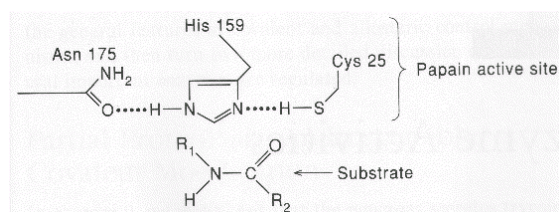
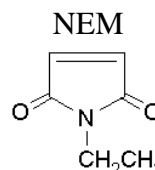


Chem 4540 Enzymology Winter 2005
Problem Set #5

1. If a lysine residue were substituted for the aspartate in the trypsin side chain binding crevice, would you expect the enzyme to be functional? If it were functional, what effect would you predict the substitution to have on substrate specificity?
2. Carboxypeptidase A preferentially cleaves C-terminal aromatic residues from proteins. When the aromatic substrate side chain is bound, water is expelled from the active site. How does the release of water stabilize binding of substrate in the active site?
3. (a) The amino acids in the active site of the protease papain are shown. Predict a feasible reaction mechanism for papain.



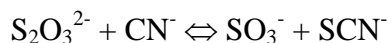
(b) N-ethylmaleimide (NEM) reacts rapidly with cysteine thiolate anion via a Michaelis addition. What is the product of the reaction between NEM and cysteine? Would you expect the rate of R-SH reaction with NEM to be more rapid at pH 5 or pH 7.5? Why?



- (c) Would you expect Cys 25 (see above scheme) to be more reactive with NEM than any of the other cysteine residues in the protein. Explain.
4. Why do structural analogues of the transition state intermediate of an enzyme inhibit the enzyme competitively and with low K_i values?
 5. Transition-state analogues of a specific chemical reaction have been used to elicit antibodies with catalytic activity. These catalytic antibodies have great promise as experimental tools as well as having commercial value. Why is it reasonable to assume that the binding site for the transition-state analogue on the antibody would mimic the enzyme active site? What difficulties might be encountered if a catalytic antibody were sought for a reaction requiring a cofactor (coenzyme)?
 6. Using site-directed mutagenesis techniques, you have isolated a series of recombinant enzymes in which specific lysine residues were replaced with aspartate residues. The enzymatic assay revealed the following:

<u>Enzyme Form</u>	<u>Activity (U/mg)</u>
Native enzyme	1,000
Recombinant Lys 21 → Asp 21	970
Recombinant Lys 86 → Asp 86	100
Recombinant Lys 101 → Asp 101	970

- (a) What might you infer about the role(s) of Lys 21, 86 and 101 in the catalytic mechanism of the native enzyme?
- (b) Speculate on the location of Lys 21 and Lys 101. Would you expect these residues to be conserved in an evolutionary sense?
- (c) Would you expect Lys 86 to be evolutionarily conserved? Why or why not?
7. The serine protease, subtilisin, is used in some laundry detergents to help remove protein-type stains.
- (a) What unusual kind of stability does this suggest for subtilisin?
- (b) Subtilisin does have a problem (weakness), in that it becomes inactivated by oxidation of a methionine close to the active site. Suggest a way to make a better subtilisin.
8. Lysozyme residues Asp 101 and Arg 114 are required for efficient catalysis, although they are located at some distance from the active site Glu 35 and Asp 52. Substituting Ala for either Asp 101 or Arg 114 does not significantly alter the enzyme's tertiary structure, but it significantly reduces its catalytic activity. Explain.
9. The enzyme rhodanese is abundant in mammalian liver and kidney tissue. It can catalyze the transfer of a sulfur atom from thiosulfate to cyanide to yield sulfite and thiocyanate:



Although this reaction is valuable for studying the mechanism of rhodanese catalysis in the laboratory, it probably does not occur to a significant extent in tissues. (Cyanide is a deadly poison). Rather, some sulfur acceptor other than cyanide serves as the major substrate in the cell.

- (a) The given reaction involves an interaction between ions with like charges. One effect that an enzyme could have would be to divide the reaction into steps so that no such unfavorable interaction would occur. It was found experimentally that when the enzyme was incubated with thiosulfate labelled with ^{35}S , a stable enzyme—sulfur compound could be isolated. Give a two-step reaction mechanism for rhodanese that involves such an enzyme—sulfur intermediate.
- (b) One might expect the negatively charged thiosulfate to bind to the enzyme electrostatically. In an effort to demonstrate such an interaction, neutral salts like NaCl were added to the enzyme assay system to determine whether they had any effect on thiosulfate binding. It was found that the addition of such salts decreased the ability of thiosulfate to bind. Does this support the hypothesis of electrostatic interaction? Explain.