(a) glutamine hydrolysis

$\text{glutamine} + H_2O \xrightarrow{\text{glutaminase}} \text{glutamate}$

(b) glutamine synthesis from glutamate glutamine synthetase

$\text{glutamate} + ATP + NH_4^+ \rightleftharpoons \text{glutamine} + ADP + P_i$

(c) glutamate oxidation using NAD+ as electron acceptor

$\text{glutamate} + NAD^+ + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+$

glutamate dehydrogenase

(d) transamination of pyruvate and glutamate glutamate aminotransferase

$\text{Pyruvate} + \text{glutamate} \rightleftharpoons \text{alanine} + \alpha\text{-ketoglutarate}$

(e) carbamoyl phosphate synthesis

$HCO_3^- + 2ATP + NH_4^+ \rightarrow NH_2-\overset{O}{\overset{\|}{C}}-O-\overset{O}{\overset{\|}{\underset{O^-}{\underset{|}{P}}}}-O^- + 2ADP + P_i$

carbamoyl phosphate synthetase

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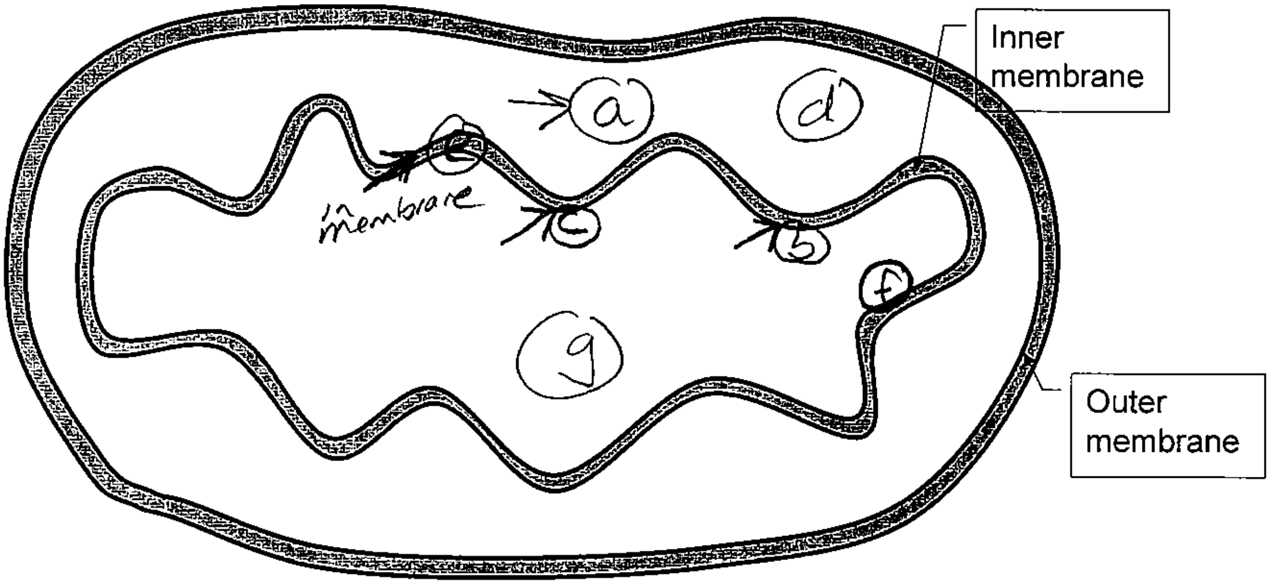
(3) (7 points) Using the indicated letters and arrows, label the mitochondrion shown below to indicate the specific area or portion of the mitochondrion where the following events take place.

(a) portion of mitochondrion where protons are pumped during electron transfer
(b) site/location of O2 reduction
(c) site/location of NADH oxidation
(d) location of water soluble cytochrome c
(e) location of ubiquinone
(f) site/location of ATP synthesis
(g) location of citric acid cycle enzymes except succinate dehydrogenase



(4) (6 points) The further oxidation of the NADH produced during glycolysis provides energy for ATP synthesis. Explain why the yield of ATP from glycolytically-produced ATP is 2ATP/NADH in muscle yet 3ATP/NADH in the liver.

