MCB 102 MIDTERM EXAM I

Tues. Sept. 30, 2003

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

POINTS ARE ***SUBTRACTED*** FOR INCORRECT TRUE/FALSE ANSWERS, SO IT IS BETTER TO NOT ANSWER AT ALL WHEN YOU ARE NOT SURE OF THE CORRECT ANSWER

Name _____

Student ID # _____

1. _____ (10)
2. _____ (4)
3. _____ (4)
4. _____ (16)
5. _____ (12)
6. _____ (6)
7. _____ (6)
8. _____ (15)
9. _____ (7)
10. _____ (8)
11. _____ (12)

Total _____

Name _____

1. (10 points) *Classify each position* in the aligned amino acid sequences (i.e., primary structures) below, according to the <u>most likely</u> of the following possibilities:
   I - invariant residue
   C- only conservative substitutions are possible
   V - widely variable types of amino acids are tolerated at these positions

Position	1	2	3	4	5	6	7	8	9	10
Class	V	V	C	I	I	C	V	C	V	C
Human	**S**	**Q**	**L**	**C**	**H**	**T**	**V**	**E**	**K**	**N**
Mouse	**A**	**L**	**I**	**C**	**H**	**T**	**I**	**E**	**K**	**Q**
Tuna	**E**	**E**	**V**	**C**	**H**	**S**	**E**	**D**	**P**	**N**

2. (4 points) The relative molecular weight (M_r) of myoglobin is about 16,700. *Approximately how many amino acids* are in this polypeptide chain? *Show how you obtained your answer.*

16,700/110 = ~152

3. (4 points) *Draw the structure* of the dipeptide formed by two alanine molecules. *Explain* why cleavage of this peptide bond by gas-phase collision (as in peptide sequencing by mass spectroscopy) produces fragments that *do not* have the same molecular weight.

Name _____

4. (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 -1 points for incorrect answers*; it is better to leave a question unanswered than to guess the answer randomly*.]

__T_ *Phenylisothiocyanate* is the key reagent that is used in the scheme developed by Edman for N-terminal sequencing of polypeptides.

__F_ The "globin fold" includes the commonly occurring "β – α – β Loop" motif as a small portion of the full tertiary structure of the protein.

__F_ Ribosomes are considered to be "soluble particles" because they remain in the supernatant fraction after centrifugation for 3 hours at 150,000g.

__F_ The "phi" and "psi" angles used in the Ramachandran plot refer to rotation about the (amide-nitrogen) - Cα bond and the (carbonyl-carbon) - (amide-nitrogen) bond, respectively.

__T_ Starch (an important dietary source of glucose) and cellulose (e.g., wood) are both built from glucose monomers, but with a stereochemically different glycosidic linkage.

__T_ The aldehyde group of glucose forms an intramolecular hemiacetal during the formation of α-D-Glucopyranose.

__T_ Some molecules that are related to fatty acids and other molecules that are related to cholesterol *both* play important roles as hormones or signaling molecules.

__T_ When the phosphatidylinositol 4, 5-bisphosphate is hydrolyzed by phospholipase C, the hydrolysis products serve as potent signaling molecules within cells (intracellular messages).

Name _____

5. (12 points) Pyruvate dehydrogenase is a protein complex that is made up of three different polypeptide chains that co-purify with each other in a mole ratio of 2:2:1. Although the molecular weights of each of the three polypeptide chains are not identical, assume that each one is approximately 30,000 +/- 5,000 Da.

   Rather surprisingly, the active enzyme complex is found in the flow through fraction during size exclusion chromatography with polymer beads that have a molecular weight cut off of 800,000.

