

Key

Biochemistry 5700
Fall, 2002
Final Exam
December 9, 2002

INSTRUCTIONS:

Do not begin until 9:30 AM. The exam must be turned in by 11:20 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: $\Delta G = \Delta G^\circ + RT \ln Q$ $\Delta E = \Delta E^\circ - \frac{RT}{nF} \ln Q$ $\Delta G' = -nFE'$

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

table 5-1

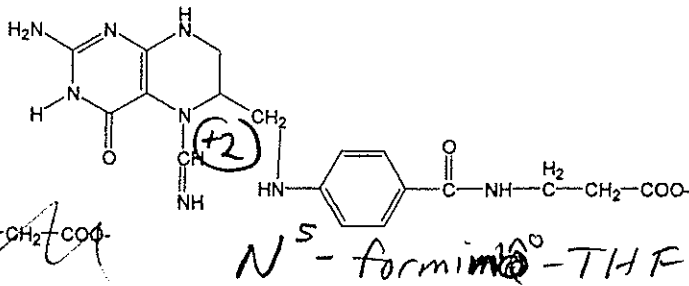
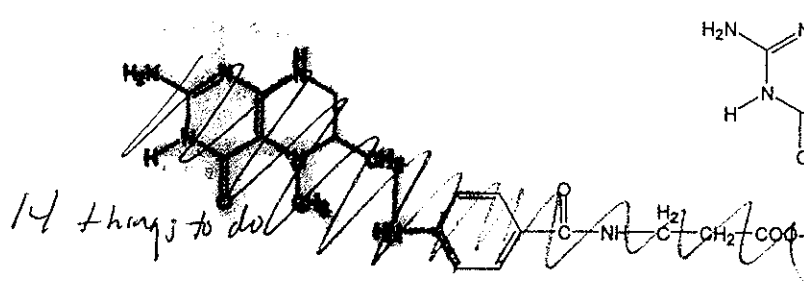
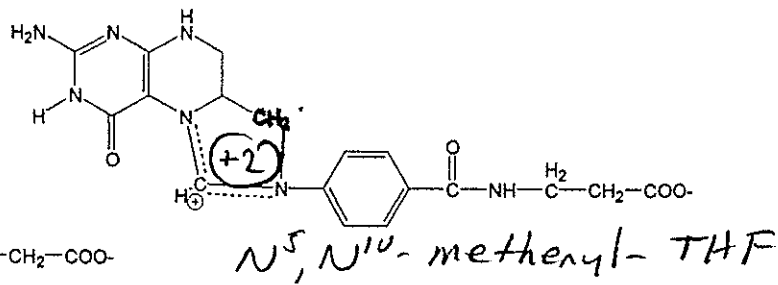
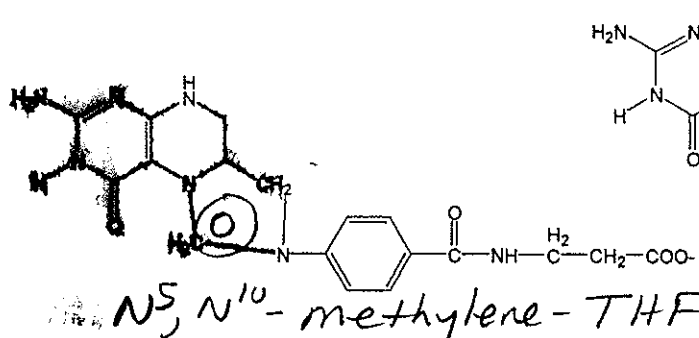
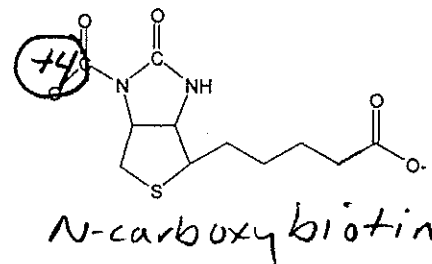
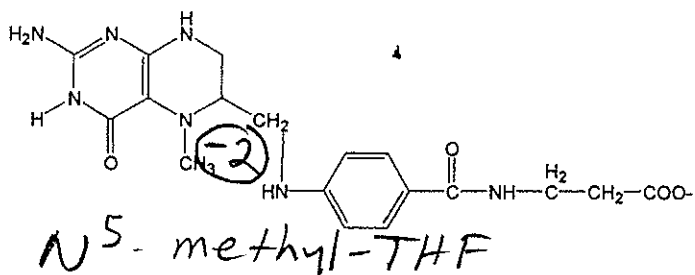
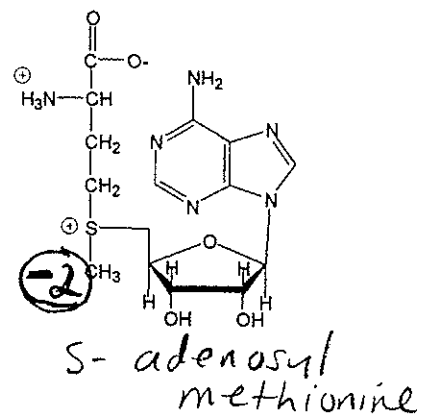
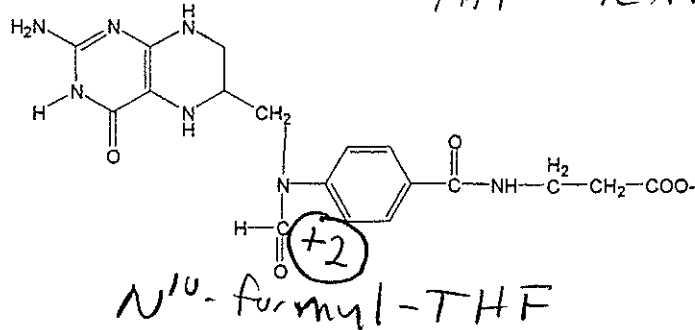
Properties and Conventions Associated with the Standard Amino Acids						
Amino acid	Abbreviated names	M_r	pK_a values			
			pK_1 ($-\text{COOH}$)	pK_2 ($-\text{NH}_3^+$)	pK_R (R group)	
Nonpolar, aliphatic R groups						
Glycine	Gly G	75	2.34	9.60		
Alanine	Ala A	89	2.34	9.69		
Valine	Val V	117	2.32	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	

