Problem Set A (PSA)

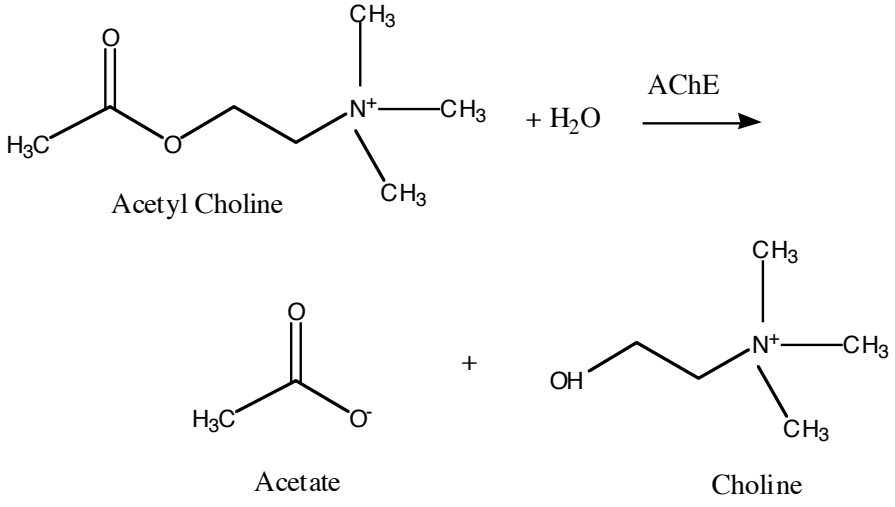
1) There are multiple serine residues in chymotrypsin, but only Ser195 reacts rapidly when the enzyme is treated with diisopropyl fluorophosphate (DFP). Explain

2) a) Identify the residues in the catalytic triad of chymotrypsin and indicate the type of catalysis mediated by each residue.
b) What additional amino acid groups are found in the oxyanion hole and what role do they play in catalysis?
c) Explain why site directed mutagenesis of Asp to Asn in the active site of trypsin decreases catalytic activity 10,000 fold.

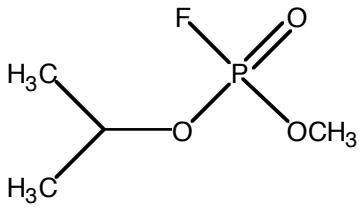
3) Catalytic triad groupings of amino acid residues increase the nucleophilic character of the active site serine, threonine, and cysteine residues present in many enzymes involved in catalyzing the cleavage of substrate amine and ester bonds. Using chymotrypsin as a model system, diagram the expected arrangement of the catalytic triads in the enzymes below.

a) Human cytomegalovirus protease: His, His, Ser
b) β-lactamase: Glu, Lys, Ser
c) Asparaginase: Asp, Lys, Thr
d) Hepatitis A Protease: Asp, (H_2O), His, Cys (the water molecule is situated between the Asp and His residues)

4) Acetyl Cholinesterase (AChE) catalyzes the breakdown of the neurotransmitter acetylcholine to acetate and choline as shown below. It has a catalytic triad with residues, His, Glu, and Ser. The catalytic triad enhances the nucleophilicity of the serine residue in a manner very similar to the situation found in trypsin and chymotrypsin. Diagram the expected arrangement of the amino acids in the catalytic triad.



5) The nerve agent Sarin is an extremely potent irreversible inhibitor of Acetylcholinesterase. Propose a mechanism for the covalent modification of acetylcholinesterase by Sarin. (Hint: Consider how DFP inhibits serine proteases.)

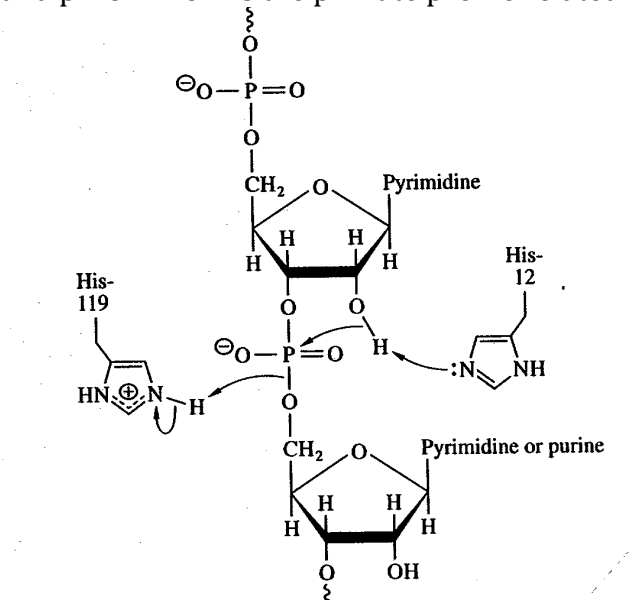


Sarin

6) The protease from Human Immunodeficiency Virus 1 (HIV-1) possesses a catalytic diad of two aspartate residues (one residue in each of two identical domains: Asp-25, pKa=3.3, and Asp-25', pKa=5.3). In the first step of peptide hydrolysis, one aspartate acts as a base, accepting a proton from water, and the other aspartate acts as an acid, donating a proton to a peptide carbonyl group. The resulting tetrahedral intermediate is not covalently bound to the enzyme. Write a mechanism for HIV-1 protease.

7) Draw the pH profile expected for HIV-1 protease (as described in problem 6).

8) Ribonuclease catalyzes the hydrolysis of ribonucleic acid (RNA). The active site of the enzyme contains two histidine residues, whose participation is shown below. The pH-rate profile is bell-shaped, with inflection points at approximately pH 5.8 and pH 6.2. How is the pH-rate profile related to the mechanism for ribonuclease?



9) A hypothetical enzyme has an active site with an effective pKa' value of 5. Draw pH-rate profiles for the following possibilities:

a) The residue acts as an acid catalyst.

b) The residue acts as a base catalyst.