Cytosolically produced NADH cannot cross inner mitochondrial membrane. Two shuttles are used to transport the reducing equivalents from cytosolic (glycolytically-produced) NADH into the mitochondrion. In the liver, the malate-aspartate shuttle is used. The red. eq. of NADH are transferred to OAA, producing malate. Malate is then transported in, & NADH reformed. Thus, there is complete conservation of NADH. In contrast, in the muscle, red. eq. from cytosolic NADH are transferred to Q in the inner mito membrane via glycerol-3-phosphate-DH, an FAD enzyme. Hence the proton-pumping step of complex I is missed for these red eq. and hence 2 net ATP are formed

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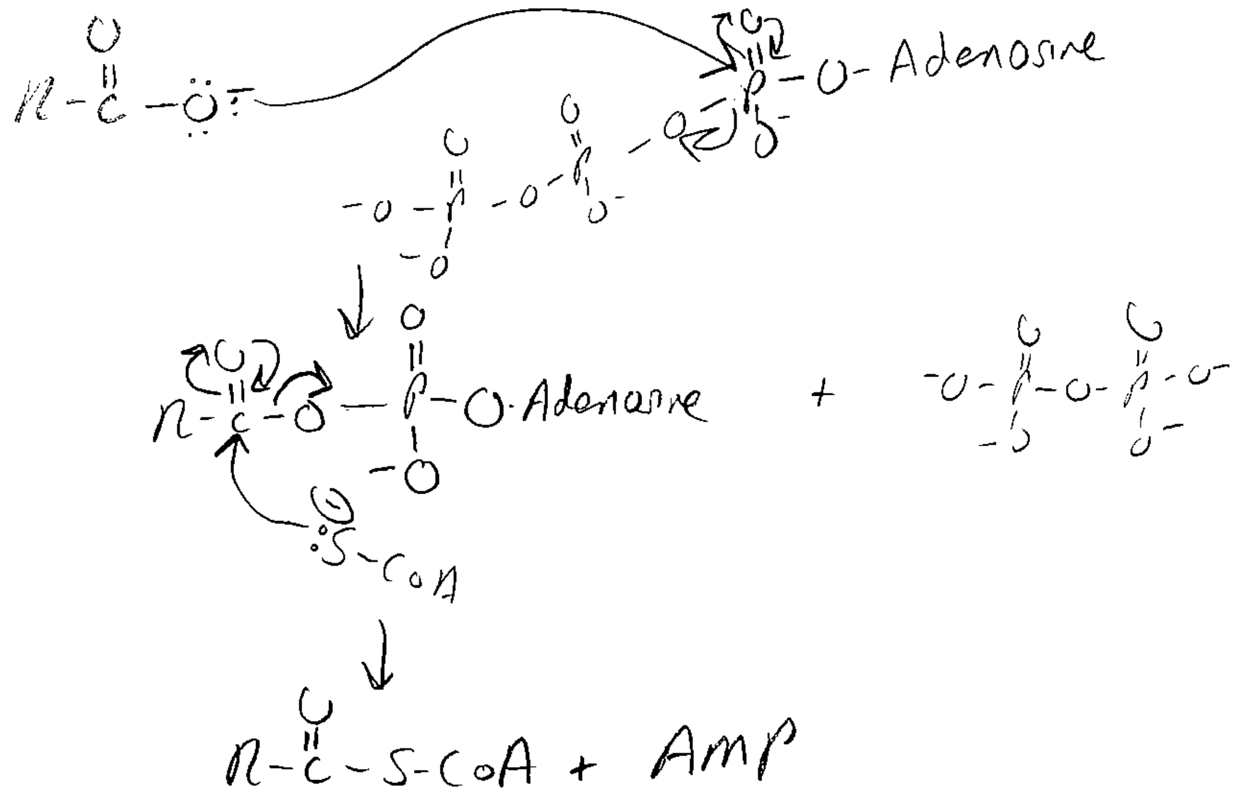
(5) (10 points) Fatty acyl-CoA synthetase catalyzes the following reaction, which proceeds through an acyl-AMP intermediate:

$$R{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}O^- + HSCoA + ATP \longrightarrow R{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}SCoA + AMP + PPi$$

(a) (7 pts) Diagram the complete catalytic mechanism for this reaction.
(b) (3 pts) Rather than occurring as shown, this reaction might be expected to proceed through an acyl-phosphate intermediate, producing ADP and Pi as products. Explain in thermodoynamic terms why AMP and PPi are formed instead.