*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.

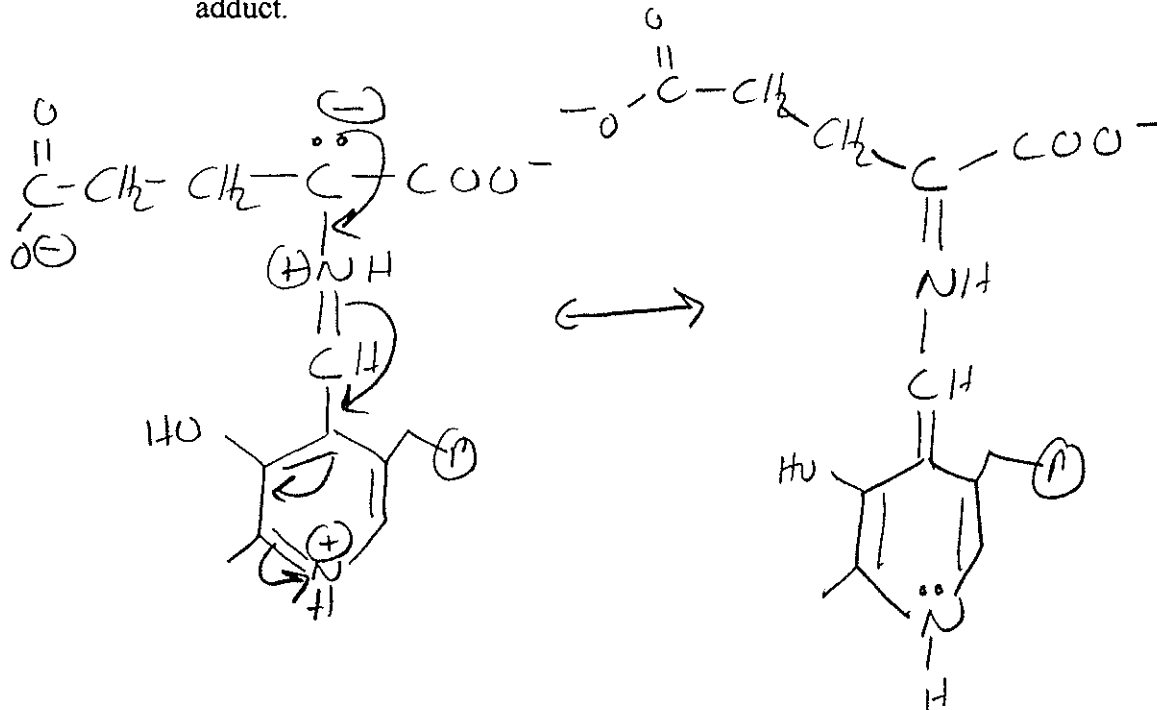
†Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

