Name__________________________

INTD 5000 Fundamentals of Biomedical Science
Biochemistry Section
(Hart)

1. The titration curve of the amino acid alanine shows the ionization of two functional groups with pKa values of 2.34 and 9.69, corresponding to the ionization of the carboxyl and the protonated amino groups, respectively. The titration of di-, tri-, and larger oligopeptides of alanine also show the ionization of only two functional groups, although the experimental pKa values are different. The trend in pKa values is summarized as follows:

Amino Acid or Peptide	pK_1	pK_2
Ala	2.34	9.69
Ala-Ala	3.12	8.30
Ala-Ala-Ala	3.39	8.03
Ala-(Ala)n-Ala n≥4	3.42	7.94

a) The value of pK_1 increases in going from Ala to an Ala oligopeptide. Provide and explanation. (3 points)

b) The value of pK_2 decreases in going from Ala to an Ala oligopeptide. Provide an explanation. (3 points)

2. Draw the structure of the hexapeptide defined by the single letter codes W-Q-T-E-P-M (12 points)

3. (12 points total)

Sketch the titration curve of the tripeptide G-H-L. ($pK_1 = 2.2$, $pK_R = 6.5$, $pK_2 = 9.7$).

Label both axes. Indicate where glutamate has no net charge. What pH does this correspond to?

4. Describe the groundbreaking experiments performed by Christian Anfinsen. What functional concept(s) did this series of experiments demonstrate? (5 points).

5. What is the pH of a 0.18 M solution of sodium formate? pKa of formic acid = 3.75 (5 points)

6. **What is the pOH of 232 mL of a 0.26 M lactic acid solution (pKa = 4.0) to which 16.7 mL of 2.4 M KOH has been added? (5 points)**

7. For the amino group of glycine: $R\text{-}NH_3^+ \longleftrightarrow R\text{-}NH_2 + H^+$ (pKa = 9.6)
How many mL of 4.0 M KOH must be added to 1.0 L of 0.7 M glycine at pH 8.5 to bring its pH to 10? (5 points).

INTD5000 EXAM
Catalysis, Enzyme kinetics, mechanism, inhibition & regulation (Sousa Lectures)

Total =50 points (question 1-4 are 6 pts. each; question 5 is 14 pts. and question 6 is 12 pts.) Work on back of exam if you need more space

Hill equation: $V=V_{max}[S]^n/(K_s^n+[S]^n)$; for M-M kinetics, n=1

1. You are characterizing a plant enzyme which catalyzes polymerization of glucose to form storage polysaccharides. You measure the rate at which this enzyme metabolizes glucose as a function of varying glucose concentration. The following are some of your (very accurate) data:

[glucose]=2 mM, V= .033 mM/sec
[glucose]=6 mM, V=.06 mM/sec
[glucose]=200 mM, V=.1 mM/sec
[glucose]=1M, V=.1 mM/sec

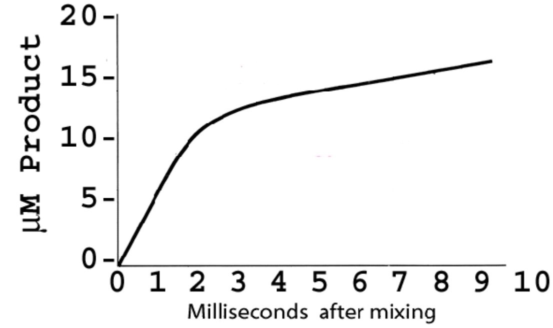
Does this enzyme obey Michaelis-Menten kinetics? How do you know? What is its K_m or K_s?

2. In the spring when this plant needs glucose for growth it produces a small molecule, X. You measure the activity of the above enzyme in the presence of 2 mM of X and obtain the following data:

[glucose]=2 mM, V= .011 mM/sec
[glucose]=6 mM, V=.02 mM/sec
[glucose]=200 mM, V=.033 mM/sec
[glucose]=1M, V=.033 mM/sec

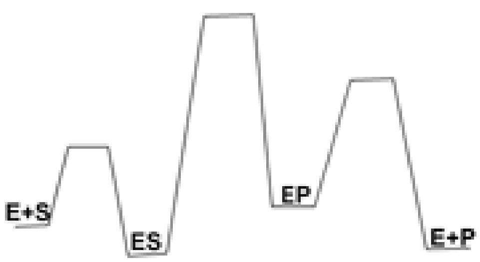
How would you describe the effects of X on the activity of your enzyme? How do these effects fit the plant's physiological requirement for more glucose in the spring? Does X bind to the enzyme, and if so, what is its dissociation constant or K_I?

