BCH 4053 **FINAL EXAM** **NAME_____KEY_____**
May 1, 2003

There are 9 pages and 9 questions on the exam.
Only five are to be answered, each worth 20 points.

Page	Points
1	____
2	____
3	____
4	____
5	____
6	____
7	____
8	____
9	____
Total	__100_

Answer two from questions 1, 2, 3, and 4

Answer three from questions 5, 6, 7, 8, and 9.

(Only five questions total will be graded. If you answer more than the required number from either group, please indicate which ones are to be graded.)

1. Following is an alphabetical list of the glycolytic and TCA cycle enzymes plus a few other enzymes we have discussed. Choose enzymes from this list that are described by the statements below, and place the number or numbers of the enzyme in the blank to the left of the statement. In most cases, more than one enzyme will apply. Given in parenthesis after the statement is the **target number** of enzymes for you to identify.

(1) aconitase
(2) aldolase
(3) citrate synthase
(4) enolase
(5) fumarase
(6) glucose-6-phosphate isomerase (phosphoglucoisomerase)
(7) glyceraldehyde-3-phosphate dehydrogenase
(8) hexokinase
(9) isocitrate dehydrogenase
(10) a-ketoglutarate dehydrogenase complex
(11) malate dehydrogenase
(12) malic enzyme
(13) phosphoenolpyruvate carboxykinase
(14) phosphofructokinase
(15) phosphoglycerate mutase
(16) phosphoglycerate kinase
(17) pyruvate carboxylase
(18) pyruvate dehydrogenase complex
(19) pyruvate kinase
(20) succinate dehydrogenase
(21) succinyl-CoA synthetase
(22) triose phosphate isomerase

(a) ___9,10,12,13,17,18_____ CO_2 is a substrate or a product. (six enzymes)

(b) ___8,13,14,16,17,19_____ ATP or GTP is a substrate or a product. (six enzymes)

(c) ___3,10,18,21_____ Coenzyme A is a substrate or a product. (four enzymes)

(d) ___7,9,10,11,18_____ NADH is a substrate or a product. (five enzymes)

(e) ___10,18_____ Thiamine pyrophosphate is a prosthetic group. (two enzymes)

(f) ___12,13,17_____ Possible anaplerotic reactions. (three enzymes)

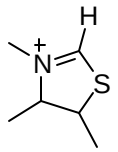
(g) ___7,9,10,11,12,18,20_____ An oxidoreductase (six enzymes)

(h) __3,8,9,10,14,17,18,19____ Operates removed from equilibrium (eight enzymes)

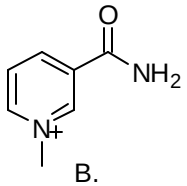
minus 0.5 for each missing enzyme, minus 0.2 for an enzyme that doesn't belong
up to maximum minus2.5 for each blank.

2. Below are the **partial** structures of seven coenzymes you have studied.

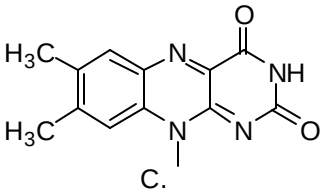
(a) Below each structure, draw an alternative form of the coenzyme to which it is converted during the course of a reaction.



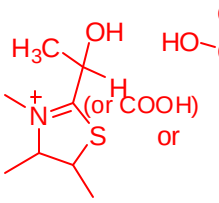
A.



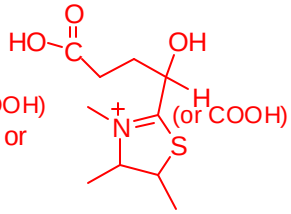
B.

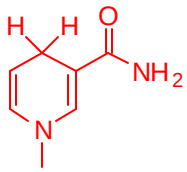


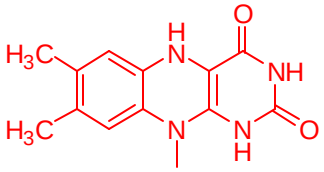
C.

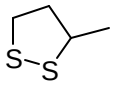


or

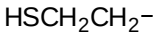




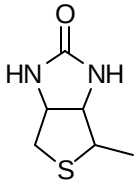




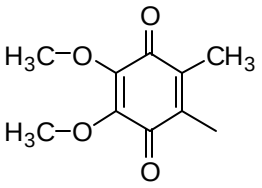
D.



