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MCB 102 MIDTERM EXAM I

Feb. 26, 2001

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

1. (16 points) Use the following table to draw the specified structures and to describe their biochemical functions.

	Draw the Molecular Structure	Describe **one** Role or Function of the underlined item, as it was discussed in class-lecture
The side chain (R-group) of histidine, unprotonated		1. THE RESIDUE ON HELIX F OF GLOBINS THAT BINDS THE HEME GROUP 2. GROUP THAT IS PROTONATED (BY "BOHR-EFFECT" PROTONS) IN THE T-to-R CONFORMATIONAL CHANGE OF HEMOGLOBIN 3. ONE OF THE RESIDUES IN THE CATALYTIC TRIAD OF CHYMOTRYPSIN
The peptide bond, ***showing all atoms and the bond angles that are designated as ϕ and ψ*** in a Ramachandran plot		1. THIS IS THE BOND THAT CONNECTS SUCCESSIVE AMINO ACIDS TOGETHER, TO CREAT THE LINEAR POLYMER, i.e. THE PRIMARY STRUCTURE OF A PROTEIN
Fructose		1. FRUCTOSE (IN THE FORM OF FRUCTOSE 6-PHOSPHATE) IS A KEY INTERMEDIATE FORMED DURING GLYCOLYSIS

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Palmitic acid		1. ONE OF THE FATTY ACIDS THAT ARE COMMONLY FOUND IN TRIGLYCERIDES (FATS) AND IN MEMBRANE LIPIDS 2. A SOURCE OF "FUEL" IN ENERGY-METABOLISM

2. (4 points) Identify the *class of protein fold* that is appropriate for each of the structures shown below. Examples of the class of fold would be α/β, or $\alpha + \beta$.

Rhodopsin, the light-absorbing protein responsible for vision ___ALL ALPHA _____
[A retinal molecule is shown in the middle of the protein]

Fab, the antibody-binding domain of immunoglobulin G (IgG) ____ ALL BETA _____

3. (26 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*, add a written note 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_ The number of chemical equations that is needed to describe a living organism can be estimated by determining the number of genes contained in its DNA.

_T_ Every copy of a protein such as myoglobin or chymotrypsin, when in its native-state, is folded up very precisely into the same 3-dimensional structure.

_F_ Tyrosine, Tryptophan, and Histidine are the only amino acids in proteins that absorb light in the "near-ultraviolet", i.e. at a wavelength of about 280nm.

_F_ The relative molecular weight, M_r, of a protein can be estimated to within an accuracy of +/- 20% by the formula 150x(number of amino acids).

_F_ A change in pH is expected to have the same effect (called the Bohr effect) on O_2-binding in hemoglobin and on myoglobin, but the effect is irrelevant for myoglobin because the tissue pH is almost constant.

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_F_ The Hill model, the concerted model, and the sequential model of cooperative binding of O_2-binding are all able to explain why the slope of a Hill plot is ~3 for hemoglobin.

_F_ Phosphatidylcholine is an important source of intracellular signal-molecules, as well as serving a structural role when forming the lipid bilayer of the cell membrane.

_F_ Prostaglandins are an important class of steroid hormones that are derived from cholesterol.

_F_ The amount of free energy released from the hydrolysis of a thioester bond, such as the one that is present in acetyl-CoA, is less than that released by the hydrolysis an oxygen ester bond, such as the ones that are present in triglycerides (e.g. dietary fats).

_T_ Many of the enzymes that remove electrons from a substrate molecule are classified as dehydrogenases.

_T_ The electron carrier FAD uses exactly the same fused ring structure to carry out electron transfer reactions as does the electron carrier FMN.

_T_ The appearance of peaks at off-diagonal positions in a 2-D NMR spectrum provides quantitative information about the distances between individual pairs of atoms, and observing enough such off-diagonal peaks ultimately allows one to tell how a protein is folded up in space.

_T_ X-ray diffraction spots (i.e. patterns of diffraction intensities) represent the raw data from which one can calculate a "picture" of the molecule that has been crystallized.

4. (6 points) Draw structures of the following:

A. The hemiacetal form of N-acetyl glucosamine.
B. Two molecules of glucose joined together by an (α 1 $\rightarrow$ 4) O-glycosidic bond.

5. (16 points) Two proteins, whose relative molecular weights (M_r) are 190,000 and 210,000 respectively, seemed to be inseparable from one another by all of the techniques that Prof. Glaeser used during his most recent effort to purify a new steroid hormone receptor, a protein suspected of playing a key role in expressing genes related to the normal development of muscle tissue [actually, he is making this up].

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A. Both proteins remained in the supernatant after very high-speed centrifugation, even though things like small membrane vesicles were then in the pellet. *Explain* what that result tells us about the proteins.

THEY ARE SO-CALLED SOLUBLE PROTEINS (OR AT LEAST A SMALL COMPLEX OF SOLUBLE PROTEINS), AND THEY ARE TOO SMALL TO BE BROUGHT DOEWN INTO THE PELLET BY THESE CONDITIONS OF CENTRIFUGATION

B. 2-D gel electrophoresis showed that the two proteins had almost identical isoelectric points. Explain why that fact made it less likely that there would be any buffer-pH at which they could be separated by ion-exchange chromatography.

