

December 12, 2001

Name \_\_\_\_\_

ID number \_\_\_\_\_

## CH461/561 Final Exam

There are **12** multiple-choice questions worth **2** points each.

There are **6** short answer problems worth **5** points each.

You must answer **5** of them.

(If you attempt all, I will drop the one on which you score the lowest.)

There are **7** problems worth **18** points each.

If you are a CH461 student, you must answer **5** problems.

If you are a CH561 student, you must answer **6** problems.

(If you attempt all, I will drop the problem(s) on which you score the lowest.)

The maximum amount of points is thus **139** (CH461) or **157** (CH561).

You are advised to look over all the problems first before starting to work them. Time should not be a factor, if you are well prepared.

Multiple choice:		/24
Short answer:		/25
Problems:	1	/18
	2	/18
	3	/18
	4	/18
	5	/18
	6	/18
	7	/18
Total		

**No notes or books of any sort may be used during the exam.**

I have neither given nor received aid on this exam.

\_\_\_\_\_  
(signature)

1. One hundred mL of 0.1 M NaOH is added to 55 mL of 0.2 M lactic acid. (The  $pK_a$  of lactic acid is 4.1.) The resulting mixture has a pH close to:
- A. 2.
  - B. 3.
  - C. 4.
  - D. 5.
  - E. 6.
2. Thr and/or Leu residues tend to disrupt an  $\alpha$  helix when they occur next to each other in a protein because:
- A. of electrostatic repulsion between the Thr and/or Leu side chains.
  - B. both amino acids are highly hydrophobic.
  - C. the R group of neither amino acid can form a hydrogen bond.
  - D. of steric hindrance between the bulky Thr and/or Leu side chains.
  - E. of the possible covalent interactions between the Thr and/or Leu side chains.
3. Which of the following statements concerning the process of *spontaneous* folding of proteins is *false*?
- A. It may be an essentially random process.
  - B. It may involve initial formation of local secondary structure.
  - C. It may involve initial formation of a highly compact state.
  - D. It may involve a gradually decreasing range of conformational species.
4. Protein S will fold into its native conformation only when protein Q is also present in solution. However, protein Q can fold into its native conformation without protein S. Protein Q therefore, may function as a \_\_\_\_\_ for protein S.
- A. supersecondary structural unit
  - B. protein precursor
  - C. molecular chaperone
  - D. structural motif
  - E. ligand

5. Compare the following sequences of three separate regions taken from four different proteins, and select the answer that best characterizes their relationships:

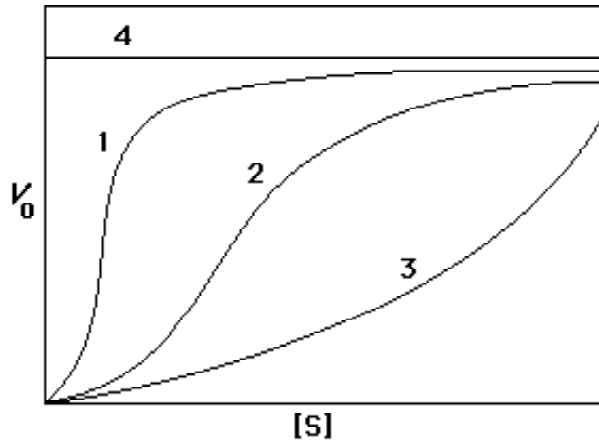
	A	B	C
1	DVEKGKKIDIMKCS	HTVEKGGKHKHTGPNLH	GLFGRKTGQAPGYSYT
2	DVQRALKIDNNLGQ	HTVEKGAKHKHTAPNVH	GLADRIAYQAKATNEE
3	LVTRPLYIFPNEGQ	HTLEKAAKHKHTGPNLH	ALKSSKDLMFTVINDD
4	FFMNEDALVARSSN	HQFAASSIHKNAPOFH	NLKDSKTYLKPVISET

- A. Protein 4 is the protein that shows the greatest overall homology to protein 1.
- B. The portions of amino acid sequence shown suggest that these proteins are completely unrelated.
- C. Based only on sequences in column B, protein 4 reveals the greatest evolutionary divergence.
- D. Comparing proteins 1 and 2 in column A reveals that these two proteins have diverged the most throughout evolution.
- E. Proteins 2 and 3 show a greater evolutionary distance than proteins 1 and 4.
6. The term *specific activity* differs from the term *activity* in that *specific activity*:
- A. refers to proteins other than enzymes.
- B. refers only to a purified protein.
- C. is measured only under optimal conditions.
- D. is the activity (enzyme units) in a milligram of protein.
- E. is the activity (enzyme units) of a specific protein.