3. You are interested in understanding the detailed reaction mechanism of this enzyme so you measure the amount of product it produces within milliseconds after mixing 10 mM of glucose with .01 mM of enzyme. You obtain the following plot:

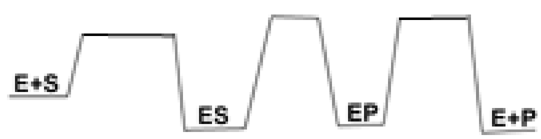


Based on this plot which of the following reaction diagrams is most likely to describe this enzyme's reaction mechanism:

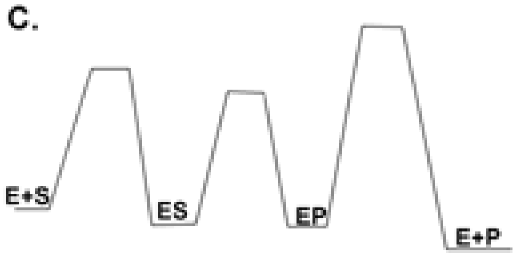
A.



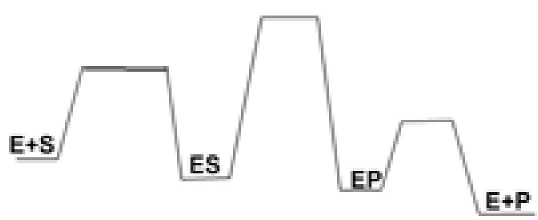
B.



C.



D.



?

4. You determine the structure of this enzyme complexed with substrate. In the active site you note the presence of aspartate and glutamate side chains positioned near the substrates as shown below. <u>Draw and describe</u> a <u>reasonable</u> mechanism showing how these two side chains catalyze attachment of the glucose to the polysaccharide chain:

5. You discover that in humans there is gene which is homologous to the gene which encodes the plant enzyme. You make the following observations:

1. The plant gene encodes a 25 kD enzyme.
2. The molecular weight of the plant enzyme, as determined from gel filtration, is 25kD.
3. The human gene encodes a 25 kD enzyme.
4. The molecular weight of the human enzyme, as determined from gel filtration, is 100kD.
5. The human and plant enzymes both catalyze an identical reaction.
6. Preparations of the human and plant enzymes display half-maximal reaction velocities at the same glucose concentration.

You measure reaction velocities for the human enzyme as a function of glucose concentration and obtain the following data:

[glucose]=2 mM, V= .0125 mM/sec
[glucose]=6 mM, V=.077 mM/sec
[glucose]=200 mM, V=.1 mM/sec
[glucose]=1M, V=.1 mM/sec

a. Does the human enzyme obey Michaelis-Menten Kinetics? Why or why not?

b. Draw a qualitative plot illustrating how the V vs. S curve for the human enzyme differs from that of the plant enzyme. What is the name for the shape of the curve for the human enzyme?

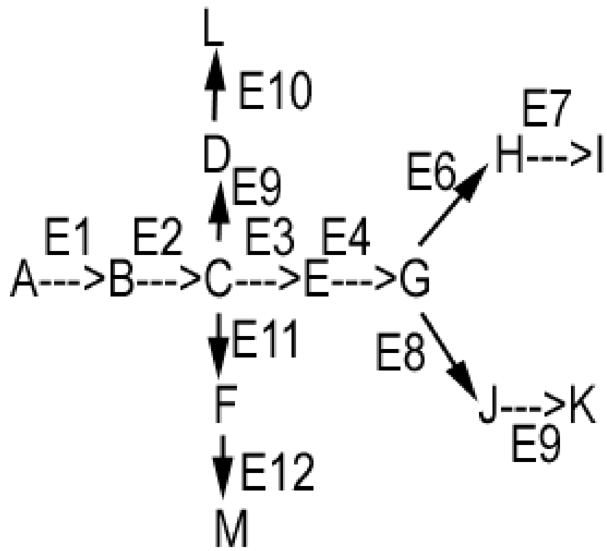
c. What is the Hill coefficient of the human enzyme?