SINCE THEY HAVE THE ;SAME ISOELECTRIC POINT, THE TWO PROTEINS MUST BOTH BE EITHER POSITIVELY CHARGED OR NEGATIVELY CHARGED (THE SIGN OF THE CHARGE DEPENDING UPON THE pH, OF COURSE), AND THEY WOULD STICK (OR NOT) TO AN ION-EXCHANGE RESIN UNDER THE SAME CONDITIONS.

C. Explain how it could be that both proteins appeared in the flow-through fraction during size exclusion (gel filtration) chromatography when beads with an M_r = 300,000 cutoff were used, but they appeared together in the same eluted-fraction when beads with an M_r = 500,000 cutoff were used.

THE PROTEINS MUST BE DIMERS, SINCE MONOMERS WOULD NOT APPEAR IN THE FLOW-THROUGH WITH BEADS HAVING AN Mr = 300 CUTOFF, WHILE TRIMERS OR HIGHER WOULD SHOP UP IN THE FLOW-THROUGH OF THE BEADS HAVING AN Mr = 300 CUTOFF.

NOTE, FOR THE NEXT QUESTION HOWEVER, THAT THE Mr VALUES OF THE TWO PROTEINS ARE TOO CLOSE TO ONE ANOTHER TO TELL IF THE DIMERS ARE MONODIMERS OR HETERODIMERS

D. Finally, however, when affinity chromatography was used (with the hormone used as the immobilized ligand), the M_r = 210,000 protein was retained on the column, while the M_r = 190,000 protein washed out in the flow-through. *How can you reconcile this result* with the information from the experiments described in A, B, and C, above? (Assume that the native structure of the proteins is retained under all conditions used for purification!)

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BOTH THE M_r = 210,000 PROTEIN AND THE M_r = 190,000 PROTEIN MUST HAVE BEEN PRESENT IN THE FORM OF HOMODIMERS, WITH IDENTICAL ISOELECTRIC POINTS, IMPROBABLY THOUGH THAT MAY SEEM. THE M_r = 210,000 PROTEIN (AS A HOMODIMER) MUST BE THE STEROID HORMONE RECEPTOR, AND IT IS JUST A COINCIDENCE THAT THE M_r = 190,000 PROTEIN HAS SIMILAR PROPERTIES. THE POSSIBILITY THAT THE TWO PROTEINS WERE CO-PURIFYING BECAUSE THEY FORMED A HETERODIMER IS "RULED OUT" BY THE FACT THAT THE M_r = 190,000 PROTEIN IS NOT RETAINED ON THE AFFINITY COLUMN UNDER NATIVE CONDITIONS.

6. (5 points) Competitive inhibitor molecules are molecules that bind very specifically to the active site of an enzyme but then do not complete the reaction that is normally catalyzed there. In this way they block (i.e. prevent) the enzyme from carrying out its usual function.

Which would be a more effective inhibitor of lipase (the enzyme that releases fatty acids from triglycerides): (A) an analog of triglycerides in which the hydrocarbon chains are connected to the glycerol group by a nonhydrolyzable ether linkage, or (B) a molecule in which the linkage involves a phosphonate, which is an analog of the transition state in the hydrolysis of an ester. *Explain your answer*.

THE MOLECULE THAT IS AN ANALOG OF THE TRANSITION STATE WOULD BE A MORE EFFECTIVE INHIBITOR BECAUSE AN ENZYME BINDS THE TRANSITION STATE MORE STRONGLY THAN IT BINDS EITHER THE SUBSTRATE OR THE PRODUCT.

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7. (9 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. A *primary-structure* motif known as a leucine zipper consists or amino acid sequences in which conserved Leu or Ile residues occur once every 6 or 7 residues, e.g. such a motif might look like …xLxxxxxxLxxxxxxLx…. , where "x" can be any amino acid.

+1/0 __**X**_ If such a sequence forms an alpha-helix, these leucine residues can all be roughly on one side of the helix

+1/-1

____ If such a sequence forms a strand of a beta-sheet, these leucine residues can all be on the same side of the sheet (ignore the matter of the twist of the beta sheet)

+1/-1

____ When this *primary-structure* motif is used to form a single alpha-helix, the distance between successive hydrophobic residues will be 5.4 Angstroms.

B. Aspartate transcarbamoylase (ATCase) is made up of 6 protomer units, each of which is a heterodimer consisting of one catalytic subunit and one regulatory subunit. ATCase catalyzes the first committed step in the pyrimidine biosynthetic pathway, and its activity is inhibited by a final product (CTP) of that pathway.

+1/0 __**X**_ Feedback inhibition occurs commonly among the enzymes that catalyze the first committed steps in metabolic pathways.

+1/-1

____ High concentrations of the inhibitor, CTP, will cause the Hill coefficient (for binding of aspartate to ATCase) to be less than one.

+1/-1

____ It is expected that ATCase will more nearly obey Michaelis-Menten kinetics in the presence of CTP, i.e. the kinetics will be more like a hyperbolic curve than they are without CTP.