(1) (10 points) Name, and write the oxidation state of the C1 unit, in each of the following C1-cofactor adducts.

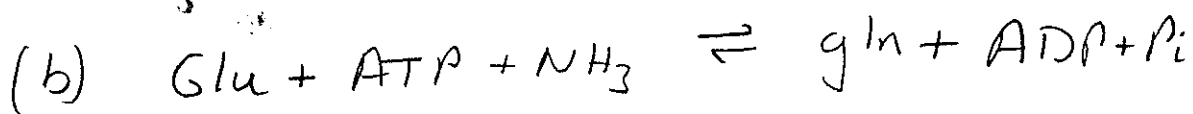
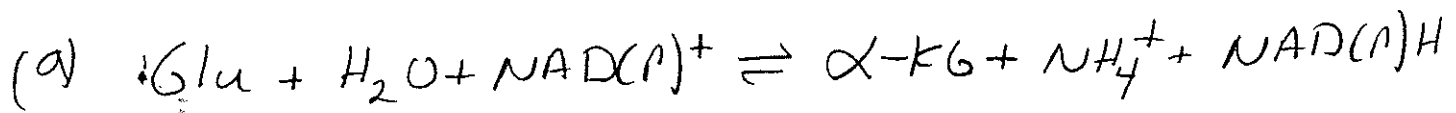
THF = tetrahydrofolate



