Name _____

MCB 102 MIDTERM EXAM I

Monday Oct. 1, 2001

REGRADE REQUESTS WILL NOT BE ACCEPTED IF THE ANSWERS ARE WRITTEN IN PENCIL OR IN ERASABLE INK

POINTS ARE ***SUBTRACTED*** FOR INCORRECT TRUE/FALSE AND MULTIPLE-CHOICE ANSWERS, SO IT IS BETTER TO NOT ANSWER THOSE QUESTIONS IF YOU ARE NOT SURE OF THE CORRECT ANSWER

Name _____

Student ID # _____

1. _____ (10)
2. _____ (20)
3. _____ (16)
4. _____ (5)
5. _____ (5)
6. _____ (5)
7. _____ (15)
8. _____ (4)
9. _____ (10)
10. _____ (10)

Total _____

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1. (10 points) Assume that the following oligopeptides were obtained from a full-length protein, and that the sequence of each oligopeptide was obtained by mass spectroscopy.

PEPTIDES PRODUCED BY CHYMOTRYPSIN	PEPTIDES PRODUCED BY V8 PROTEASE
M-K-A-P-Y	G-M-K-E
R-T-E-G-M-K-E	G-H-S-D
E-G-H-S-D-A-R-W	A-R-W-R-T-E
	M-K-A-P-Y-E

What is the sequence of the full-length protein?

M-K-A-P-Y - E-G-H-S-D-A-R-W - R-T-E-G-M-K-E

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2. (20 points) Propose explanations that account for each of the following facts that were observed during purification of a dehydrogenase that converts succinate to fumarate. ***Read the entire question and then go back and use the open spaces to explain or describe what information is provided by each statement.***

   Assume that the enzyme is NOT DAMAGED by any of the purification steps described below.

A two-step purification scheme is used that starts with (1) a sizing gel (i.e. gel filtration chromatography) and follows with (2) affinity chromatography [in which the coenzyme FAD is used as the immobilized ligand]. This scheme produces an enzyme preparation with a very high specific activity of ~150,000 units/mg.

- Thinking that the protein was almost 100% pure, it was subjected to four cycles of Edman degradation. This step resulted in the following yield of amino acids (all values are normalized to 1.0 lysine residues obtained in the first cycle). [Note: one must expect some experimental error, and some loss of material in successive cycles.]
  1. 2.1 glycine plus 1.0 lysine
  2. 0.9 alanine plus 1.9 aspartate
  3. 2.7 leucine
  4. 0.8 histidine plus 1.8 tryptophan

THE PURIFIED PROTEIN SEEMS TO CONSIST OF TWO POLYPEPTIDE CHAINS. THE N-TERMINAL SEQUENCES ARE G-D-L-W and K-A-L-H, RESPECTIVELY

THERE APPEARS TO BE TWICE AS MUCH OF ONE TYPE OF CHAIN AS THERE IS OF THE SECOND TYPE OF CHAIN

- For solutions containing 100 micrograms of protein per milliliter in both cases, the absorbance at 280 nanometers increased from 0.8 (for the sample prepared by the 2-step purification method) to 1.2 for a sample that was collected in the flow-through fraction [i.e. the material that was *not* retained on the column] of a **third** chromatographic step, which used ion-exchange chromatography on negatively charged beads.

ONE OF THE TWO PROTEINS IS POSITIVELY CHARGED, AND IS RETAINED ON THE COLUMN, WHILE THE OTHER PROTEIN IS NEGATIVELY CHARGED AND WAS NOT RETAINED ON THE COLUMN

THE PROTEIN THAT IS NOT RETAINED ON THE COLUMN APPEARS TO BE ENRICHED IN AROMATIC RESIDUES, COMPARED

Name _____

TO THE PROTEIN MIXTURE THAT WAS OBTAINED AFTER THE INITIAL, 2-STEP PURIFICATION METHOD

- The specific activity of the sample collected in the flow-through of this third step remained at ~150,000 units/mg, within experimental error.

ALTHOUGH SOME MATERIAL WAS REMOVED (i.e. RETAINED) DURING ION EXCHANGE CHROMATOGRAPHY), THE FACT THAT THE SPECIFIC ACTIVITY OF WHAT CAME OUT IN THE FLOW-THROUGH WAS NOT INCREASED SUGGESTS THAT THE MATERIAL RETAINED WAS ALSO AN ENZYME THAT HAD THE SAME SPECIFIC ACTIVITY FOR THIS REACTION. (This is not impossible, since the protein that was removed also binds to FAD during affinity chromatography, and thus it could be a related succinate dehydrogenase, but one with a distinct N-terminal sequence.)

- Explain whether you believe that anything further would be learned by repeating the N-terminal sequencing experiment with the fraction obtained in the flow-through from the ion-exchange chromatography step.

N-TERMINAL SEQUENCING SHOULD SHOW JUST ONE SEQUENCE AT THIS POINT.

IF THIS DOES HAPPEN, THEN N-TERMINAL SEQUENCING SHOULD ALSO SHOW WHICH OF THE TWO ENZYMES, WITH IDENTICAL SPECIFIC ACTIVITY, IS THE ONE THAT APPEARED IN THE FLOW-THROUGH OF THE THIRD STEP, AND WHICH ONE WAS RETAINED ON THE ION-EXCHANGE COLUMN

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3. (16 points) Write "T" to identify the statements that are true, and "F" to identify the statements that are false.