b) The removal of the product pyrophosphate by inorganic pyrophosphatase shifts the equilibrium for the rxn to the right, providing driving force for fatty acyl CoA synthesis under cellular conditions.

$$FA + CoA + ATP \rightleftharpoons FA\text{-}CoA + AMP + PPi$$

remove product, shift eq. to R

$PPi \downarrow 2Pi$

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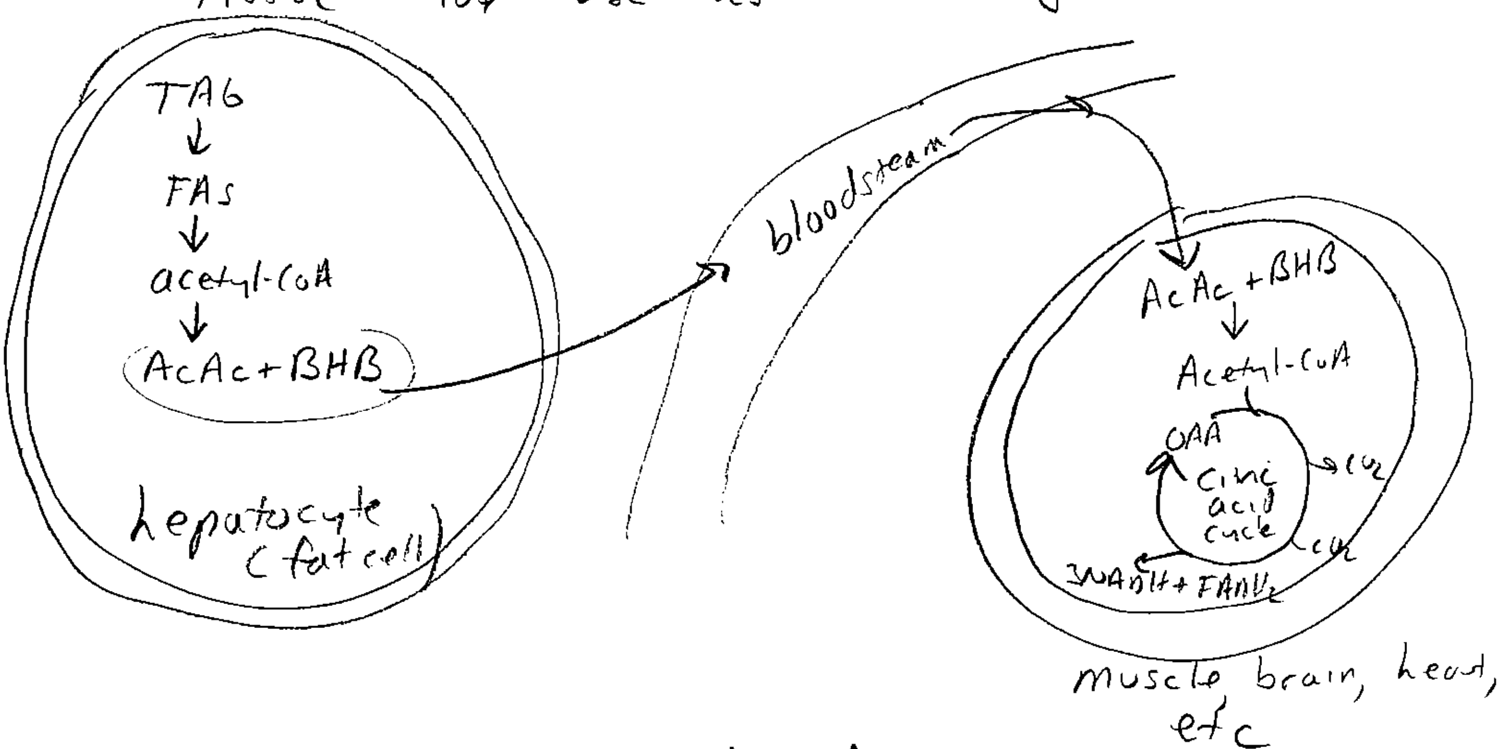
(6) 14 points.
(a) (6 points) Draw the structures and name the three compounds collectively referred to as "ketone bodies.
(b) (8 points) What is the purpose of ketone body formation and breakdown in mammals? In answering your question, explain where ketone bodies are produced, where they are ultimately metabolized, and how they get from the site of production to the ~~site of consumption~~.

