C484 – Fall 2001 Due 12/3/01
Problem Set 6

PLEASE WRITE YOUR ANSWERS ON A SEPARATE SHEET

Topics: Enzyme Regulation
Hemoglobin/Myoglobin
Enzyme Mechanisms
Serine Proteases and Lysozme
(Motor Proteins)
Unknown assignment

A recent paper in the journal *Nature* from Steven Withers' laboratory at UBC made a startling claim that is in direct contradiction with your textbook (Nature, 2001, 412, 835-838 – available on-line at www.nature.com from any computer with an IU IP address). Answer the following questions about this paper and the nature of the current lysozyme controversy.

1. Draw out schematically the mechanism of lysozyme from your textbook showing all of the postulated intermediates. Identify the general modes of catalysis that are relevant to any given step in the mechanism, and where ever possible, the amino acid residue(s) assisting in this process.

2. How does the Withers mechanism differ from the one in your text? Be specific and relate your answer to the scheme you drew for question 1.

3. Enzymology is a field that relies heavily on comparisons between enzymes that perform similar reactions. Vocadlo et al. make a comparison between lysozyme and a group of enzymes called "retaining β-glycosidases". This terminology is to contrast it with another group of enzymes called inverting β-glycosidases. What is being retained or inverted and why is it relevant to the enzyme mechanism?

4. There are so many glycosidases that people have divided up this class of enzymes into smaller groups of more closely related proteins called families. Families usually act on a common substrate and with a similar mechanism. They are also related to one another based on sequence homology more closely than they are to members of different families. The most common form of lysozyme studied is HEW (Hen egg white). Look up this enzyme on ExPASy (organism = Chicken). To which family of glycosidases does lysozyme belong (it is on the NiceZyme page)?

5. Use the link under the comments section labeled "**glycosyl hydrolases**" to find information on many other known glycosyl hydrolases. How many families of glycohydrolases have been identified?

6. In question 3, you identified two major classes of glycosyl hydrolases – retaining and inverting. Identify two families of glycosidases that work on the same substrate but are members of the retaining and inverting glycosyl hydrolases, respectively.

7. In question 1, you should have identified an Asp and a Glu residue participating in one of the steps of the reaction. Analyze the output on the different classes of glycosyl hydrolases from question 5 above. What other pairs of reactive functionalities are observed in glycosyl hydrolases taking the place of these two side chains?

8. In any enzymatic system that generates a transient intermediate, what conditions must be met if the intermediate is to be experimentally observed (or observable)?

9. In order to meet the criteria you just described in question 7, what did Vocadlo et al. do to lysozyme and why was it effective? Be as specific as possible.

10. What fundamental tenet of mechanistic enzymology does the controversy between the Phillips Mechanism, the Koshland Mechanism and new Withers Mechanism illustrate? Please explain.

11. Use any of the resources available to you (On-line databases, Biochemistry textbooks or primary research articles found through Pub-Med) to propose a catalytic mechanism for your unknown enzyme. Identify elements of the mechanism that fall into our general classes of catalytic rate enhancement (i.e. general acid/base catalysis, transition state stabilization, metal ion catalysis, proximity effects, etc.) If you have trouble finding information on your exact mechanism, use the tool of mechanistic analogy to help you propose a mechanism. Be as specific as possible and use the tools of molecular visualization (i.e. Protein Explorer) to help if necessary to postulate roles for individual side chains in the active site.