   A. *Explain why one might have expected the protein to elute from this column much later, rather than appearing in the "flow through" fraction.*

THE SIMPLEST 2:2:1 COMPLEX WOULD HAVE A M_r = 150,000 WHICH IS SMALL ENOUGH THAT THE PROTEIN COMPLEX SHOULD BE RETAINED IN A SIZE EXCLUSION GEL WITH A CUT OFF OF 800,000

   B. *Propose a simple explanation for the fact that the enzyme complex is not retained during size exclusion chromatography.*

GIVE FULL CREDIT FOR ANSWERS THAT SUGGEST EITHER:

- THE COMPLEX MUST CONSIST OF 6 OR MORE COPIES OF THE BASIC 2:2:1 COMPLEX
- THE 2:2:1 COMPLEX HAS SUCH A LONG, ROD-LIKE SHAPE THAT IT IS NOT RETAINED BY THE PORES OF THE STATIONARY PHASE MATRIX, EVEN THOUGH ITS MOLECULAR WEIGHT IS LOW ENOUGH THAT A MORE GLOBULAR PROTEIN OF THE SAME MOLECULAR WEIGHT WOULD HAVE BEEN RETAINED

   C. Once this protein complex has been purified to homogeneity, *how many bands will be seen in an SDS gel electrophoresis experiment?*

THREE

Name _____

6. (6 points) *Complete the following sentences* by filling in the blank spaces:
   a) In order to obtain an x-ray diffraction pattern for a newly purified protein, it is first necessary to grow __CRYSTALS__ of the protein.
   b) Each type of protein produces its own unique pattern of ___DIFFRACTION SPOTS ***or*** BRAGG REFLECTIONS ___ when it is illuminated by a monochromatic, collimated x-ray beam.
   c) Mathematical computations transform this pattern into a density map, which must be ___INTERPRETED___ by building an atomic model of the protein that fits all of the observed features of the density map.
   d) Information about which amino acids in a native protein structure (fold) are very close to one another is contained in the peaks that are off the ___ DIAGONAL___ in a 2-dimensional NMR spectrum.
   e) The larger the number of [**circle the correct answer**] DISTANCE CONSTRAINTS / PSI AND PHI ANGLES / H-BONDED INTERACTIONS that one is able to obtain by NMR spectroscopy, the better one is able to build a model of the 3-dimensional structure of a protein (i.e. in the way that Prof. Glaeser demonstrated in lecture with a piece of rope.)
   f) The native, folded state of a protein is said to be only *marginally stable* because the change in free energy when the polypeptide chain goes from the unfolded to the folded structure is only about __-20 to -60 kJ/mole__.

7. (6 points) Use the known values of the Hill constant to decide which of the following "formal" chemical equations

   a) $Hb + 2.5O_2$ $Hb\text{-}(O_2)_{2.5}$

   b) $Hb + 3O_2$ $Hb\text{-}(O_2)_3$

   c) $Hb + 4O_2$ $Hb\text{-}(O_2)_4$

   provides the closest approximation to the oxygen-binding data for hemoglobin (Hb)

   - at "physiological" concentrations of bisphosphoglycerate (BPG)

   b)

   - when all BPG has been removed

   a)

Name _____

8. (15 points) All chemical reactions in cells are catalyzed, and the catalytic activity is very often regulated.

A. If K_M of an enzyme that obeys Michaelis-Menten kinetics is *increased* by (reversible) phosphorylation of a particular serine residue, will the specific activity of the enzyme be increased or decreased? *Explain.*

ACTIVITY IS PROPORTIONAL TO $[S]/\{K_M + [S]\}$
THUS, IF K_M INCREASES, THEN ACTIVITY MUST DECREASE. AND IF
ACTIVITY DECREASES, SO WILL THE SPECIFIC ACTIVITY

B. Explain why it is valuable for aspartate transcarbamolyase to be a "smart enzyme" in the sense that it can be both activated by ATP and inhibited by CTP. [*Explain* what purpose is served by activation in the one case and what purpose is served by inhibition in the other case.]