a) $CH_3-\overset{O}{\overset{\|}{C}}-CH_2-\overset{O}{\overset{\|}{C}}-O^-$ acetoacetate (AcAc)

$CH_3-\underset{H}{\overset{OH}{C}}-CH_2-\overset{O}{\overset{\|}{C}}-O^-$ β-hydroxybutyrate (BHB)

$CH_3-\overset{O}{\overset{\|}{C}}-CH_3$ acetone

b) when carbohydrate reserves are low, ketone bodies are produced from fat in adipocytes, then exported in the blood stream to other tissue for use as an energy source



acetone is formed from the spontaneous decarboxylation of acetoacetate, and is a toxic, non-desired compound

AcAc ⟶ acetone + CO_2

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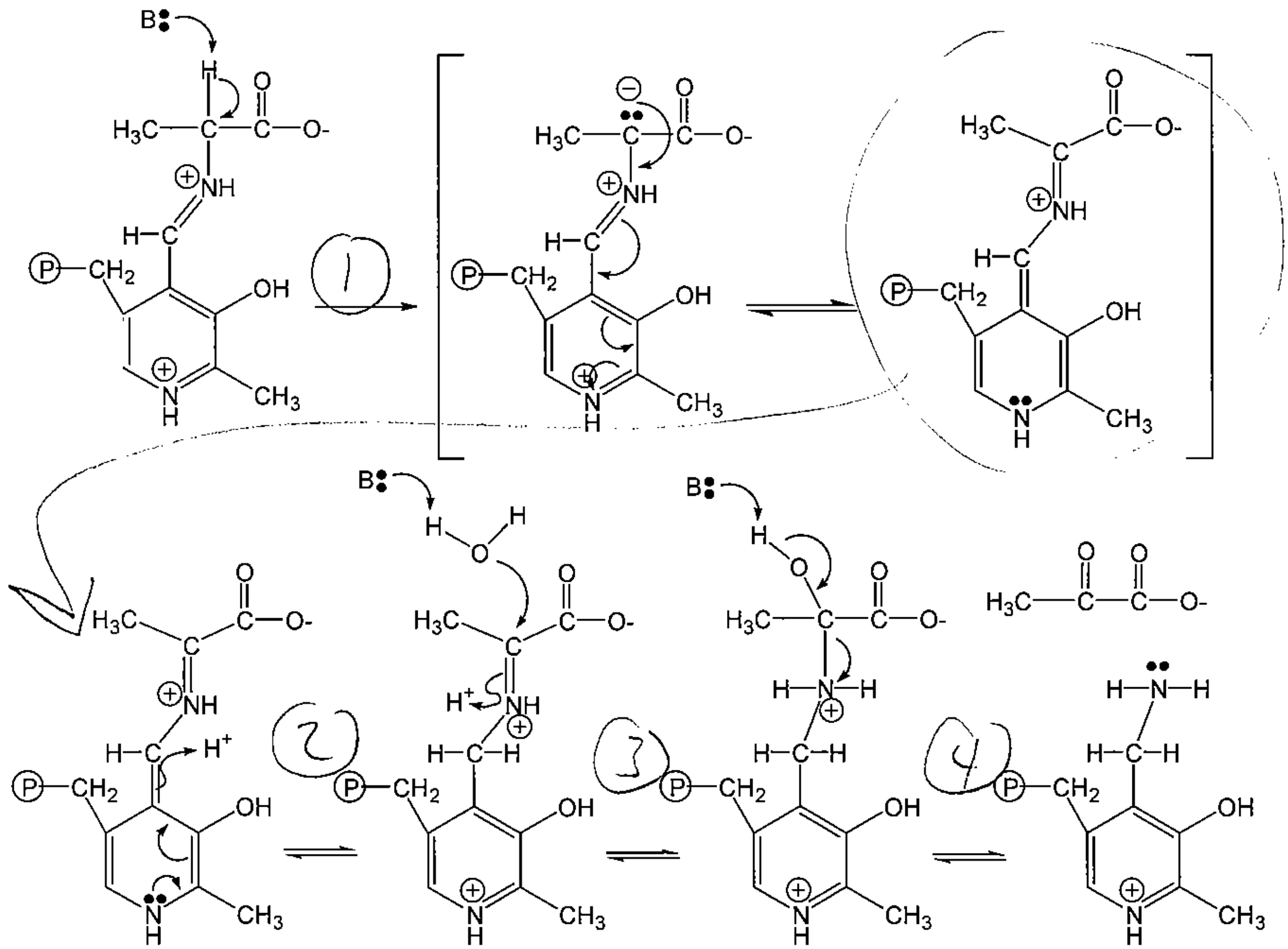
(7) (10 points) Starting with the alanine-PLP adduct shown below, diagram the necessary mechanistic steps required to convert this intermediate to pyridoxamine and pyruvate. Show all intermediates, important resonance structures, and general acids/bases in the mechanism.