d. May the larger molecular weight of the human enzyme (as measured by gel filtration) be important for why it display kinetics different from the plant enzyme? Why or why not?

e. It is known that in humans it's important to keep blood glucose within a narrow range (not too high, not too low). How might the distinct kinetic properties of the human enzyme be important for this?

f. You identify mutations in the human enzyme which can result in diabetes (aberrant blood glucose regulation). These mutations leave enzymatic activity intact. However, when you measure the molecular weight of the mutant enzymes by gel filtration you find it to be 25 kD. How might the mutations be causing disease?

6. A set of 12 enzymes (E1-E12) catalyzes a series of reactions starting with substrate A and produces several intermediate metabolites and 4 final metabolites (I, K, L, M) required by a cell:



a. Which one enzyme should be feedback inhibited by which one metabolite to specifically regulate production of I?

b. Which one enzyme should be feedback inhibited by which one metabolite to specifically regulate production of K?

c. Which one enzyme should be feedback inhibited by which one metabolite to specifically regulate production of both I and K?

d. Is feedback inhibition of E2 by L a good strategy to prevent synthesis of too much L? Why or why not?

Name ______________________________________

INTD 5000 - Carbohydrate and Energy Metabolism (Henn)

Exam 3 - October 15, 2008

1. [7 points] Glycolysis is part of Stage _______ of metabolism. In the absence of oxygen, glycolysis can supply sufficient ____________ to support the ________________ pathways in Stages ____________ as long as there is sufficient ________________ available and as long as there is a mechanism for reoxidation of _________. In the absence of such a mechanism, glycolysis would be blocked at the level of what enzyme (or reaction)? _______________________

2. [5 points] Draw the ring form of glucose (stick figure is fine):

Indicate with the letters (a) to (e) the carbon of glucose that fits each description below. Note: There may be more than one correct answer. There may be no correct answer.

(a) determines the anomeric form of glucose.

(b) forms a high-energy bond with a nucleotide as a precursor for glycogen synthesis.

(c) has a different configuration in the common epimer of glucose, galactose.

(d) is lost in the oxidative branch of the hexose monophosphate pathway.

(e) is involved in formation of long chains of glucose residues in glycogen.

3. [5 points] What is the structural or functional relationship between the following pairs of intermediates in glycolysis?

(a) dihydroxyacetone phosphate and glyceraldehyde-3-phosphate ________________

____

(b) phosphoenolpyruvate and 1,3 bisphosphoglycerate __________________________

____

The rate of flux through the pathway of glycolysis is controlled primarily at the level of the reaction catalyzed by ______________________. Flux is increased when cellular concentrations of __________________ are low and ________________ (increased or decreased) when cellular concentration of oxygen are low.

4. [4 points] Mammalian liver requires substantial amounts of NADPH to support many biosynthetic pathways and antioxidant functions, and some but much less ribose-5-phosphate. Explain what this means in terms of flux through the two branches of the hexose monophosphate pathway (= pentose phosphate pathway):

____

____

Even when concentrations of glucose in the blood are low, similar patterns of flux would be observed in these branches. In this case, however, what would you expect would be the final destination of carbon flowing through this pathway? What effect would glucagon have on this process?

____

____

5. [2 points] Name, and explain the function of two enzymes or proteins primarily or only expressed in the liver that control the concentration of glucose-6-phosphate in liver cells and the concentration of glucose in the bloodstream.

____

____

6. [6 points] Name the enzyme (or enzymes) in the TCA cycle that best fits each of the following descriptions. Abbreviations (αKGD, MDH, CIT, SCS, FUM, IDH, SDH, ACO) are fine.

