

Name: _____

Biochemistry Test 2

In class portion

1. (10 points) X-ray crystallography and NMR are the two major techniques used to determine protein three dimensional structure. Compare these methods in the following areas: Size of protein, physical state of protein when structure is determined, how a model of the protein is fit to the experimental data.

X-ray - Can be applied to proteins of any size, including intact virus. Is performed on crystals of the protein so you are dealing with solid phase. Through the diffraction experiment you derive an electron density map, and the model fitting occurs as you move models of amino acids to fit the electron density of the 'heavy' atoms in that amino acid.

NMR - Can only be applied to protein of 300 residues or less. Is performed in the aqueous phase, so you are dealing with a protein in solution (usually a VERY concentrated solution). The data derived from NMR is distanced between protons, so the model fitting involved moving amino acids around to fit the distance constraints

2. (10 points) Describe, at the molecular level, the binding of oxygen to myoglobin, and how the protein structure helps to lower the heme's ability to bind CO.

O₂ and CO are both bound by an Fe²⁺ ion, that is held by 4 coordination bonds to the hem protoporphyrin ring. A 5th coordinate bond to the Fe is made by a histidine, leaving the 6th coordinate bond for the O₂ or CO. Ordinarily Fe²⁺ readily oxidizes to Fe³⁺ in aqueous solution, but the heme-Fe system is buried in the protein's hydrophobic interior so it is in a non-aqueous environment that prevents this oxidation from occurring. The Electron structure of O₂ has lone pairs of electron in sp² orbitals at 120°. Thus O₂ wants to bind to the heme at a 120° angle, and the binding pocket is structures to enhance this binding angle. CO, on the other hand, has its electrons in an so orbital at 180°, so it prefers to bind pointing straight out form the heme. Since the pocket is not made this way, CO binding is much weaker. (Diagrams help in this explanation, but are hard to get on this computer file)

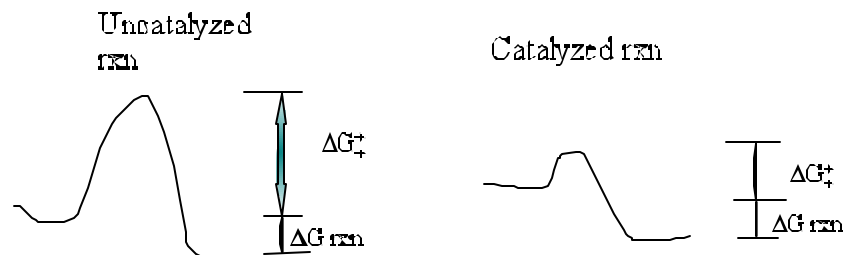
3. (10 points) What is the Bohr effect? What is the molecular explanation for the Bohr effect?

The Bohr effect refers to the lower binding affinity between hemoglobin and O_2 at low pH. This effect is advantageous for organisms using hemoglobin for transport of oxygen because in the lungs, where the CO_2 content is low, the pH is high (7.6), and the affinity of hemoglobin for O_2 is high so the hemoglobin bind more oxygen. In the peripheral tissue, where the CO_2 concentration is higher, CO_2 is reacted with water for form H^+ and HCO_3^- , and the pH drops to about 7.2 and due to the ionization state of mainly His 146 of the β hemoglobin chain, the hemoglobin molecules is pushed toward the T (tense) low affinity conformation. This forces the hemoglobin to release even more O_2 than it would have if the pH had remained constant.

(Diagrams of θ vs O_2 at different pH values helps in this discussion)

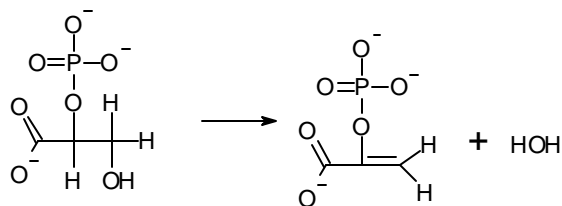
4. Make a reaction coordinate diagram for an uncatalyzed reaction and an enzyme catalyzed reaction. Use these diagrams to explain how catalysis works. In your discussion include such terms as transition state, ΔG_{rxn} , ΔG^\ddagger , activation energy, and explain the difference between the rate of a reaction and the

K_{eq} of a reaction.