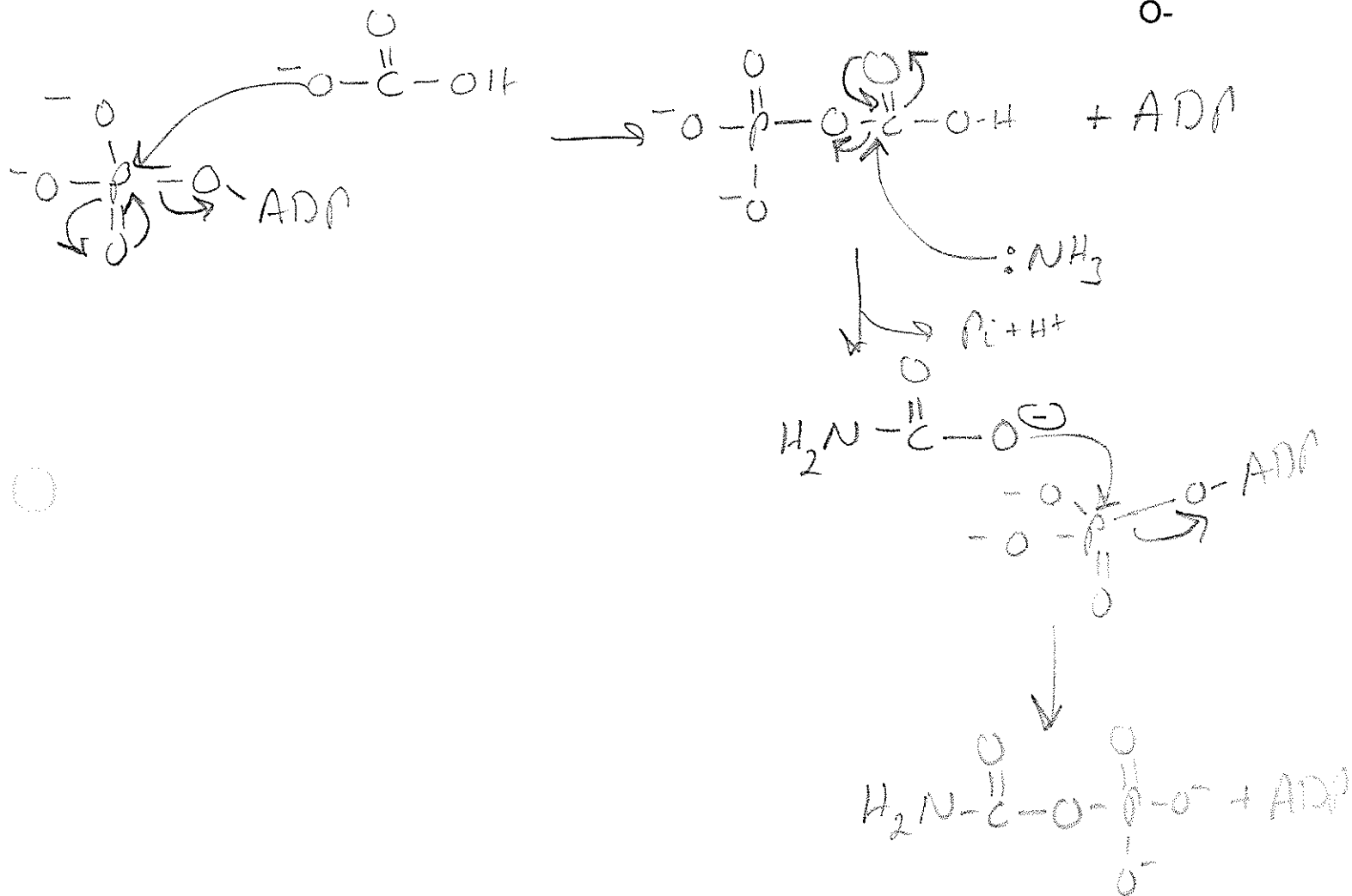
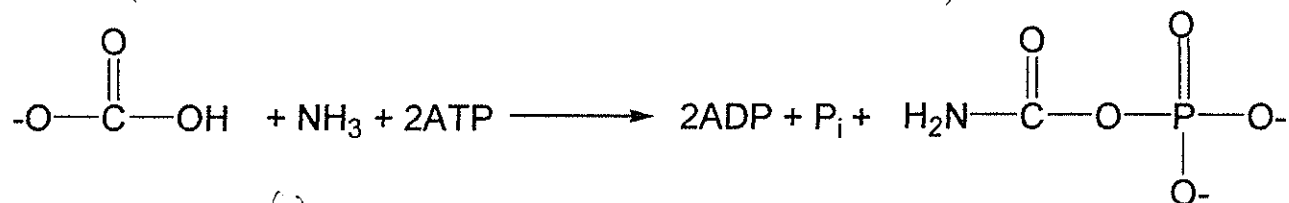
(2) (10 points) Draw the two resonance structures for the alpha carbanion of the glutamate-PLP adduct.



(3) (9 points) Write the balanced chemical equation for the reactions catalyzed by (a) glutamate dehydrogenase, (b) glutamine synthetase, and (c) glutaminase



(4) (10 points) Draw a complete reaction mechanism for the carbamoyl phosphate synthetase reaction. (Recall that carbamate is an intermediate in the overall reaction)



(5) Definitions (20 points)

Provide a proper enzyme name for the electron transfer complex I	NADH dehydrogenase
Provide a proper enzyme name for the electron transfer complex II	Succinate dehydrogenase
Provide a proper enzyme name for the electron transfer complex III	Ubiquinone: cytochrome oxidoreductase
Provide a proper enzyme name for the electron transfer complex IV	Cytochrome oxidase
Name an electron transfer cofactor that is membrane soluble and not tightly bound to an enzyme	ubiquinone
Name an electron transfer cofactor that is water soluble and not tightly bound to an enzyme	NAD(P) ⁺
Name a metal-free organic electron transfer cofactor that is always found associated with a protein	FAD, FMN
Which amino acid is preferentially transported in the bloodstream?	gln
Name an inorganic metal electron transfer cofactor that is always found associated with a protein	iron-sulfur cluster
Name the transporter that brings ATP into the mitochondrion	adenine nucleotide translocase
Where in the mitochondrion does NADH oxidation occur?	matrix side of inner mem.
Which cytosol to mitochondrion shuttle system is most efficient at conserving the chemical energy of NADH?	malate-aspartate shuttle
Where in the mitochondrion is cytochrome C located?	intermembrane space
Where in the mitochondrion is ATP synthesized?	matrix side of inner mem.
Into what portion of the mitochondrion are protons pumped?	intermembrane space
What is the alpha-keto acid counterpart of alanine?	pyruvate
In which organ does the majority of amino acid catabolism occur?	liver
Which citric acid cycle intermediate is a direct product of the reactions of the urea cycle?	fumarate
The N atoms converted into urea come from what two nitrogenous urea cycle substrates?	(1) carbonyl phosphate
	(2) aspartate