___________ catalyzes formation of a nucleotide triphosphate.

___________ is also part of the electron transport chain.

___________ catalyzes a reaction needed for gluconeogenesis from pyruvate.

___________ is structurally and functionally similar to the pyruvate dehydrogenase complex.

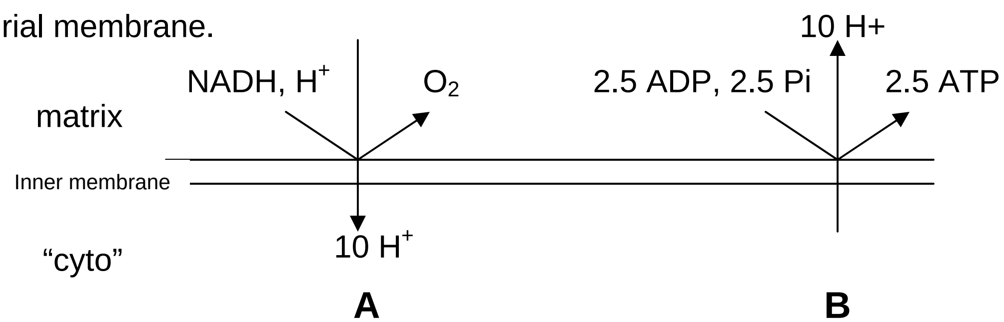
Answer True or False:

The tricarboxylic acid cycle:

______ exhibits rapid rates of flux in the presence of high levels of NADH.

______ exhibits rapid rates of flux in red blood cells due to high levels of oxygen.

7. [8 points] The following diagram illustrates two major processes involving the inner mitochondrial membrane.



Name the enzymes or pathways that catalyze the processes.

(A)________________________________ (B)___________________________________

Name a membrane-mobile component of process A, and explain how its redox properties relate to those of other components.

____

____

(continued next page)

The two processes (A and B above) are coupled, i.e. flux through each is dependent on flux through the other. Explain what this means.

____

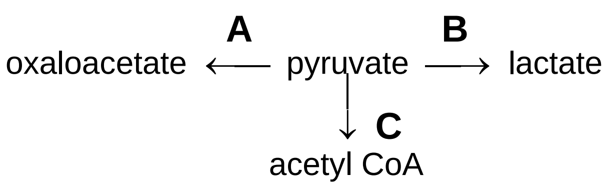
____

____

This interdependence has been shown using isolated mitochondria with plenty of the necessary metabolites present to support both processes. When a chemical that can inhibit process A is added, this results in loss of both ___________ and ___________ If an uncoupling chemical is then added, this results in ________________________

____

8. [7 points]



Pyruvate has many different fates in the cell, including the conversions shown in the partial reactions labeled A, B, and C in the diagram above. Indicate which reaction applies to the following statements:

______ requires NAD^+.

______ requires hydrolysis of ATP.

______ requires CoASH (= coenzyme A).

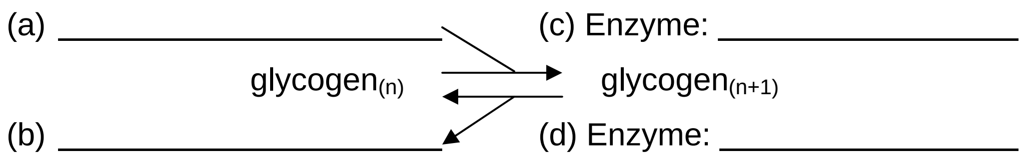
______ requires acetyl CoA.

______ marks a commitment to aerobic metabolism.

______ occurs primarily under anaerobic conditions.

______ can serve as an anaplerotic reaction.

9. [6 points] Fill in the blanks in the diagram below, i.e. name the substrate (a) or product (b) and the enzymes, (c) and (d),involved in extending and shortening the ends of non-reducing ends of glycogen chains.



Indicate the state of phosphorylation and activity of the two enzymes under conditions of insulin stimulation in the liver.

____

____

Name ____

INTD 5000 - Nitrogen Metabolism (Shiio)

Exam 3 - October 15, 2008

Choose the right words from the list to complete the sentences that describe nitrogen metabolism. (A word can be used more than once.) Write the number of your choice in the appropriate parenthesis.

1. The most important source of nitrogen for living organisms is ( ), which is converted to ( ) by ( ). ( ) is used by bacteria and plants to synthesize the amino groups of amino acids. The carbon skeletons of amino acids are derived from the following three sources: ( ), ( ), and ( ). Animals can synthesize only about half of 20 common amino acids in enough amounts. These amino acids are called ( ). The amino acids that need to be obtained from food are called ( ). In addition to the 20 common amino acids, some proteins contain uncommon amino acids such as 4-hydroxyproline, 6-*N*-Methyllysine, and desmosine. These are synthesized by ( ). The exception to this rule is ( ), which is incorporated during ( ).