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(8) (10 points) You have just eaten a big juicy, fatty hamburger. Explain (feel free to diagram) how the portion of the hamburger that is fat eventually arrives in your muscle cell (i.e., what is the biochemical/molecular path from intestine to muscle?

- intestinal lipases degrade TAGs → fatty acids + glycerol
- FAs are taken up by intestinal mucosa cells
- FAs are reesterified to TAGs
- TAGs are incorporated w/ cholesterol + proteins into chylomicrons, surrounded by a phospholipid bilayer
- chylomicrons are exported to lymph, blood stream
- lipoprotein lipases in blood stream hydrolyze TAGs in chylomicrons, releasing free FAs
- fatty acids are transported into muscle cells

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COMPREHENSIVE SECTION 3 points per problem. Mark answers on scantron.

(1) You have an aqueous 0.10 M solution of the amino acid histidine at pH 6.4. Determine the concentration of the protonated form of histidine in this solution.

(a) 0.028 M
(b) 0.040 M
(c) 0.060 M
(d) 0.072 M
(e) 0.090 M

$pH = pKa + \log\frac{A^-}{HA}$; $6.4 = 6.0 + \log\frac{A^-}{HA}$; $\log\frac{A^-}{HA} = 0.4$

$\frac{A^-}{HA} = 2.512$; $A^- + HA = 0.10$; $3.512\,HA = 0.10$; $HA = 0.028$

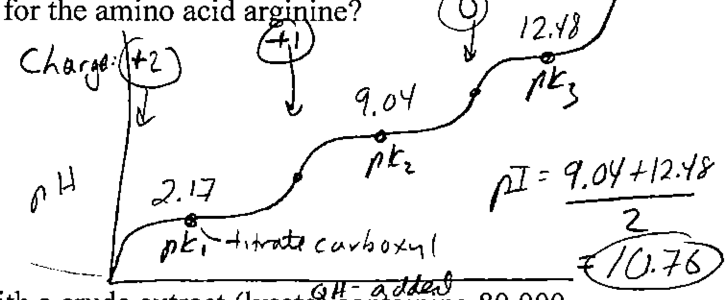
$A^- = 2.512\,HA$

(2) Given the information on page 1, what is the pI for the amino acid arginine?

(a) 5.60
(b) 7.32
(c) 7.90
(d) 10.8
(e) 12.5

Start in form

$HOOC-\overset{H}{\underset{R^{+}}{C}}-NH_3^{+}$



$pI = \frac{9.04 + 12.48}{2} = 10.76$

(3) During the purification of a protein, you start with a crude extract (lysate) containing 80,000 units of enzyme activity and 3000 mg of protein. Your final purified enzyme preparation contains 5,000 units of activity and 1.2 mg of protein. What is the fold-purification of your purified enzyme?

(a) 0.0625
(b) 16
(c) 156
(d) 2500
(e) 4167

$SA_{crude} = \frac{80{,}000\,u}{3000\,mg} = 26.7\,u/mg$

$SA_{pure} = \frac{5000\,u}{1.2\,mg} = 4167\,u/mg$

$fold = \frac{SA_{pure}}{SA_{crude}}$

$fold = \frac{4167}{26.7} = 156$

(4) Referring to question (3), given that your protein is a monomer with a turnover number of 2000 s^{-1}, what is the molecular weight of your protein?