(6) (16 points) What effects will the following compounds have on the rates of ATP synthesis and O_2 consumption (no effect, slow down, stop completely) in isolated mitochondria oxidizing succinate? Explain the chemical basis for their effects.

- (a) a protonophore
- (b) a potassium ionophore
- (c) rotenone (an inhibitor of complex I)
- (d) cyanide (inhibitor of complex IV)

(a) O_2 consumption - no effect, ATP ^{stop} syn: slow $\rightarrow$ stop
 no effect a protonophore allows free movement of H^+ across membrane, dissipating the H^+ motive force necessary for ATP synthesis

(b) O_2 ^{no effect} consumption - no effect, ATP syn - slow down, but not stop. A K^+ ionophore will dissipate the electrical potential ($\Delta\psi$) component of the H^+ motive force, but will not affect the H^+ gradient itself, so some ATP synthesis can continue

(c) no effect on either. e^- from succinate enter the e^- transfer chain thru complex II, bypassing complex I.

(d) will stop both ATP synthesis & O_2 consumption.
 As e^- carriers upstream of complex IV get fully reduced, e^- transfer & H^+ pumping will cease

COMPREHENSIVE SECTION

(1) What is the formula for a multimeric protein consisting of 25 and 50 kDa subunits, present in a ratio of 3:2, which has a native molecular weight of 525 kDa? Note- α refers to the small subunit while β refers to the large subunit.

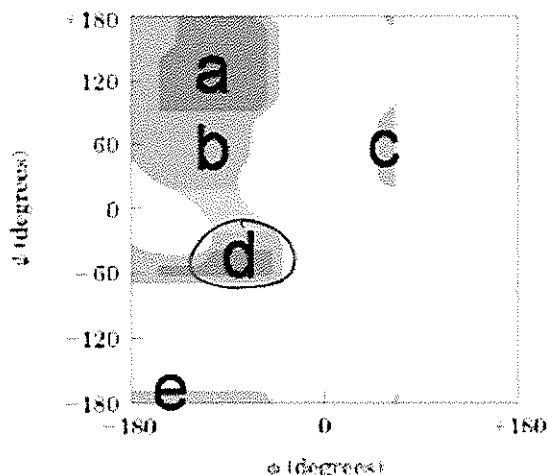
- (a) $\alpha_3\beta_2$
 (b) $\alpha_4\beta_3$
 (c) $\alpha_6\beta_4$
 (d) $\alpha_9\beta_6$

$(25)_3 (50)_2 = 175 \text{ kDa protomer}$
 $525 / 175 = 3 \text{ protomers / oligomer}$
 so $(25)_9 (50)_6$

(2) What would be a "conservative" amino acid substitution for an internal valine residue in a protein?

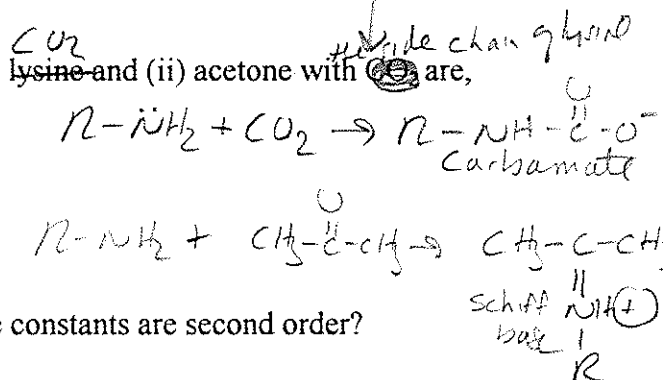
- (a) glu
 (b) arg
 (c) ile
 (d) phe

(3) Which letter on the following Ramachandran plot corresponds to the approximate region where an α -helix would be found?