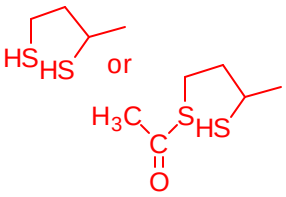
E.

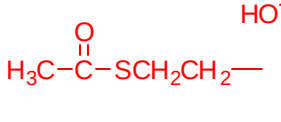


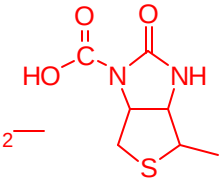
F.

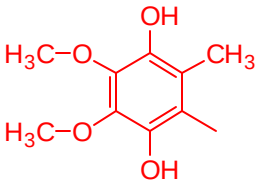


G.









(b) Complete the following table by giving the name of the coenzyme, identifying it as a **cosubstrate** or **prosthetic group**, and give the name of an enzyme that it reacts with.

Structure	Coenzyme	Cosubstrate or Prosthetic Group?	Enzyme (only one needed)
A	thiamine pyrophosphate	Prosthetic group	pyruvate dehydrogenase (DH) or alpha-ketoglutarate DH
B	NAD	Cosubstrate	glyceraldehyde-3-P DH, lactate DH, isocitrate DH, alpha-ketoglutarate DH malate DH, pyruvate DH, Complex I
C	FAD or FMN	Prosthetic group	succinate DH (aka Complex II), Complex I glycerol-3-P DH
D	lipoic acid	Prosthetic group	pyruvate DH, alpha-ketoglutarate DH
E	coenzyme A	Cosubstrate	pyruvate DH, citrate synthase, alpha-ketoglutarate DH, succinyl-CoA synthase
F	biotin	Prosthetic group	pyruvate carboxylase
G	coenzyme Q (or ubiquinone)	Cosubstrate	Complex I, Complex II, Complex III

(1.5 pt. each structure, 0.5 pt. each column entry)

3. Glycerol, formed from hydrolysis of triglycerides, can be provide energy by oxidation via glycolysis and the TCA cycle. It must first be activated by the enzyme **glycerol kinase**, which catalyzes:

glycerol + ATP → glycerol-3-phosphate + ADP

Glycerol phosphate dehydrogenase converts glycerol-3-phosphate to dihydroxyacetone phosphate.