In both the catalyzed and uncatalyzed reaction the overall ΔG of the reaction is supposed to be the same, indicating the catalyst does not affect the K_{eq} or overall ratio of product to reactants. What catalysis does affect is the rate at which the reaction can occur. This is through the ΔG^\ddagger or the height of the energy hump between products and reactants. The rate of a reaction is proportional to $e^{-\Delta G^\ddagger/RT}$ so as the hump gets smaller, the rate gets faster.

5. Enolase catalyzes the reaction:



Below is a partial mechanism for this enzyme taken from your text.

See figure in test for enolase, not copied here

(10 points) In this mechanism point out as many examples as possible of general acid or base catalysis, specific acid or base catalysis, covalent catalysis, or metal ion catalysis. While this mechanism does not include any binding interactions, discuss how binding interactions also could be used by this enzyme to enhance the reaction rate.

Lys 345 acts as a general base

Glu 211 acts as a general acid

2 Mg²⁺ ions act as metal catalysts

NO covalent catalysis (no covalent bonds formed between enzyme and substrate)

NO specific acid or base catalysis (no H⁺ or OH⁻ in mechanism)

Some binding interactions that might take place. Arg or Lys in + charged form used to bind PO₃²⁻

COO⁻ of asp or glu used to hold metal ions

Shape of pocket made to fit transition state, not product or reactant

Binding interaction can enhance a reaction in several ways. One is to reduce the rotational freedom within the molecule to push the molecule toward the correct transition state. Another way is by desolvating the molecules and removing water groups that may be interfering in a reaction pathway.

Another is by have a binding interaction that favors the transition collapse toward the correct product, rather than back toward the reactants.

6. (10 points) What are the differences between a competitive inhibitor, an uncompetitive inhibitor and a suicide inactivator. How are their mechanisms different? How could you tell them apart experimentally?

Competitive inhibitors bind reversibly to the active site of the enzyme. Uncompetitive inhibitors bind reversibly to some other site on the enzyme, and produce a change in the enzyme's structure that in turn alters the enzyme's kinetics. A suicide inhibitor is a compound that permanently binds to an enzyme's active site, thus permanently destroying the enzyme's activity.

The easiest way to differentiate a reversible inhibitor from a suicide inactivator is to treat the enzyme with the inhibitor, then dialyze the solution to remove the inhibitor, then do kinetic experiments with the enzyme. The reversible inhibitors will simply dialyze away at step 2, and the enzyme will regain full activity. Since the suicide inactivator permanently destroys the enzyme, the enzyme will not regain activity after dialysis.

Competitive and Uncompetitive inhibitors can be differentiated using a Lineweaver-Burke analysis of V_o data obtained under different inhibitor concentrations. Since a competitive inhibitor can be competed off by high substrate concentrations, all lines obtained at different [S] concentrations will converge to the same V_{max} value (all lines will have the same Y intercept). Uncompetitive inhibitors, however, affect both K_m and V_{max} , so in this plot you typically see a series of parallel lines for each [S]. (Again a diagram is better than words, but hard to do for this computer file)

7. (10 points) I have isolated a Martian enzyme that has some curious properties. When I isolate the enzyme quickly, I get an enzyme with a kinetic profile like that of A below, and the enzyme behaves like it is in a tetramer form. If I let the preparation sit around a few days before I isolate the enzyme, I get a kinetic profile like that in B below, and the enzyme behaves like a monomer. When I double check the composition of the enzyme after it has sat around I find that the only change that has occurred is that 2 sulfate groups (SO_4^{2-}) have been attached to my enzyme on serine residues. Can you explain what might be happening?

Diagram is V_o vs [S] the curve for B is to the left of the curve for A, and shows no cooperativity, while the curve for A is to the right of the curve for B and shows cooperativity.

When quickly isolated the enzyme is in the A form which is a tetramer, and shows the cooperativity. After the preparation sits around the enzyme is sulfated, and this sulfation seems to break the tetramer apart and make the enzyme non-cooperative. I wanted the student to see that this might be a form of enzyme control through covalent modification. Similar to the covalent modification of many enzymes with phosphate using kinases to attach phosphates and phosphatases to remove phosphates. If you made this connection then you could build an entire 'Martian' control system that would parallel the phosphate control system found in many terrestrial organisms and use this system to discuss many of the aspects of covalent control systems.