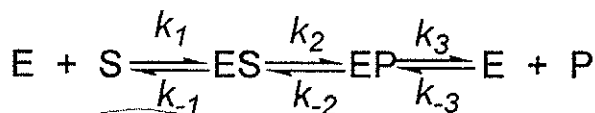


(4) The products of the reactions of the side chain of (i) lysine and (ii) acetone with CO_2 are, respectively:

- (a) (i) a Schiff base and (ii) a Schiff base
 (b) (i) a Schiff base and (ii) an amide
 (c) (i) a carbamate and (ii) an amide
 (d) (i) a carbamate and (ii) a Schiff base



(5) For the kinetic mechanism shown below, which rate constants are second order?



- (a) k_1 and k_3
 (b) k_2 and k_2
 (c) k_3 and k_{-1}
 (d) k_1 , k_2 , and k_3

(6) 10 points. For the purification table shown below, what is the total fold purification following step 5?

- (a) 450
(b) 1500
(c) 4500
(d) 15,000

Sample 5-5

A Purification Table for a Hypothetical Enzyme*

Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)	Fold purification
1. Crude cellular extract	1,400	10,000	100,000	10	1
2. Precipitation with ammonium sulfate	280	3,000		32	
3. Ion-exchange chromatography	90		80,000	200	
4. Size-exclusion chromatography	80	100	60,000	600	
5. Affinity chromatography	6	3	45,000	15,000	

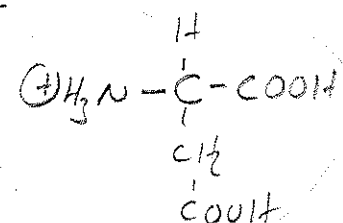
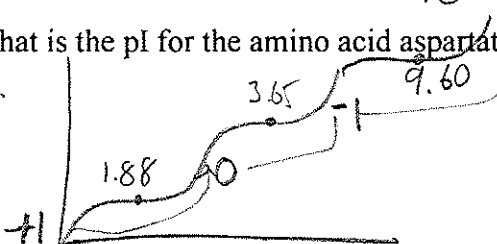
*All data represent the status of the sample after the designated procedure has been carried out. Activity and specific activity are defined on page 137.

$$\frac{15,000}{10} = 1,500$$

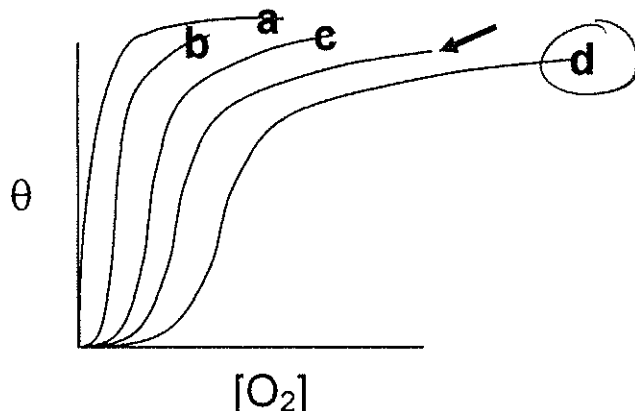
(7) Given the information on page 1, what is the pI for the amino acid aspartate?

- (a) 2.76
(b) 6.62
(c) 5.74
(d) 5.04

$$pI = \frac{1.88 + 3.65}{2}$$



(8) Given that the line indicated by the arrow corresponds to the normal hemoglobin O₂ dissociation curve, which line corresponds to the effect of increasing [2,3-DPG] by a factor of two?



increase DPG decrease O₂ affinity shift Right

(9) An enzyme produces protons as the product with specific activity 5 U/mg. If the reaction was carried out with 20 mg protein in 0.1 liter of 0.100 M Tris buffer (pK_a = 8.10) starting at pH 8.10, what would be the pH of the solution after 30 minutes of reaction?