1. ammonia
2. anemia
3. atmospheric nitrogen
4. γ-carboxyglutamate
5. CO_2
6. essential amino acids
7. fungi
8. glycerol
9. glycolysis
10. gout
11. ion channels
12. nitrogen-fixing bacteria
13. non-essential amino acids
14. pentose phosphate pathway
15. photosynthesis
16. porphyria
17. post-translational modification
18. second messenger
19. selenocysteine
20. sphingolipids
21. steroid hormones
22. TCA cycle
23. thermophilic bacteria
24. transcription
25. translation
26. urea cycle
27. vincristine

2. During degradation of amino acids, the amino groups are first collected in the form of (   ) by a reaction called (   ). Then (   ) releases its amino group as (   ). This reaction is called (   ). The product of this reaction is toxic to the brain and needs to be converted to (   ) or (   ) for transport to the (   ), where the (   ) generates the end product of amino group catabolism, (   ).

The carbon skeletons of amino acids are degraded to (   ) and (   ), which are then converted to (   ), (   ), or (   ).

In addition to proteins and degradation products described above, amino acids are precursors of the following biomolecules: (   ), (   ), (   ), (   ), (   ), (   ), and (   ).

1. acetyl-CoA
2. acyclovir
3. alanine
4. allopurinol
5. ammonia
6. CO_2
7. creatine
8. dopamine
9. fluorouracil
10. GFP
11. glucose
12. glutamate
13. glutamine
14. glutathione
15. HIV
16. hydrochloric acid
17. ketone bodies
18. kidney
19. liver
20. nitric oxide
21. nucleotides
22. oxidative deamination
23. porphyrin
24. RNAi
25. sodium hydroxide
26. spermidine
27. TCA cycle intermediates
28. transamination
29. transfection
30. urea
31. urea cycle
32. uric acid
33. wortmannin

3. Nucleotides have three characteristic components: ( ), ( ), and ( ). 4 main functions/roles of nucleotides are: ( ), ( ), ( ), and ( ).

There are two types of synthesis pathways for nucleotides: ( ) pathways begin with the metabolic precursors whereas ( ) pathways recycle the free bases and nucleosides.

The precursor of de novo purine nucleotide synthesis is ( ). ( ) provide the nitrogen atoms in this pathway. The first purine nucleotide to be synthesized in this pathway is ( ), which is then converted to ( ) or ( ).

De novo pyrimidine nucleotide synthesis has two precursors: ( ) and ( ).
( ) provides the sugar and phosphate. ( ) is a precursor of ( ) in this pathway.

The degradation product of purine nucleotides is ( ) whereas pyrimidine nucleotides are catabolized to ( ). Gout is caused by excess ( ).

1) acid
2. adenosine
3. amino acids (Gly, Gln, Asp)
4. AMP
5. aspartate
6. azotobacter
7. base
8. carbamoyl phosphate
9. components of enzyme cofactors
10. CTP
11. de novo
12. energy carriers
13. Gertrude Elion
14. GMP
15. IMP
16. interferon
17. Lesch-Nyhan syndrome
18. oxidative phosphorylation
19. pentose
20. phosphate
21. PRPP (5-phosphoribosyl 1-pyrophosphate)
22. recycle
23. reducing agents
24. salvage
25. second messengers
26. secretory
27. subunits of DNA and RNA
28. transcriptional coactivators
29. telomerase
30. urea
31. uric acid
32. UTP

Name ____

INTD 5000 - Lipids (Weintraub)

Exam 3 - October 15, 2008

PLEASE WRITE LEGIBLY. THERE IS PLENTY OF ROOM FOR EACH ANSWER. IF YOUR ANSWER ISN'T READABLE, YOU WON'T RECEIVE CREDIT FOR IT. FOR THE "SHORT-ANSWER" QUESTIONS, ONLY A SENTENCE OR TWO IS NEEDED—NOT AN ESSAY. PLEASE ANSWER THE QUESTION BEING ASKED AND DO NOT LIST EVERYTHING YOU KNOW ON THE TOPIC. - *thanks*

(1 pt)

1. a. In what direction do phospholipids move freely in cell membranes?

   b. What type of movement is extremely slow or does not take place for phospholipids in a membrane unless the process is mediated by an enzyme?