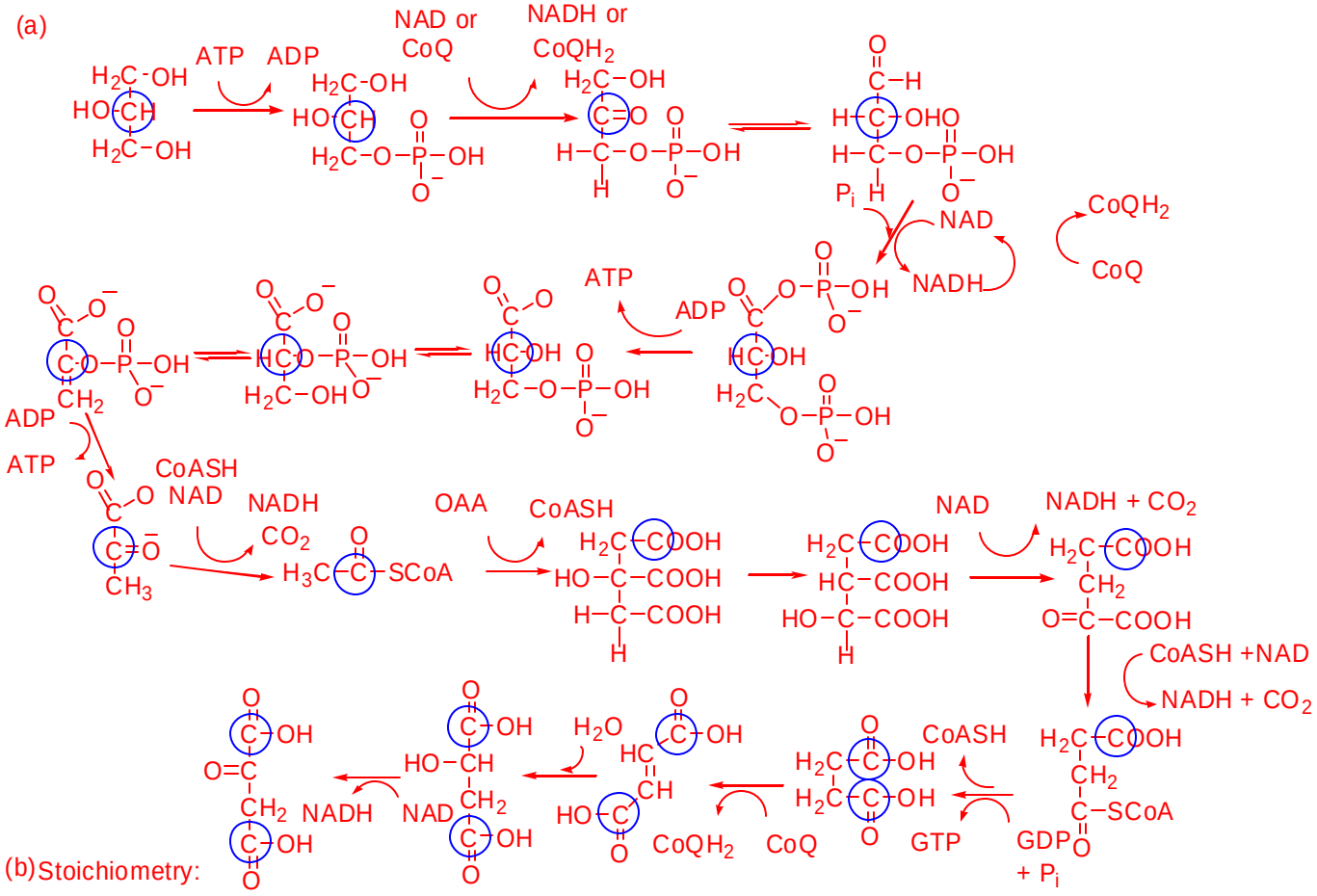
(a) Starting with [2-^{14}C]-glycerol (the middle carbon), trace the radioactive carbon through glycolysis and the **first turn** of the citric acid cycle, showing how oxaloacetate would be labeled at the end of the first turn. To do so, give the structure of each intermediate in the pathway, and circle the carbon atom(s) of each intermediate which derive from carbon-2 of glucose.

(b) Indicate each step that **uses** or **produces** an NADH, $CoQH_2$, ATP, or GTP, and calculate the overall stoichiometry for the reaction:

glycerol → 3 CO_2

(c) Assuming 2.5 ATP per NADH, 1.5 ATP per $CoQH_2$, and 1.5 ATP per cytoplasmic NADH, what is the total ATP yield from oxidation of 1 mole of glycerol?

(a)



(b) Stoichiometry:

glycerol + 4 NAD + 3 CoQ + ADP + GDP + 2 P_i → 3 CO_2 + 4 NADH + 3 $CoQH_2$ + ATP + GTP

(2 of the $CoQH_2$ from cytoplasmic NADH)

(c) ATP yield:

4 NADH x 2.5 = 10 ATP
3 $CoQH_2$ x 1.5 = 4.5 ATP
GTP + ATP = 2 ATP
Total = 16.5 ATP

0.5 each labeled intermediate = 9 pts
2 pts each coenzyme stoich. = 8 pts
Total ATP calculation = 3 pts

4(A) Following is an alphabetical list of the intermediate electron carriers found in the mitochondrial electron transport chain. Identify the carriers that fit each description on the right by placing the letter of the carrier(s) in the blank next to the description. A carrier may be used more than once.
1 pt each blank; partial credit units of 0.2

Electron Carrier

(a) Coenzyme Q
(b) Cu_A
(c) Cu_B
(d) cytochrome a
(e) cytochrome a_3
(f) cytochrome b_H
(g) cytochrome b_L
(h) cytochrome c
(i) cytochrome c_1
(j) FAD
(k) Fe/S center
(l) FMN

Description

A component of Complex I ____l, k, (accept a)_____
A component of Complex II ___j, k_____
A component of Complex III ___f, g, i, k_(accept a)_____
A component of Complex IV ___b, c, d, e_____
Carries electrons from Complex II to Complex III ___a____
Carries electrons from Complex III to Complex IV __h____
Forms a binuclear center for oxygen reduction ___c, e_____
Accepts electrons directly from succinate ____j_____
Accepts electrons directly from NADH _____l_____