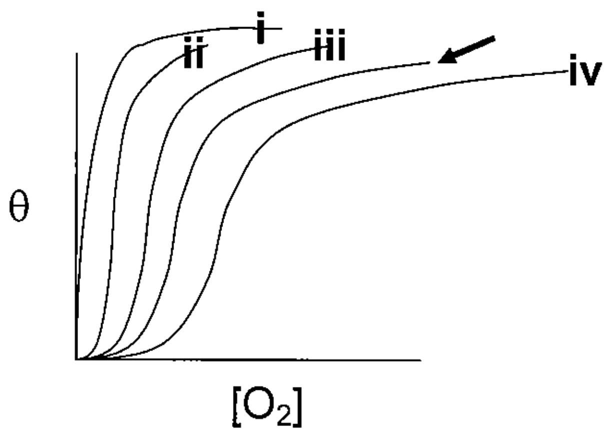
(a) 28,800
(b) 34,700
(c) 125,000
(d) 1,730,000
(e) 8,330,000

$\left(\frac{1000\,\cancel{umol}}{mmol}\right)\left(\frac{1\,mg\ protein}{4167\,u}\right)\left(\frac{1\,u}{1\,\cancel{umol\ product}/\cancel{min}}\right)\left(\frac{60\,\cancel{s}}{\cancel{min}}\right)\left(\frac{2000\,\cancel{umol\ product}}{umol\ enzyme \cdot \cancel{s}}\right)$

$= 28{,}797 \Rightarrow 28{,}800$

(5) Given that the line indicated by the arrow corresponds to the normal hemoglobin O_2 dissociation curve at pH 7.6, which line(s) might represent the result of decreasing the pH to 7.0?

decrease pH, increase $[H^+]$, shift curve to right as T form is stabilized



(a) line (i) only
(b) either line (i), (ii), or (iii)
(c) either line (ii) or (iii) only
(d) line (iv) only

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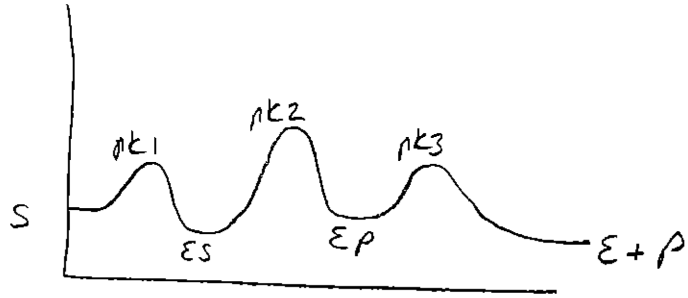
(6) For the kinetic mechanism shown below:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

If you were to draw the reaction coordinate diagram for this enzyme-catalyzed reaction, how many peaks would be present between S and P?

(a) 1
(b) 2
(c) 3
(d) 4

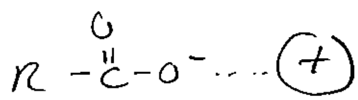


(7) Rotation about the bond between which two atoms in a polypeptide chain defines the bond angle phi?

(a) R- C_α
(b) N-C_α
(c) $C_{(carbonyl)}$-C_α
(d) $C_{(carbonyl)}$-N

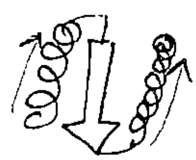
(8) Which amino acid would be most likely to form a salt bridge with Asp at pH 7?

(a) Arg
~~(b) Cys~~
(c) Glu
(d) Pro



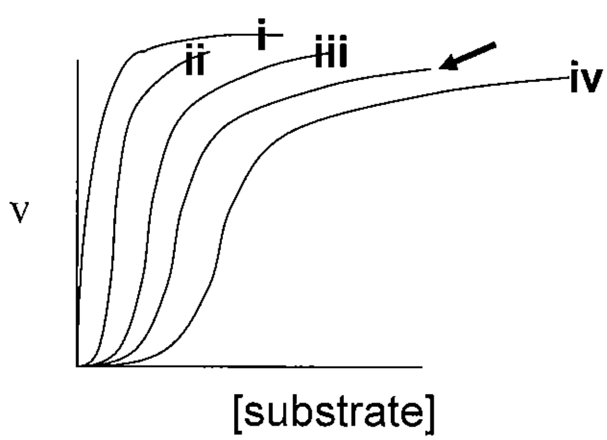
(9) What type of motif is most likely to allow two adjacent alpha helices to be largely parallel (not anti-parallel) to each other?

