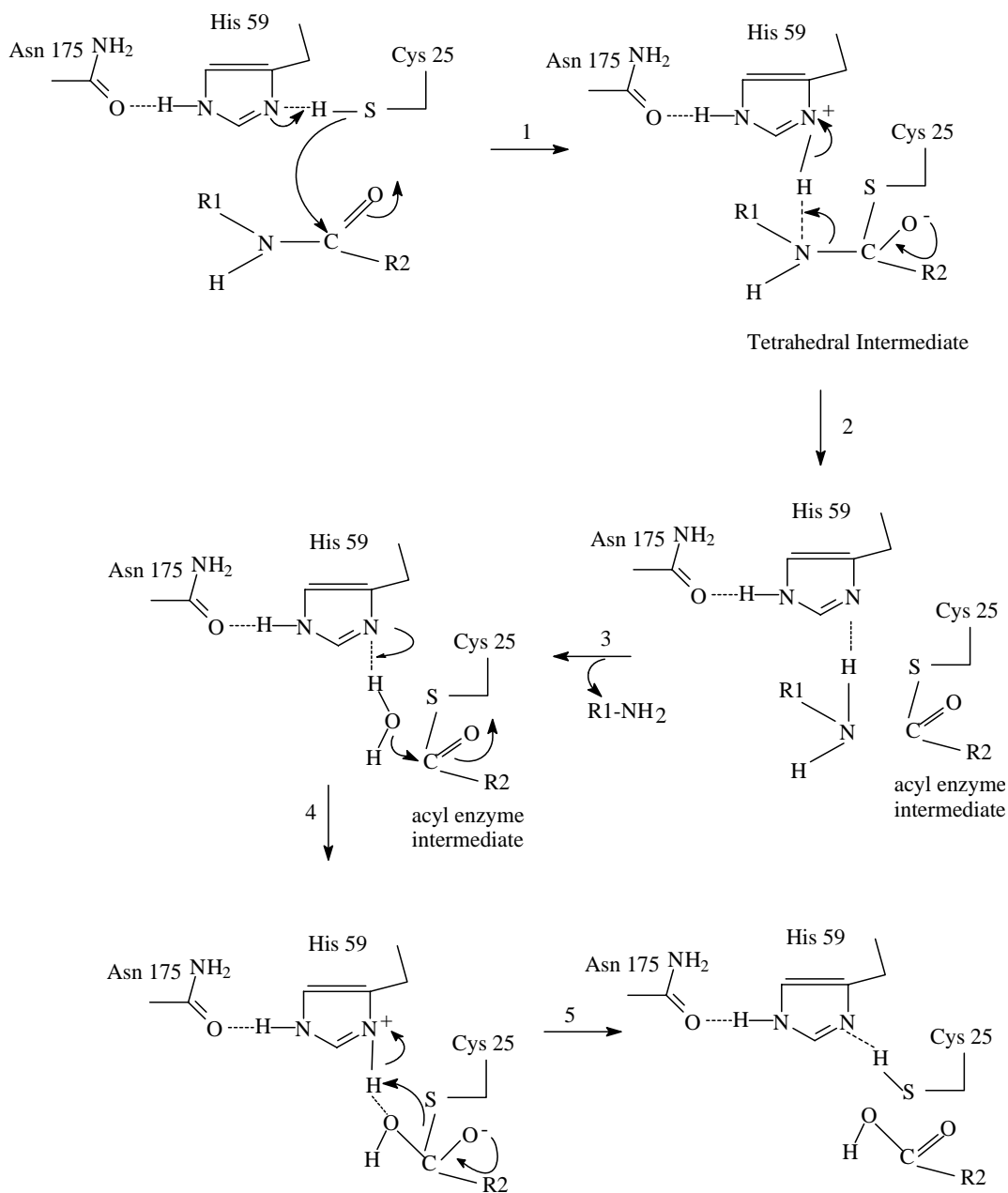


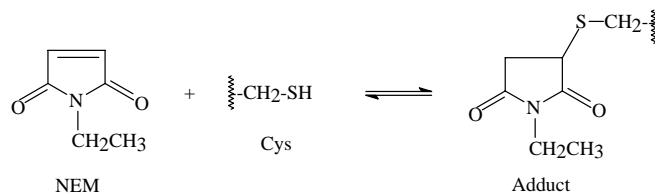
Chem 4540 Enzymology Winter 2005
Problem Set #5 ANSWERS

1. It may be functional depending upon whether there is space for the side chains of substrate proteins or peptides to nestle into. Certainly substrate specificity would change where from positively charged side chains to smaller, more negatively charged residues.
2. Often the release of water during the conformational change initiated by binding the substrate provides a hydrophobic environment to stabilize the nonpolar side chain of the substrate.

3.



(b)

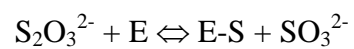


One would expect the reaction of R-SH to be more rapid at pH 7.5 because at the more alkaline pH the concentration of the R-S⁻ species is higher (the reactive species).

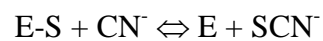
(c) It would be expected that Cys 25 is more reactive with NEM than the other residues within the protein because His 159 acts as a base to deprotonate Cys 25 and shift the equilibrium to a higher concentration of the more reactive species (R-S⁻).

4. The structure of the transition-state analogue is complementary to the structure of the active site. The analogue thus binds tightly to the active site.
5. Because the antibody was raised against the transition-state complex and so should mirror its structure. In this way, the antibody should reflect at least part of the active site of the enzyme. If a cofactor or coenzyme is required, then the antibody will not have this factor to provide the needed chemistry and hence will likely not exhibit enzymatic activity for these cases.
6. (a) Substitution of Asp for Lys 86 markedly decreases activity and several explanations are possible: The lysine is a critical residue either at or near the active site or is essential for maintaining a catalytically competent conformation of the enzyme.
(b) Lysines 21 and 101 are probably outside the catalytic site and may not be evolutionarily conserved. Their replacement with aspartate yielded no great change in enzymatic activity.
(c) Lysine 86 is essential for enzymatic activity and would be conserved.
7. (a) The enzyme must be stable both to the presence of detergents and to moderately high temperatures.
(b) Replace the methionine, by site-directed mutagenesis, with another residue. since methionine is quite hydrophobic, a hydrophobic replacement would seem appropriate. A single base change in the codon would yield Phe, Leu, Ile, or Val.
8. It must be that Asp 101 and Arg 114 form H-bonds with the substrate molecule. Replacement with Ala result in a change whereby the H-bonds are eliminated so the substituted enzyme is less active likely due to its inability to orient the substrate properly (or as well as the wild-type enzyme).

9. (a) Step 1:



Step 2:



(b) Yes, it does support the hypothesis of electrostatic interaction. If an interaction between a negatively charged thiosulfate and a positively charged functional group on the enzyme occurred, the addition of a neutral salt would weaken the interaction by competition.