7. A heterotropic modulator of an allosteric protein:

- A. is the same as its normal ligand.
- B. is not the same as its normal ligand.
- C. always increases the activity of the protein.
- D. always decreases the activity of the protein.
- E. does not affect the activity of the protein.

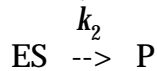
8. Below is a plot of  $V_0$  vs.  $[S]$  for a specific allosteric enzyme under different conditions:



Which of the following best describes the graph?

- A. Adding a positive modulator to #1 would result in curve 2.
- B. Curve 3 represents the effect of a negative modulator added to #2.
- C. Adding a positive modulator to #2 would result in curve 3.
- D. Curve 1 represents maximum inhibition.

9. For enzymes in which the slowest (rate-limiting) step is the reaction



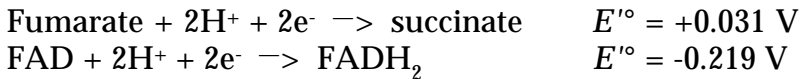
$K_m$  becomes almost equivalent to:

- A. the  $[S]$  where  $V_0 = V_{max}$ .
- B. the turnover number.
- C.  $k_{cat}$ .
- D. the dissociation constant,  $K_d$ , for the ES complex.
- E. the maximal velocity.

10. The conversion of 1 mol of fructose-1,6-bisphosphate to 2 mol of pyruvate by the glycolytic pathway results in a net formation of:

- A. 1 mol of  $NAD^+$  and 2 mol of ATP.
- B. 1 mol of NADH and 1 mol of ATP.
- C. 2 mol of NADH and 2 mol of ATP.
- D. 2 mol of NADH and 4 mol of ATP.
- E. 2 mol of  $NAD^+$  and 4 mol of ATP.

11. The standard reduction potentials ( $E'^{\circ}$ ) for the following half reactions are given.



If you mixed succinate, fumarate, FAD, and FADH<sub>2</sub> together, all at 1 M concentrations and in the presence of succinate dehydrogenase, which of the following would happen *initially*?

- A. Succinate would become oxidized, FAD would become reduced.
- B. Succinate would become oxidized, FADH<sub>2</sub> would be unchanged because it is a cofactor, not a substrate.
- C. Both fumarate and succinate would become oxidized; both FAD and FADH<sub>2</sub> would become reduced.
- D. Fumarate would become reduced, FADH<sub>2</sub> would become oxidized.
- E. No reaction would occur, because all reactants and products are already at their standard concentrations.
12. The enzyme acetate thiokinase (acetyl-CoA synthetase) along with pyrophosphatase cause this overall transformation:  

$$\text{acetate} + \text{ATP} + \text{CoA-SH} \rightarrow \text{acetyl-CoA} + \text{AMP} + 2 \text{P}_i$$
What is the most *plausible* sequence of events?
- A. ATP is the phosphate donor to activate both acetate and CoA-SH:  

$$\text{acetate} + \text{CoA-SH} + \text{ATP} \rightarrow \text{acetyl-P}_i + \text{CoA-S-P}_i + \text{AMP}.$$
Acetyl-CoA synthetase then catalyzes formation of acetyl-CoA:  