(a) ~~globin fold~~ — perpendicular
(b) alpha-beta barrel — parallel
~~(c) helix-turn-helix~~ — anti parallel
(d) beta bend — anti parallel



(10) Given that the line indicated by the arrow is the v vs. S plot for an allosteric enzyme, which line(s) might represent the result of adding a positive heterotropic modulator to the enzyme?

shift to L, retain allostery



(a) line (i) only
(b) either line (ii), (iii), or (iv)
(c) either line (ii) or (iii) only
~~(d) line (iv) only~~
(e) either line (i) or (ii) only

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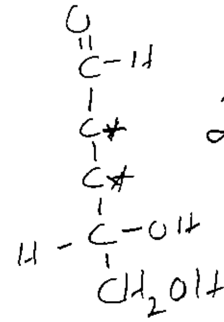
(11) What type(s) of carbohydrate linkage(s) are present in glycogen?

(a) α (1→4) only
(b) β (1→4) only
(c) α (1→4) and α (1→6)
(d) β (1→4) and β (1→6)

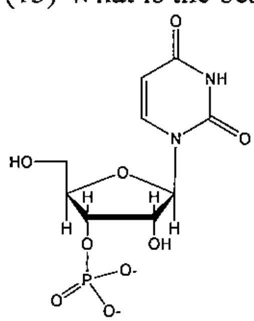
(12) How many stereoisomers are possible for the family of D-aldoses with five carbon atoms?

(a) 2
(b) 4
(c) 8
(d) 16



$2^2 = 4$

(13) What is the best name for the compound shown below?

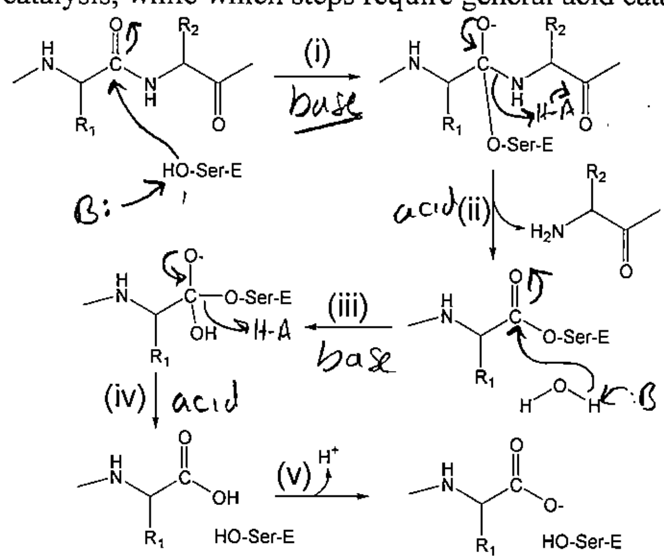


(a) uracil 3'-monophosphate
(b) uridine 3'-monophosphate
(c) cytosine 3'-monophosphate
(d) cytidine 3'-monophosphate
(e) thymidine 3'-monophosphate

(14) In aqueous solutions, large concentrations of fatty acids associate to form structures called called micelles, large concentrations of sphingolipids associate to form structures called liposomes, and large concentrations of glycerophospholipids associate to form structures called liposomes.

(a) micelles, micelles, liposomes
(b) liposomes, micelles, micelles
(c) micelles, liposomes, micelles
(d) micelles, liposomes, liposomes
(e) micelles, liposomes, micelles

(15) Referring to the mechanistic scheme shown below, which step(s) require general base catalysis, while which steps require general acid catalysis?



	Gen. base steps	Gen. acid steps
(a)	(i), (iii)	(ii), (iv)
(b)	(ii), (iv)	(i), (iii)
(c)	(i), (iv)	(ii), (iii)
(d)	(i), (ii)	(iii), (iv)
(e)	(iii), (iv)	(i), (ii)

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(16) Which polysaccharide is most likely to be a structural polysaccharide in mammals?

(a) glycogen Storage
(b) glycosaminoglycan mammals
(c) chitin invertebrates
(d) cellulose plants
(e) peptidoglycan bacteria

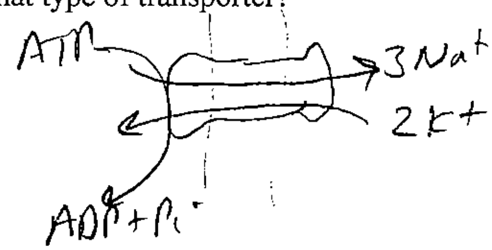
(17) The reaction of the side chain of lysine with CO_2 would produce what type of compound?