(1 pt)

2. How are the double bonds arranged relative to one another in naturally-occurring polyunsaturated fatty acids such as linoleic or arachidonic acid?

(1 pt)

3. Why is it necessary for lipids to be digested?

(1 pt)

4. Name one (1) essential fatty acid. Why is it "essential?"

(2 pts)

5. a. What must be done chemically to a fatty acid before it can be esterified in an enzymatically-controlled biological reaction?

   b. What is the driving force for the reaction?

(3 pts)

6. a. Name one (1) ketone body.

   b. How are ketone bodies *normally* utilized in the body?

   c. What is the interrelationship between ketone body formation and the citric acid cycle? In your answer, please indicate how the level of a specific TCA cycle intermediate influences the production of ketone bodies. (*Be sure to give the name of the TCA cycle intermediate, explain what happens to the level under certain metabolic states and why.*)

(3 pts)

7. Complete the following comparison between fatty acid synthesis and β-oxidation in eukaryotes. *Select your answers from the choices given below in italics.*

   a. Location
   (*cytosol, endoplasmic reticulum, nucleus, mitochondria, golgi apparatus, lysosome*)

   β-Oxidation:

   Fatty acid synthesis:

   b. State of intermediates
   (*free fatty acids, acyl-CoA, covalently bound to enzyme, triglycerides, phospholipids*)

   β-Oxidation:

   Fatty acid synthesis:

   d Enzyme form
   (*tightly-associated enzyme complex, dimer of identical multi-functional polypeptide chains, membrane-bound proteins, separate soluble proteins, ribosomal proteins*)

   β-Oxidation:

   Fatty acid synthesis:

(2 pts)

8. What critical role does malonyl-CoA play that links fatty acid synthesis with control of β-oxidation?

(4 pts)

9. For the following processes, (a) name the key enzyme(s) **regulated by phosphorylation/dephosphorylation** and (b) indicate whether the enzyme(s) is activated or inactivated by phosphorylation.

   a. fatty acid synthesis

   b. fatty acid liberation/β-oxidation

   c. cholesterol synthesis

   d. phosphatidyl choline synthesis

(2 pts)

10. What is the major role of the following lipoproteins? List what directs each to its target location?

    a. Chylomicrons

    b. VLDL

    c. LDL

    d. HDL

(2 pts)

11. a. What is the common non-hormone precursor to all mammalian steroid hormones?

b. What is the rate-limiting step in steroid hormone synthesis?

c. Where in steroidogenic cells do the major steps in steroid hormone synthesis take place?

d. Name one of two processes required for steroid hormone biosynthesis that is controlled by the level of intracellular cAMP in steroidogenic cells.

e. How do steroid hormones travel through the bloodstream?

f. From the following list, which word best describes the type of control exhibited by steroid hormones?

post-translational, migrational, longitudinal, transcriptional, recreational, translational

(3 pts)

12. a. What enzyme catalyzes the committed first step in the biosynthesis of prostaglandins, prostacyclins and thromboxanes?

b. List two catalytic activities associated with this enzyme.

c. What isoforms of this enzyme have been identified?

(question 12, cont.)

d. What chemical reaction takes place between aspirin and this enzyme?

e. How is the activity of this enzyme affected by the chemical reaction with aspirin?

f. Briefly explain why chronic treatment with a low dose of aspirin is beneficial in prevention of heart attacks and strokes.

(2 pts)

13. a. What two (2) second messengers are produced by the hydrolysis of phosphatidyl inositol-4,5-bis phosphate?

b. Name two (2) enzymes that are activated as a consequence of the actions of these second messengers.

(2 pts)

14. a. List two (2) short-term mechanisms for control of cellular levels of cholesterol in cells where cholesterol is not synthesized.

    b. List two (2) short-term mechanisms for control of cholesterol synthesis in the liver.

(2 pts)

15. Name four (4) proteins that are regulated together during long-term control of cholesterol biosynthesis.

    a.

    b.

    c.

    d.

(2 pts)

16. What aspect of control does carnitine palmitoyl transferase I (CPTI; fatty acid oxidation) have in common with the stAR protein (steroid hormone synthesis)?