4(B). Mitochondria or submitochondrial particles can carry out the following coupled reaction:

$$2 \text{ cyt c (red)} + \tfrac{1}{2} O_2 + ADP + P_i \rightarrow 2 \text{ cyt c (ox)} + H_2O + ATP$$

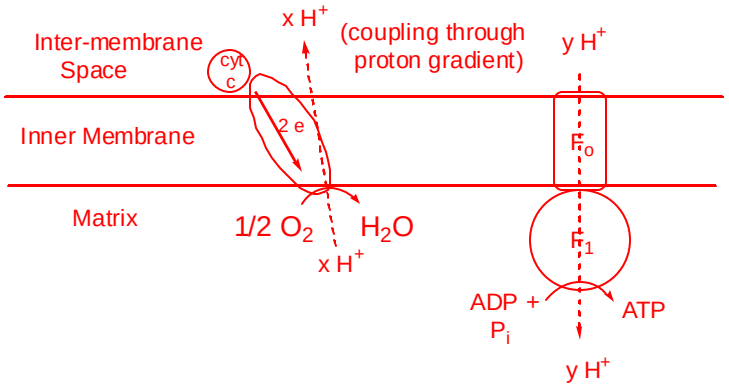
(a) Calculate the overall $\Delta G^{o'}$ for this coupled process. (E_o' for cyt $c_{(ox)}$/cyt $c_{(red)}$ is +0.25 V, E_o' for ½ O_2/H_2O is +0.82 V, R = 8.314 J-mol^{-1}-K^{-1}, F = 96.5 kJ-mol^{-1}V^{-1}, T = 298 K $\Delta G^{o'}$ for ATP hydrolysis = -30.5 kJ-mol^{-1})
(b) Diagram the orientation of the two complexes in the inner mitochondrial membrane that carry out this coupled reaction, illustrating in the diagram how the coupling occurs.
(c) Explain how the proposed coupling leads to the proposed stoichiometry in the equation (1 ATP made per two cyt c's reduced).

(a) (6 pts. 2 pt ΔE, 2 pt $\Delta G^{o'}$ for redox; 2 pt for overall $\Delta G^{o'}$)

$$\Delta E_o' = E_o'_{reduction} - E_o'_{oxidation} = 0.82 - 0.25 = 0.57 \text{ V}; \quad \Delta G^{o'} = -nF\Delta E_o' = -(2)(96.5\frac{kJ}{mol-V})(0.57 \text{ V}) = -110\frac{kJ}{mol}$$

$$\Delta G^{o'}_{overall} = \Delta G^{o'}_{redox} + \Delta G^{o'}_{ATPformation} = -110\frac{kJ}{mol} + 30.5\frac{kJ}{mol} = -79.5\frac{kJ}{mol}$$

(b) (3 pts. 1 pt each complex, 1 pt showing proton movement for coupling)



(c) The cytochrome oxidase (complex IV) is believed to pump 4 protons per pair of electrons passed to oxygen (x = 4). The ATP synthase is believed to pump 3 protons per ATP made (y = 3). In addition one proton is used for the antiport of ATP and ADP across the mitochondrial membrane (not shown) and the import of P_i, for a total of 4 protons needed per ATP produced. Therefore the stoichiometry in the equation is 1:1 for ATP made per pair of cytochrome c's oxidized. (2 pts. 1 pt proton stoichiometry of each complex)

5. One of the proton dissociations of hemoglobin occurs near neutral pH. This dissociation is affected by the binding of oxygen to Hb. Assume that the dissociations for oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb) can be treated as a simple monoprotic acids as follows:

$HHbO_2 \rightleftarrows H^+ + HbO_2$, pK = 6.6; and $HHb \rightleftarrows H^+ + Hb$, pK = 8.2;

(a) Assume that blood is 3.0 mM in hemoglobin. At the plasma pH of 7.4, and the hemoglobin in the lungs fully oxygenated, what are the concentrations of the protonated ($HHbO_2$) and unprotonated (HbO_2) forms?

(b) In tissues, the pressure of O_2 drops, and the oxygen dissociates from hemoglobin. The deoxyhemoglobin is a stronger base, and must be titrated with protons for the pH to remain constant at 7.4. Assuming the oxygen were completely dissociated, how many protons would be required per liter to produce the proper Hb/HHb ratio for pH 7.4?

