

Name _____

Score _____

Exam 3, BICH 440, Monday, November 20, 2006

You MUST sign the following academic integrity statement:

On my honor, as an Aggie, I have neither given nor received unauthorized aid on this academic work. Signed: _____

Write concise answers to demonstrate effectively your mastery of the subject. Show your work in order to receive maximum credit where applicable.

gas constant R 8.315 J/mol-K

Faraday constant F 96.5 kJ/mol-volt

- 1) (15 pts) Draw the mechanism of ribonuclease A. Show the structures of the phosphodiester backbone of RNA at atomic resolution, the structures of the sidechains of the important amino acid residues at the active site and the movement of electrons and protons during the mechanism.

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2) (5 pts) By what factor will a reaction at 25°C be accelerated if a catalyst reduces the activation free energy of the transition state by 10 kJ/mole?

3) (8 pts) A sample of highly-purified chromatin contains only double-stranded DNA and core histones in the amounts of 135 ng of DNA and 132 ng of core histones. What is the average spacing of nucleosomes on this DNA (i.e., the number of bp of DNA per core particle). Assume the following molecular weights of core histones: H2A: 14,000; H2B: 14,000; H3: 15,000; H4: 11,000.

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- 4) (15 pts) For the mechanism of chymotrypsin, draw the steps in the acylation phase (from the start to the formation of the acyl-enzyme intermediate). At the beginning of your diagram draw the entire side chains of the amino acid residues in the catalytic triad. For the remaining steps draw only the pertinent side chain structures involved in the chemistry of the reactions. Show the movement of electrons and protons in the reaction mechanism. DO NOT draw the entire reaction mechanism, just the ACYLATION phase (or you will lose some points).

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- 5) (15 pts) Penicillin is hydrolyzed and made inactive by the enzyme penicillinase that is found in some resistant bacteria. The molecular weight of this enzyme in *S. aureus* is 29,400. The amount of penicillin hydrolyzed in 1 minute in a 10 mL solution containing 10 ng of penicillinase was measured at various concentrations of penicillin:

| <u>[penicillin] (micromolar)</u> | <u>amount hydrolyzed (nmoles per minute)</u> |
|----------------------------------|--|
| 1 | 0.11 |
| 3 | 0.25 |
| 4 | 0.34 |
| 10 | 0.45 |
| 30 | 0.58 |
| 50 | 0.61 |

A) Using the graph paper on the next page, generate a double reciprocal plot of this data. Does penicillinase follow Michaelis-Menten kinetics? What is the K_m (units of Molar)?

B) What is the V_{max} (units of moles/min)?

C) What is the turnover number (k_{cat}) under these experimental conditions if there is one active site per enzyme molecule (units of sec^{-1})?

6) (15 pts) Given the following sequence of a double-stranded segment of DNA:

5' -CCGAATGTCGGAACTATAGCCTAGCTGGGGTTACACATCG-3'
3' -GGCTTACAGCCTTGATATCGGATCGACCCAATGTGTAGC-5'

Using an oligodeoxynucleotide primer of sequence: 5' -GTAACCCAGCTAGGC-3' for a dideoxy sequencing reaction with DNA polymerase, write the complete set of DNA fragments that are synthesized for each reaction to which the designated nucleotides are added. You do not have to write the entire primer sequence for each DNA fragment; just write "5'-primer" followed by the rest of DNA in that fragment.

- A) dATP, dGTP, dTTP, dCTP, plus a lower concentration of ddCTP
- B) dATP, dTTP, dCTP, ddGTP
- C) dATP, dTTP, dGTP, plus a lower concentration of ddTTP
- D) dATP, dGTP, dTTP, dCTP

7) (27 pts) Shorter answer questions.

A) (5 pts) Does lowering the Na^+ concentration of a solution of double-stranded DNA increase or decrease its melting temperature? Why?

B) (4 pts) Does the linking number of a DNA molecule increase, decrease, or stay the same when a segment is converted to Z-form DNA from B-form DNA, and the number of supercoils remains constant? EXPLAIN your answer.

C) (5 pts) For HIV protease, what is the quaternary structure of this enzyme? What residue(s) are essential for the catalytic mechanism? Name a specific competitive inhibitor of this enzyme.

D) (2 pts) What type of double-helical structure is found in the stem regions of the structure of transfer RNA?

E) (2 pts) Write an expression that describes the inhibition constant K_i in terms of the concentrations of E, EI, and I.

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F) (2 pts) Name two enzymes that are irreversibly inhibited by DIFP (diisopropylfluorophosphate).

G) (3 pts) Draw the structure of the covalent intermediate proposed to form between lysozyme and its substrate. You need to draw the entire sidechain of the amino acid and a very abbreviated version of the linked carbohydrate substrate.

H) (4 pts) Sketch the Lineweaver/Burk double-reciprocal plots (on one set of axes) for an enzyme with no inhibitor and with an uncompetitive inhibitor. Be sure to label the axes on your plot.