(a) Schiff base
(b) ester
(c) carbamate
(d) thioether

$R-NH_2 + CO_2 \rightarrow R-NH-\overset{O}{\overset{\|}{C}}-O^-$ carbamate

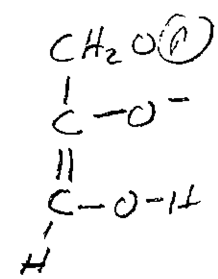
(18) The sodium-potassium ATPase represents what type of transporter?

(a) primary active, antiport
(b) primary active, symport
(c) passive, antiport
(d) passive, symport
(e) secondary active, symport



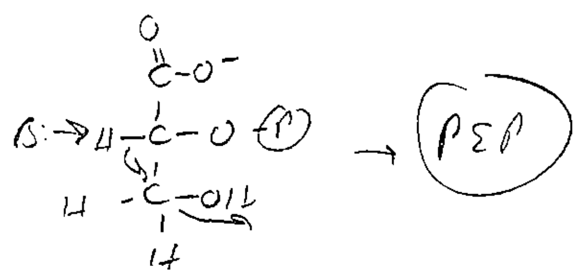
(19) For which of the following enzymes is a cis-ene-diolate an important intermediate in the reaction?

(a) alcohol dehydrogenase no
(b) citrate synthase no
(c) aldolase uses Schiff base
(d) triosephosphate isomerase — intermediate is
(e) enolase no



(20) Which of the following reactions of glycolysis results in the formation of a high energy compound?

(a) aldolase no
(b) phosphoglycerate mutase no
(c) phosphohexose isomerase no
(d) enolase yes



(21) Which of the following enzymes forms a Schiff base intermediate during catalysis?

(a) enolase
(b) citrate synthase
(c) aldolase
(d) triosephosphate isomerase
(e) both (b) and (c)

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(22) In the hormonal activation of glycogen breakdown, protein kinase is activated by cAMP; allostery while phosphorylase kinase is activated by phosphorylation; cov. mod

(a) allostery, allostery
(b) allostery, covalent modification
(c) covalent modification, allostery
(d) covalent modification, covalent modification

(23) How many of the reactions of glycolysis and the citric acid cycle result in substrate level phosphorylation to form a nucleoside triphosphate?

(a) 2
(b) 3
(c) 4
(d) 5
(e) 6

1) phosphoglycerate kinase
2) pyruvate kinase
3) succinyl-CoA synthetase

(24) Some chemoautotrophic bacteria gain all their energy from the oxidation of nitrite to nitrate (a **two electron** process). In theory at least (biochemical standard state conditions), the free energy provided by the **oxidation of** **one mol** **nitrite** in the presence of O_2 is sufficient to form how many mols of ATP?.
($E^{\circ\prime}$= +0.421 V for the NO_3^-/NO_2^- couple, $E^{\circ\prime}$= +0.816 V for the O_2/H_2O couple, $\Delta G^{\circ\prime}$ = -30.5 kJ/mol for ATP hydrolysis)

(a) 0.83
(b) 1.2
(c) 2.5
(d) 3.9
(e) 7.8

$NO_2^- \rightarrow NO_3^- + 2e^-$ $\quad \mathcal{E}^{o\prime} = -0.421$

$2e^- + \frac{1}{2} O_2 \rightarrow H_2O$ $\quad \mathcal{E}^{o\prime} = +0.816V$

$\mathcal{E}^{o\prime} = 0.395V$

$\Delta G^{o\prime} = -nF\mathcal{E}^{o\prime} = -(2)(96.5 \frac{kJ}{V \cdot mol})(0.395V) = -76 \frac{kJ}{mol}$

$76.2 \frac{kJ}{mol\ NO_3^-} \Big/ 30.5\ kJ/mol\ ATP = 2.5\ ATP/NO_2^-$

(25) Which of the following organic cofactors is used to stabilize a carbanion formed during catalysis?

(a) thiamine pyrophosphate
(b) biotin
(c) lipoamide
(d) NAD^+
(e) cofactors are never used to stabilize carbanions