(a) $\dfrac{[HbO_2]}{[HHbO_2]} = 10^{pH-pK} = 10^{7.4-6.6} = 10^{0.80} = 6.31$

$[HbO_2] + [HHbO_2] = 3.0 \text{ mM}$

$6.31[HHbO_2] + [HHbO_2] = 3.0 \text{ mM}$

$[HHbO_2] = \dfrac{3.0 \text{ mM}}{7.31} = 0.41 \text{ mM}$

$[HbO_2] = 3.0 \text{ mM} - 0.41 \text{ mM} = 2.59 \text{ mM}$ (8 pts. to here, 4 pts. ratio, 4 pts. conc.)

(b) $\dfrac{[Hb]}{[HHb]} = 10^{pH-pK} = 10^{7.4-8.2} = 10^{-0.80} = 0.158$

$[Hb] + [HHb] = 3.0 \text{ mM}$

$0.158[HHb] + [HHb] = 3.0 \text{ mM}$

$[HHb] = \dfrac{3.0 \text{ mM}}{1.158} = 2.59 \text{ mM}$

$[Hb] = 3.0 \text{ mM} - 2.59 \text{ mM} = 0.41 \text{ mM}$ (8 pts. to here, 4 pts. ratio, 4 pts. conc.)

To go from 0.41 mM protonated (as $HHbO_2$) to 2.59 mM protonated (as HHb) one needs 2.59-0.41 = 2.18 mM protons (2.18×10^{-3} moles per liter) (4 pts. calculation)

6. In 2010 the Mars shuttle returned to earth with a sample of a Martian single-celled organism.. Not surprisingly, extracts of the organism catalyzed the hydrolysis of ATP, showing Michaelis-Menten kinetics with a $\mathbf{K_m}$ of 3.5 x 10^{-5} M and a $\mathbf{V_m}$ of 90 µmoles-min^{-1}-mg protein^{-1}.
   (a) Give the **Michaelis-Menten** equation.
   (b) Calculate the velocity of the ATPase reaction at the following ATP concentrations:
   S = 0.75 x 10^{-6} M; S = 2.5 x 10^{-4} M; S = 0.035 M
   (c) What would $\mathbf{V_m}$ be in the presence of 0.0015 M concentration of a competitive inhibitor of ATPase that had a $\mathbf{K_I}$ of 0.0015 M?
   (d) The ATPase activity was stimulated by the addition of Ca^{2+} ions. What does this suggest about the function of the ATPase?
   (e) Using GTP as a substrate, the kinetic parameters were a $\mathbf{K_m}$ of 4.9 x 10^{-3} M and a $\mathbf{V_m}$ of 230 µmoles-min^{-1}-mg protein^{-1}. Would you consider GTP or ATP the "better" substrate? Why?

(a) $v = \dfrac{V_m S}{K_m + S}$

(b) $v = \dfrac{(90 \frac{\mu mol}{min\text{-}mg})(0.75 x 10^{-6}\ M)}{(3.5 x 10^{-5}\ M + 0.75 x 10^{-6}\ M)} = 90(0.021) = 1.9 \frac{\mu mol}{min\text{-}mg}$

$v = \dfrac{(90 \frac{\mu mol}{min\text{-}mg})(2.5 x 10^{-4}\ M)}{(3.5 x 10^{-5}\ M + 2.5 x 10^{-4}\ M)} = 90(0.877) = 79 \frac{\mu mol}{min\text{-}mg}$

$v = \dfrac{(90 \frac{\mu mol}{min\text{-}mg}(0.035 M)}{(3.5 x 10^{-5}\ M + 0.035 M)} = 90(0.999) = 90 \frac{\mu mol}{min\text{-}mg}$

(c) V_m would be $90 \frac{\mu mol}{min\text{-}mg}$ (no change in V_m for a competitive inhibitor

(d) Probably a calcium pump

(e) for ATP, $\frac{V_m}{K_m} = \frac{90}{3.5 x 10^{-5}} = 2.5 x 10^6$; for GTP, $\frac{V_m}{K_m} = \frac{230}{4.9 x 10^{-3}} = 4.7 x 10^4$

ATP would be the "better" substrate with the higher $\frac{V_m}{K_m}$ ratio.