C. The expression "the catalytic triad" of chymotrypsin refers to:

+1/-1

____ three amino acid R-groups that approach the peptide bond from three different sides, so as to each lower the activation barrier for hydrolysis

+1/-1

____ the three intermediate steps in the hydrolysis of a peptide bond

+1/-1 ____ the three polypeptide chains that assemble to form the active, quaternary structure of the enzyme

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8. (6 points) $\Delta G'^{0}$ for hydrolysis of ATP to ADP and P_i is -30.5 kJ/mole. Is the Gibbs free energy ΔG available to *E. coli* cells from this hydrolysis (a) at least 5 kJ/mole more negative than this value, (b) within +/- 5 kJ/mole of this value, or (c) at least 5 kJ/mole more positive than this value? Assume that the pH is 7.0, [ATP] = 7.9mM, [ADP] = 1.0mM, and $[P_i]$ = 7.9mM. *Explain your answer.*

$\Delta G = \Delta G'^{0} + RT \ln \{ [ADP] [P_i] \} / [ATP]$

$= -30.5$ KJ/mole $+ RT \ln 10^{-3}$

$= -30.5$ KJ/mole $- 3\ RT \ln 10$

ΔG AVAILABLE TO E.COLI IS MORE NEGATIVE THAN $\Delta G'^{0}$, BY MORE THAN 3RT = ~7.5 KJ/MOLE. THE CORRECT ANSWER IS THEREFORE (a).

9. (6 points) Lactic acid is generated in muscle tissue during intense activity, when the amount of oxygen available is limited. The reaction that produces lactate is

$$\text{pyruvate} + \text{NADH} + H^+ \qquad \text{lactate} + NAD^+$$

Calculate $\Delta G'^{0}$ for the formation of lactate from pyruvate; numerical values needed to do this calculation are given on the last page of the exam.

USE THE FOLLOWING INFORMATION FROM THE ACCOMPANYING TABLE:

$PYRUVATE + 2\ H^+ + 2\ e^-$ _____> LACTATE $E'^{0} = -0.185$

NADH _____> $H^+ + 2\ e^- + NAD^+$ $E'^{0} = +0.320$

ADD THE TWO HALF-CELL REACTIONS TO OBTAIN

PYRUVATE + NADH + H^+ _____> LACTATE + NAD^+ $E'^{0} = +0.135$

THEN CALCULATE:

$\Delta G'^{0} = -n\ F\ E'^{0} = -$ 2x100KJ/V-mole x 0.135 volt

$= -27$ kJ/mole

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10. (6 points) The concentration of 2,3-bisphosphoglycerate (BPG), an allosteric modulator of oxygen binding, remains constant inside of the hemoglobin-carrying red blood cells as they circulate back and forth between muscle tissue and the lungs.

A. *Identify* (1) a heterotropic modulator of hemoglobin, other than BPG, and (2) a molecule that is responsible for reversible covalent modification of hemoglobin, whose concentrations *do change* between the two environments (i.e. muscle and lung).

(1) = HYDROGEN IONS

(2) = CARBON DIOXIDE

B. Even though the concentration of BPG remains constant, the amount of BPG bound to hemoglobin is not the same when a red blood cell is circulating through muscle tissue and when it is in the lung. *When is more BPG bound to hemoglobin? Explain* why that is the case. {Hint: it may help to use the concerted model when you give your explanation.]

BPG CAN ONLY BIND TO HEMOGLOBIN WHEN IT IS IN THE "T" (DEOXY) STATE. SINCE THE NUMBER OF MOLECULES IN THE T STATE WILL BE MUCH HIGHER WHEN THE RED BLOOD CELL IS CIRCULATING THROUGH MUSCLE TISSUE, IT FOLLOWS THAT THERE WILL ALSO BE MORE BPG BOUND TO HEMOGLOBIN AT THAT TIME. ON THE OTHER HAND, WHEN THE RED BLOOD CELL REACHES THE LUNG, THE HEMOGLOBIN WILL BIND OXYGEN (BECAUSE OF THE HIGH CONCENTRATION OF DISSOLVED OXYGEN THAT IS PRESENT IN THE LUNG), THEREBY INCREASING THE FRACTION OF HEMOGLOBIN MOLECULES THAT ARE IN THE "R" (OXY) STATE. THE AMOUNT OF BPG THAT CAN BE BOUND TO HEMOGLOBIN WHILE THE RED BLOOD CELL IS IN THE LUNG WILL THEREFORE BE PROPORTIONATELY REDUCED.

[THE POINT OF THIS QUESTION IS TO SHOW THAT (1) MOLECULAR OXYGEN, A GAS AND (2) BOHR PROTONS BOTH ACT AS HETEROTROPIC MODULATORS OF THE BINDING OF BPG, AT CONSTANT CONCENTRATION OF BPG; CABRON DIOXIDE, ON THE OTHER HAND, MODULATES THE BINDING OF BPG THROUGH REVERSIBLE COVALENT MODIFICATION OF THE PROTEIN.]