- (a) 8.07
(b) 8.05
(c) 7.50
(d) 1.52

$$\text{at pH } 8.1, [\text{HA}] = [\text{A}^-] = 0.05 \text{ M}$$

$$[\text{H}^+] \text{ produced} = \left(\frac{5 \text{ } \mu\text{mol H}^+}{\text{min} \cdot \text{mg}} \right) (30 \text{ min}) (20 \text{ mg}) = 3,000 \text{ } \mu\text{mol}$$

$$\left(\frac{3,000 \text{ } \mu\text{mol}}{0.1 \text{ L}} \right) \left(\frac{1 \text{ mol}}{10^6 \text{ } \mu\text{mol}} \right) = 0.03 \text{ M H}^+ \text{ produced}$$

$$[\text{HA}] = 0.05 + 0.03 = 0.08$$

$$[\text{A}^-] = 0.05 - 0.03 = 0.02$$

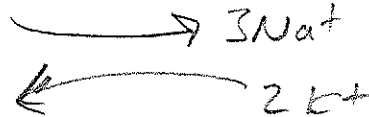
$$\text{pH} = \text{pK}_a + \log \frac{0.02}{0.08} = 8.1 + -0.6 = 7.50$$

(10) What is the repeating sugar unit and linkage in peptidoglycan?

- (a) β 1,4-NAM-NAG
- (b) β 1,4-NAG
- (c) β 1,4-glucopyranose
- (d) α 1,4-glucopyranose
- (e) α 1,4-NAM-NAG

(11) What type of transporter is the sodium-potassium ATPase?

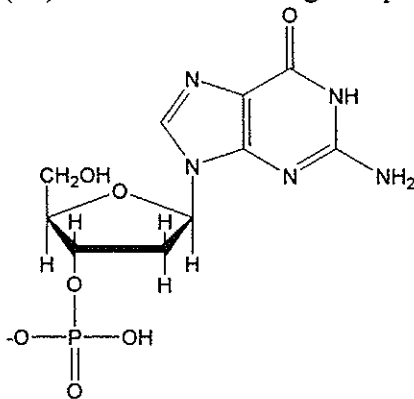
- (a) primary active symport
- (b) primary active antiport
- (c) secondary active symport
- (d) passive antiport
- (e) passive symport



(12) Which one of the following features applies, in general, to an ion channel but not a transporter?

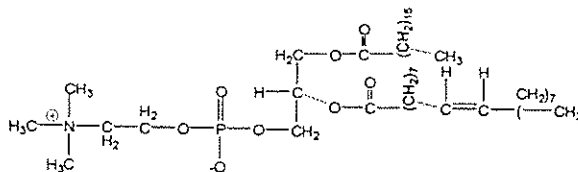
- (a) selectivity for substrate
- (b) saturable with substrate
- (c) not a protein
- (d) gated regulation

(13) Name the following compound properly:



- (a) deoxyguanosine-3'-monophosphate
- (b) deoxyadenosine-3'-monophosphate
- (c) deoxyadenine-3'-monophosphate
- (d) adenosine-3'-monophosphate
- (e) deoxyadenosine-5'-monophosphate

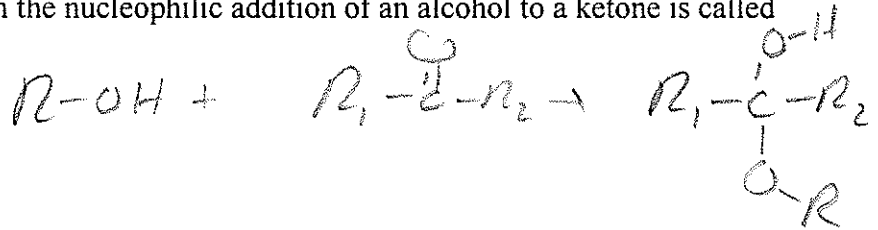
(14) What type of lipid is diagrammed here?



- (a) triacylglycerol
- (b) sphingolipid
- (c) glycerophospholipid
- (d) sphingomyelin
- (e) ether lipid

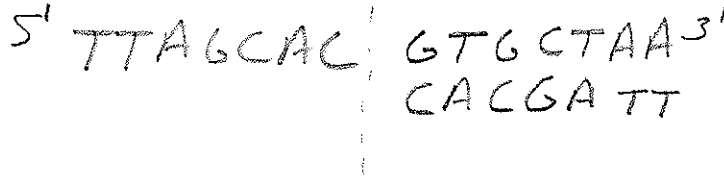
(15) The compound resulting from the nucleophilic addition of an alcohol to a ketone is called what?