3 pts (a), 3 pts each velocity in (b), 3 pts each (c) and (d), 2 pts (e)

7. Aldolase catalyses the following reaction of glycolysis:

fructose-1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate

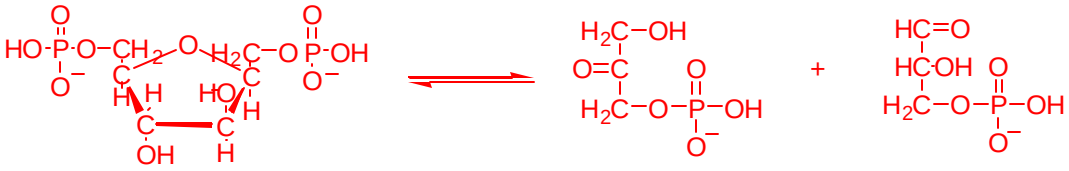
(a) Give the structures of the reactant and products of this reaction.

(b) **?G°'** for this reaction is +23.9 kJ mol^{-1}. Calculate **K'**, the equilibrium constant. (R=8.314 J/mol-K; assume T = 37 °C or 310 K)

(c) Calculate **Q'** and **?G** for the reaction when fructose-1,6-diphosphate is 1.0x10^{-4} M, dihydroxyacetone phosphate is 4.0x10^{-5} M, and glyceraldehyde-3-phosphate is 2.5 x 10^{-6} M.

(d) Fructose metabolism bypasses this reaction of glycolysis. Describe how Fructose is metabolized to glycolytic intermediates.

(a) (6 pts. 2 pts each structure)



(b) (4 pts. all or none)

$$K' = e^{-\frac{\Delta G^{o\,'}}{RT}} = e^{-\frac{23.9\frac{kJ}{mol}x10^{3}\frac{J}{kJ}}{8.314\frac{J}{mol-K}x310\ K}} = e^{-9.27} = 9.4x10^{-5}$$

(c) (3 pts. Q', 3 pts ΔG)

$$\Delta G = \Delta G^{o\,'} + RT\ln Q'$$

$$Q' = \frac{[DHAP][GA3P]}{[FBP]} = \frac{(4x10^{-5})(2.5x10^{-6})}{(1x10^{-4})} = 10^{-6}$$

$$\Delta G = 23.9\frac{kJ}{mol} + 8.314\frac{J}{mol-K}x10^{-3}\frac{kJ}{J}x310\ K\ln 10^{-6}$$

$$\Delta G = 23.9\frac{kJ}{mol} - 35.6\frac{kJ}{mol}$$

$$= -11.6\frac{kJ}{mol}$$

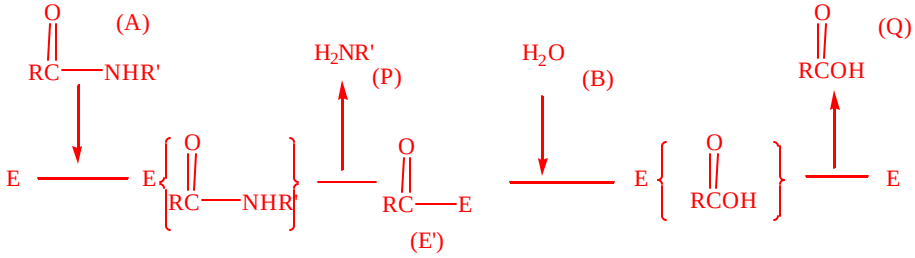
(d) Fructose is activated to Fructose-1-phosphate by fructokinase. A special aldolase cleaves this to dihydroxyacetone phosphate and free glyceraldehyde. A triose kinase converts glyceraldehyde to glyceraldehyde-3-phosphate, and both dihydroxyacetone phosphate and glyceraldehydes-3-phosphate enter glycolysis.

(4 pts. Can be words or diagrams.)

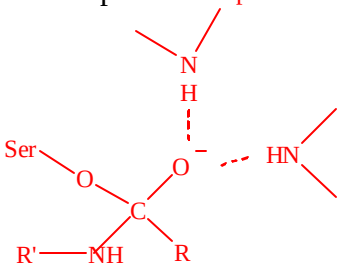
8. The mechanism of chymotrypsin illustrates several of the factors that are believed to contribute to the rate acceleration obtained by enzymes. Describe each of the following aspects of the chymotrypsin mechanism.