$$\text{acetyl-P}_i + \text{CoA-S-P}_i \rightarrow \text{acetyl-CoA} + 2 \text{P}_i.$$
- B. Acetyl-CoA synthetase catalyzes adenylation of acetate to activate it ( $\text{acetate} + \text{ATP} \rightarrow \text{acetyl-AMP} + \text{PP}_i$ ). The product is held tightly by the enzyme, which then catalyzes nucleophilic attack of the thiol of CoA-SH on the carbonyl carbon, with AMP as the leaving group ( $\text{acetyl-AMP} + \text{CoA-SH} \rightarrow \text{acetyl-CoA} + \text{AMP}$ ). Meanwhile, pyrophosphatase hydrolyzes pyrophosphate ( $\text{PP}_i + \text{H}_2\text{O} \rightarrow 2 \text{P}_i$ ) to make the first reaction irreversible.
- C. Acetyl-CoA synthetase catalyzes pyrophosphorylation of CoA to activate it ( $\text{CoA-SH} + \text{ATP} \rightarrow \text{AMP} + \text{CoA-S-PP}_i$ ). The product is held tightly by the enzyme, which then catalyzes nucleophilic attack of acetate on the S atom, with PP<sub>i</sub> as the leaving group ( $\text{CoA-S-AMP} + \text{acetate} \rightarrow \text{acetyl-CoA} + \text{AMP}$ ). Pyrophosphatase hydrolyzes pyrophosphate ( $\text{PP}_i + \text{H}_2\text{O} \rightarrow 2 \text{P}_i$ ) to make the first reaction irreversible.
- D. Acetyl-CoA synthetase catalyzes:  $\text{acetate} + \text{CoA-SH} \rightarrow \text{acetyl-CoA}$   
Meanwhile, pyrophosphatase hydrolyzes 2 phosphates from ATP ( $\text{ATP} + 2 \text{H}_2\text{O} \rightarrow \text{AMP} + 2 \text{P}_i$ ) and the free energy is beamed to acetyl-CoA synthetase via subspace frequencies.

## Answer Key

1. D
2. D
3. A
4. C
5. C
6. D
7. B
8. B
9. D
10. D
11. D
12. B



- 4) List 3 different methods used to **purify** proteins in significant quantities (i.e. not just analyze them) and briefly describe the principle each one uses to separate proteins.
- 5) Briefly describe why it is generally thought that the structure of a protein (and therefore its function) is determined solely by its amino acid sequence. Which experiment(s) provided support for this idea?
- 6) The following is a catabolic sequence:  $A \rightarrow B \rightarrow C$   
To reverse it, this anabolic sequence is used:  $C \rightarrow X \rightarrow Y \rightarrow A$   
A  $\rightarrow$  B is the first committed step of the catabolic sequence, and one of the two catabolic reactions is coupled to phosphorylation of ADP. Which one is it most likely to be and why? Would this enzyme be regulated? Should the anabolic sequence consume ATP? If so, at least how many (i.e. how many moles of  $ATP \rightarrow ADP + P_i$  per mole of A made)? Justify your answer.

## Problems

1) Draw out the reactions of **glycolysis** from glucose to pyruvate, using the next 2 pages of paper. You should draw the chemical structure of the intermediate **and** give its name for full credit. (You may draw carbohydrates in either the ring or open chain form, as you wish.) You should indicate the name of the enzyme that catalyzes each reaction by writing its name next to the arrow(s) representing the reaction. You must also indicate any co-substrates used during the reaction (such as H<sub>2</sub>O or CO<sub>2</sub>) or co-factors. For the latter, you may use the following abbreviations:

ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
GTP	guanosine triphosphate
GDP	guanosine diphosphate
GMP	guanosine monophosphate
P <sub>i</sub>	inorganic phosphate
PP <sub>i</sub>	pyrophosphate
NAD <sup>+</sup>	nicotinamide adenine dinucleotide (oxidized)
NADH	nicotinamide adenine dinucleotide (reduced)
FAD	flavin adenine dinucleotide (oxidized)
FADH <sub>2</sub>	flavin adenine dinucleotide (reduced)
CoA-SH	Coenzyme A





2) Draw out the reactions of the **TCA cycle**, and include the pyruvate dehydrogenase and pyruvate carboxylase reactions (i.e. "pyruvate partition"). You should draw the chemical structure of the intermediate **and** give its name for full credit. You should indicate the name of the enzyme that catalyzes each reaction by writing its name next to the arrow(s) representing the reaction. You must also indicate any co-substrates used/produced during the reaction (such as H<sub>2</sub>O or CO<sub>2</sub>) or co-factors (see problem 1 for abbreviations).