- (a) hemiacetal
- (b) acetal
- (c) hemiketal
- (d) ketal



(16) What is the correct palindromic sequence, on the same strand and downstream, for the sequence 5'TTAGCAC3'?

- (a) 5'GTGCTAA3'
- (b) 5'CACGATT3'
- (c) 5'TTAGCAC3'
- (d) 5'AATCGTG3'



(17) How many stereoisomers are possible for the family of D-ketoses with seven carbon atoms?

- (a) 4
- (b) 8
- (c) 16
- (d) 32

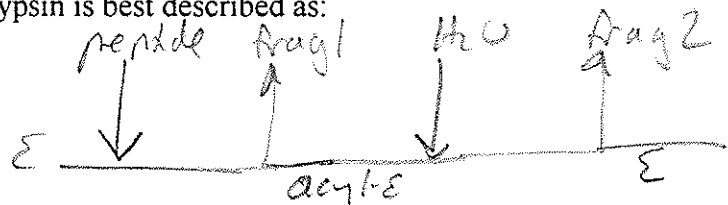
$$2^{n-3} \text{ total ketoses, } 2^{7-3} = 2^4 = 16 \text{ total, } 8 \text{ of which are D}$$

(18) Linear sugars that are structurally identical except for the orientation of the hydroxyl and hydrogen at one position are called what?

- (a) anomers
- (b) conformers
- (c) epimers
- (d) aldomers

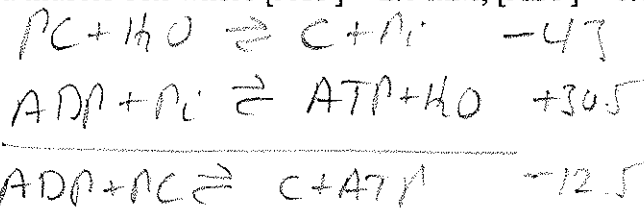
(19) The kinetic mechanism of chymotrypsin is best described as:

- (a) ordered sequential
- (b) random sequential
- (c) ordered ping pong
- (d) random ping pong
- (e) collegiate rugby



(20) The free energy of hydrolysis of creatine phosphate is -43 kJ/mol, while that of ATP is -30.5 kJ/mol. What would be the concentration of phosphocreatine in equilibrium with ATP, creatine, ADP, and phosphate in a muscle cell where [ATP] = 2.0 mM, [ADP] = 0.05 mM, and [creatine] = 0.5 mM?

- (a) 0.13 mM
- (b) 7.7 mM
- (c) 1.3 mM
- (d) 0.77 mM



$$\Delta G^\circ = -RT \ln K_{eq} \quad K_{eq} = e^{-\Delta G^\circ / RT} = e^{-(-12.5 \text{ kJ/mol}) / (8.314 \text{ J/mol} \cdot \text{K}) (298 \text{ K})}$$

$$K_{eq} = 1.55 = \frac{[C][ATP]}{[ADP][PC]} \quad [PC] = \frac{[C][ATP]}{[ADP] \cdot 1.55} = \frac{(0.5 \times 10^{-3}) (2 \times 10^{-3})}{(0.05 \times 10^{-3}) (1.55)} = 0.00013 \text{ M}$$

(21) Which of the following dehydrogenases uses FAD rather than NAD^+ as electron acceptor?

- (a) malate dehydrogenase
- (b) glyceraldehydes-3-phosphate dehydrogenase
- (c) lactate dehydrogenase
- (d) acyl-CoA dehydrogenase

(22) Which of the following enzymes catalyzes a claisen condensation reaction?

- (a) succinyl-CoA synthetase
- (b) aldolase
- (c) citrate synthase
- (d) aconitase

(23) Which of the following glycolytic enzymes catalyzes substrate level phosphorylation?

- (a) phosphofructokinase
- (b) phosphoglycerate kinase
- (c) glyceraldehydes-3-phosphate dehydrogenase
- (d) enolase

(24) Which of the following enzymes catalyzes a reaction involving a cis-enediolate intermediate?

- (a) phosphoglucosomerase
- (b) aldolase
- (c) enolase
- (d) pyruvate kinase

(25) What is the high energy intermediate of fatty acyl-CoA synthetase?

- (a) fatty acyl triphosphate
- (b) fatty acyl pyrophosphate
- (c) fatty acyl phosphate
- (d) fatty acyl-AMP