***If you think that a question is ambiguous*, <u>add a written note</u> to explain why you chose to answer it as either T or F.**

2 points for each correct answer, zero points for unanswered questions, and -2 points for incorrect answers*; it is better to leave a question unanswered than to guess the answer randomly*.]

__T_ Cholesterol is not only important as being one of the major types of lipid that is present in some types of cell membranes, it also is further metabolized to produce a range of hormones that regulate the expression of various kinds of genes.

__F_ Sequencing of the 3 billion base pairs in the human genome suggests that the combined biological activity of all human cell types, when these are added up, consists of more than 1 million types of chemical reactions.

__F_ Hydrolysis of a peptide bond produces two peptide groups (or amino acids) plus one molecule of water.

__T_ If a "typical" molecular weight of a protein is about 50,000 Dalton, then the length of a typical protein would be about 450 amino acid residues.

__F_ While many inorganic minerals are crystalline in structure, it is not possible for something as complicated as a protein to be crystallized.

__T_ Hemoglobin is a homodimer of protomers, and each protomer is a heterodimer.

__F_ The slope of the straight line on a Lineweaver-Burk plot (double reciprocal plot) provides a quantitative measure of the degree of cooperativity that is shown by a multi-subunit enzyme.

__F_ If a protein consists of two distinct, recognizable domains (e.g. each domain is folded according to a different motif), it is extremely unlikely that the primary structure that makes up just one domain would be able to fold to its native, 3-dimensional structure without the presence of the second domain.

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4. (5 points) Peptide bonds that involve a glycine residue are able to occupy a much larger range of torsion angles (i.e. ψ and ϕ) than is possible for all other amino acids. Explain what is different about glycine that might account for this fact.

GLYCINE DOES NOT HAVE A BETA-CARBON [OR, IT HAS ONLY A HYDROGEN ATOM FOR AN R-GROUP]. THIS SMALLER R-GROUP MEANS THAT THERE IS LESS TO GET IN THE WAY, i.e. LESS TO CAUSE STERIC HINDRANCE FOR ROTATION ABOUT THE ψ and ϕ TORSION ANGLES

5. (5 points) Identify all of the glycosidic bonds in the structure of ____ shown below.

6. (5 points) Draw (or write) the correct structure of the fatty acid that would be designated by the standard notation as $16{:}2(\Delta^{7,12})$.

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7. (15 points) *Mark all correct choices*. Some of the following may have no correct choices, and some may have more than one correct choice that you are expected to mark. *Negative points will once again be applied for incorrect responses*.

A. The rate of a given enzyme-catalyzed reaction depends upon $-\Delta G^{\ddagger}$ as follows:

____ linearly.

____ logarithmically.

__X__ exponentially.

B. The first committed step of a biochemical pathway is likely to be carefully regulated because this regulation

__X__ can allow the reaction to continue at the appropriate rate, even when the substrate concentration has increased or decreased.

__X__ prevents the wasteful synthesis of molecules that are intermediates compounds in the pathway, in the event that there is already enough of the final product.

____ will increase the rate of the reaction by the mechanism of feedback inhibition, when the amount of final product falls to a low level.

C. Identify which of the following are known to represent biological roles of carbohydrate molecules:

____ used to form polymers, such as glycogen, that provide long-lasting supplies (days or weeks) of energy

__X__ allosteric ligands

__X__ used as an element in nucleic acid structure

__X__ enzymatically added to certain membrane proteins, i.e. used to form a covalent modification of the protein

____ function as coenzymes, i.e. provide active-site (i.e. catalytic) residues

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8. (4 points) Give an example of a kind (i.e. type) of covalent modification (of the structure of a newly synthesized protein) that is used quite frequently to activate an enzyme, in which the covalent modification is not reversible.

PROTEOLYSIS IS USED TO ACTIVATE ZYMOGENS

9. (10 points) The cartoon below indicates residues in the primary structure of a protein that have been identified (by a 2-D NMR experiment) to be in physical contact with each other, i.e. to be 4A apart. In other words, one square is in contact with the other square, one circle is in contact with the other circle, etc.

have THREE squares and 3 circles

Make a drawing ("cartoon") of one possible way in which the protein must be folded up, such that your proposed "3-dimensional" structure would account for the distance constraints that had been obtained from the NMR experiment.

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10. (10 points) Use the coordinate axes of a graph provided here to make a sketch of what the Hill model would predict a plot of experimental data should look like for cooperative binding of a ligand, "L", to a protein with five subunits (i.e. a symmetrical pentamer). *Be sure to specify the value of the slope of any linear regions on this graph.*

Identify 2 distinct ways in which real experimental data are likely to differ from the prediction of Hill's model of cooperativity, when they are plotted on this graph.

1. THE SLOPE OF THE HILL PLOT IS LIKELY TO BE 1.0 AT THE EXTREME ENDS OF THE RANGE OF SUBSTRATE CONCENTRATION (i.e. VERY LOW AND VERY HIGH SUBSTRATE CONCENTRATION)
2. EVEN IN THE MIDDLE OF THE RANGE, WHERE THE SLOPE IS THE MOST STEEP, IT IS LIKELY THAT THE HILL CONSTANT WILL BE LOWER THAN 5, IN FACT IT IS LIKELY TO BE 4 (OR LESS)
3. ALSO ACCEPT AN ANSWER THAT SAYS THE SLOPE DOES NOT NEED TO BE AN INTEGER