ACTIVATION BY HIGHER THAN NORMAL LEVELS OF ATP, A PURINE, TELLS ATCase THAT IT IS A GOOD IDEA TO INCREASE PYRIMIDINE BIOSYNTHESIS (to catch up with the high levels of purine synthesis, or to take advantage of the high availability of energy)

INHIBITION BY CTP TELLS THE ENZYME THAT IT IS MAKING MORE CTP THAN THE CELL IS ACTUALLY USING, SO IT WOULD BE A GOOD IDEA TO SLOW DOWN

C. *Explain* why the enzyme that catalyzes the first committed step of a pathway (such as "threonine dehydratase" or "ATCase") normally must be allosterically inhibited rather than competitively inhibited by the final product of that pathway.

THE FINAL PRODUCT (MOLECULE) NORMALLY WILL HAVE LITTLE OR NO STEREOCHEMICAL SIMILARITY TO THE INTIAL REACTANTS, AND THUS THE FINAL PRODUCT (MOLECULE) WOULD NOT INTERACT WELL ENOUGH WITH THE ACTIVE SITE TO BE AN EFFECTIVE, COMPETITIVE INHIBITOR

Name _____

9. (7 points) *Draw* the transition state structure that is proposed to be stabilized after the active-serine oxygen atom (in chymotrypsin and other serine proteases) attacks the carbonyl carbon of a polypeptide substrate molecule. *Be sure to show (or explain) how the energy required to form this tetrahedral oxyanion transition state structure* is decreased due to (a) favorable electrostatic interactions with the protonated form of an acid-base residue and (b) formation of at least one new H-bond that is not formed by the initial substrate molecule.

10. (8 points) Carbohydrates

A. *Draw the structures of* glyceraldehyde and dihydroxyacetone.

B. *Draw the structure of maltose*, and indicate the (α 1 4) glycosidic bond that links the two glucose molecules together to make this disaccharide.

Name _____

11. (12 points) Lipid bilayers make it possible to establish fairly stable differences in ion concentration, pH differences, voltage differences, etc. between the inside and the outside of a cell.

A. *Explain* why phospholipid molecules such as dipalmitoyl phosphatidylcholine are well-suited to form such permeability barriers.

THE LIPID BILAYERS THAT ARE MADE BY SUCH MOLECULES HAVE A RELATIVELY THICK (i.e. ~30A THICK), CONTINUOUS LAYER OF NON-POLAR MATTER. THE ENERGY-COST FOR IONS OR POLAR SOLUTES TO ENTER THIS HYDROPHOBIC LAYER IS SO GREAT THAT THEY WILL DIFFUSE ACROSS THAT LAYER ONLY VERY RARELY

B. *What fundamental difference* must there be in the primary structure of an "all-α " membrane protein, such as a pump (e.g., bacteriorhodopsin) or a permease, as compared to an all-α, globular (soluble) protein? *How is this fundamental difference used* to identify genes that are likely to code for membrane proteins? [Hint: What is unique about the hydropathy profile of an all-α membrane protein?]

TRANSMEMBRANE HELICES ARE GENERALLY MADE UP OF A STRING OF 20 OR SO HYDROPHOBIC, OR AT LEAST RELATIVELY NON-POLAR AMINO ACIDS. THESE REGIONS OF THE PRIMARY STRUCTURE CAN BE QUITE EASILY RECOGNIZED IN A PLOT (GRAPH) OF THE AVERAGE HYDROPHOBICITY OF A SMALL STRETCH OF THE PRIMARY STRUCTURE.

THE HELICES IN GLOBULAR PROTEINS, ON THE OTHER HAND, ARE FREQUENTLY *AMPHIPATHIC*, i.e. POLAR ON ONE SURFACE AND HYDROPHOBIC ON THE OTHER SURFACE, AND AS A RESULT THEY DO NOT STAND OUT IN ANY WAY WHEN THE SEQUENCE IS SHOWN ON SUCH A *HYDROPATHY PROFILE*