(a) A reaction model that shows ping-pong kinetics. (i.e., specify the identity of A, B, P, Q, E, and E', in the following scheme:)

2 pts each for A, B, P, Q, and E' 10 total



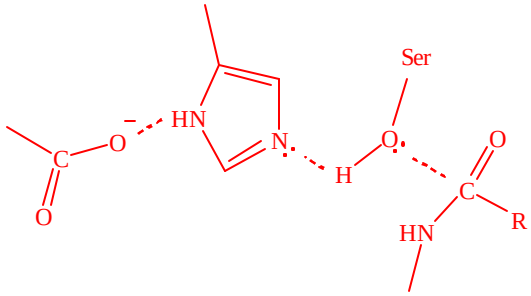
(b) Transition state stabilization by bonds formed between the enzyme and the transition state that are not found in the binding of substrate or product. 2 pts



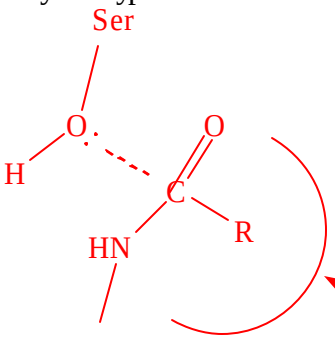
binding of O^- of transition state to two peptide NH groups in the "oxyanion hole"

(c) Acid-base catalysis mediated through a "catalytic triad". Describe how the triad assists in the formation of the covalently bound intermediate.

3 pts (1 ea triad member)



(d) Substrate specificity provided by the nature of the substrate binding site. (Explain how chymotrypsin differs from trypsin in the binding site.) 2 pts



hydrophobic binding pocket binds large bulky R groups in chymotrypsin. In trypsin, the bottom of the pocket has a negatively charged aspartate residue, which binds positively charged lysine or arginine.

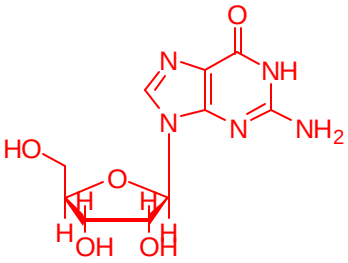
(e) Regulation of chymotrypsin activity in the digestive tract.

3 pts.

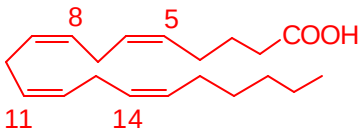
Inactive chymotrypsinogen cleaved by trypsin to π-chymotrypsin, then self digestion of π-chymotrypsin to a-chymotrypsin.

9. Draw the structure of **5** of the following: (4 pts each structure)

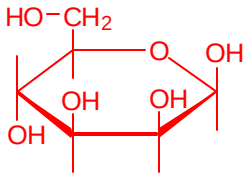
(a). Guanosine



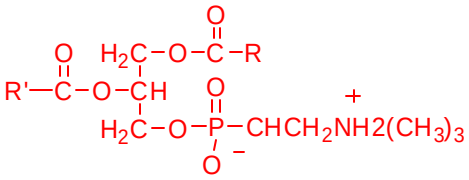
(b). Arachidonic acid



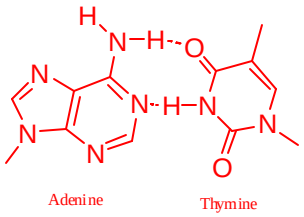
(c). Mannose (Haworth projection)



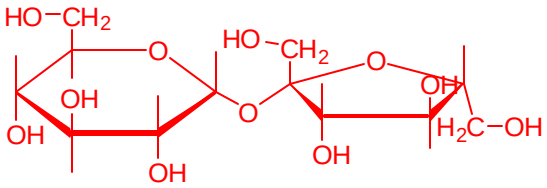
(d). Phosphatidyl choline



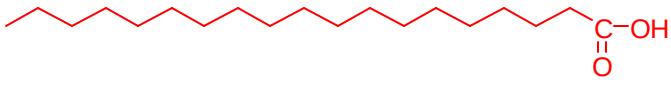
(e). AT base pair



(f). Sucrose (Haworth projection)



(g). Stearic acid



(h). Cholesterol