3) The researchers who originally traced out the glycolysis and TCA pathways used **metabolic tracers**. These are molecules that feed into the pathways and that are isotopically labeled at specific positions. Using your knowledge of these pathways, show what the products would be and which atoms would be labeled. If more than one atom/product is labeled, then indicate the percentage of labeling at that position. (e.g. if two positions are equally labeled, then put "50%" by each one.)

Starting compound(s)	Situation	Labeled product(s)
[ <sup>14</sup> C]-galactose labeled at C-3	Anaerobic fermentation to lactate	
[ <sup>14</sup> C]-succinate labeled on only one of the carboxylates	Aerobic metabolism in the presence of an inhibitor of $\alpha$ -ketoglutarate dehydrogenase	
Glucose & [ <sup>32</sup> P]-ATP labeled on $\gamma$ -P <sub>i</sub>	Glycolysis in presence of inhibitor of phosphoglycerate kinase	
citrate (unlabeled)	TCA cycle in presence of inhibitor of citrate synthase and [ <sup>18</sup> O]-H <sub>2</sub> O	
[ <sup>14</sup> C]-pyruvate labeled at C-3	Conditions that favor gluconeogenesis in liver. Only products excreted from the liver cells into the blood are collected and analyzed.	
[ <sup>14</sup> C]-pyruvate labeled at C-3	Conditions that favor aerobic catabolism in the presence of an inhibitor of succinyl-CoA synthetase	
[ <sup>14</sup> C]-pyruvate labeled at C-3	Conditions that result in the withdrawal of $\alpha$ -ketoglutarate as a starting point for other syntheses. In order to maintain this, the pyruvate partition is run such that pyruvate dehydrogenase and pyruvate carboxylase have similar rates.	( $\alpha$ -ketoglutarate)

4) **Gluconeogenesis and glycogen**

- (a) Which enzymes that you already drew in problem 1 and 2 are used by gluconeogenesis to convert 2 molecules of pyruvate to 1 molecule of glucose-6-P<sub>i</sub>? List them here:

Now draw the reactions that are unique to gluconeogenesis and name the enzymes used:

- (b) Draw the reactions that describe the synthesis of glycogen, using glucose-6-P<sub>i</sub> as a starting point. (Include the branching reaction schematically.) For the step in which a new residue is added to the glycogen chain, draw out the reaction mechanism for full credit.

(c) Draw the reactions that describe the breakdown of glycogen, using glucose-6-P<sub>i</sub> as an end point. (Include the debranching reaction schematically.)

(d) Briefly describe how the enzyme in parts (c) and (d) are coordinately regulated so that only one pathway is working at a time.

5) Based on the mechanism of pyruvate dehydrogenase, write out a plausible mechanism for the enzyme  **$\alpha$ -ketoglutarate dehydrogenase**. Draw out the reactions carried out; for full credit, you must draw out the structures of all covalent intermediates, including the functional parts of coenzymes. For each co-factor, write 1-3 sentences describing its properties that make it suitable for that particular reaction step.

- 6) **Analysis of reactions and mechanisms:**  
a) List every enzyme of glycolysis and the TCA cycle from problems 1 and 2. Classify the reaction that each enzyme catalyzes using the standard list (use the name; e.g. "lyase" rather than "4").

**International Classification of Enzymes\***

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group-transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

b) Look over your list of enzymes. Pick at least 3 pairs of enzymes in which the enzymes are catalyzing essentially the same kind of reaction (**not** the same class).

c) Pick any enzyme from your list in part (a) and write out a mechanism for this catalyzed reaction. It should incorporate as many facts as you know (from the course), but can also include plausible elements or steps based on good chemical sense and examples from other enzyme mechanisms.

7) **Drawing biomolecules**

In each case, pick a molecule that fits the description, draw its complete chemical structure and give its name.

a) a ribonucleoside triphosphate

b) a tri(deoxyribo)nucleotide  
(must have 3 different bases)

c) a phospholipid with a net charge of -1 at pH 7.0  
(draw as it would be at pH = 7.0)

- d)** a phospholipid containing an unsaturated fatty acid  
(indicate *cis* or *trans* and use different headgroup than in c)
- e)** an amino acid with a polar but uncharged side chain
- f)** a dipeptide with a net charge of +1 at pH 7.0  
(draw as it would be at pH = 7.0; both should be different from the one in e)