

Solutions to PSA

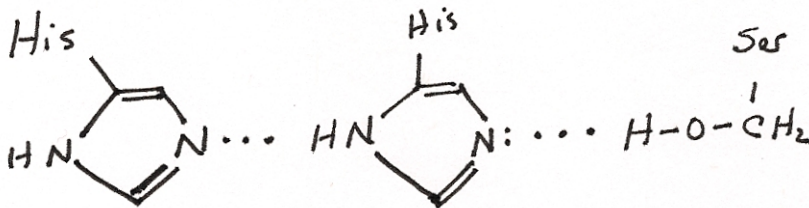
1) Ser195 is the only serine residue in the enzyme that participates in the catalytic triad at the active site of chymotrypsin. This results in a significant increase in the nucleophilic character of Ser195's Oxygen compared to other serines. This increase in nucleophilic character allows for Ser195 to react with DFP.

2) a) The catalytic triad is composed of Asp, His, and Ser. His acts as a general acid-base catalyst, removing a proton from serine to make serine a more powerful nucleophile in the initial step. Asp stabilizes the transition state via a low barrier H-bond with the His and because it's negative charge stabilizes the positive charge on the His in the transition state. The acid catalyst, His donates a proton to generate the leaving amine group.

b) the oxyanion hole contains backbone-NH- groups that form H-bonds with the negatively charged oxygen of the tetrahedral intermediate. The oxyanion hole stabilizes the transition state since it binds more tightly to the transition state than it binds to the substrate.

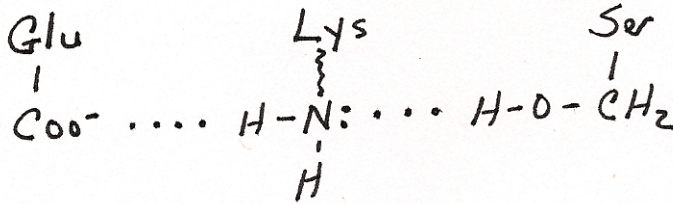
c) During trypsin catalysis, Asp is negatively charged stabilizing the imidazolium (protonated) form of the His. Asn lacks the carboxylate and therefore does not carry a negative charge, it therefore does not stabilize the transition state and there is a dramatic decrease in enzyme activity for the mutated enzyme.

3a) Human cytomegalovirus protease: His, His, Ser



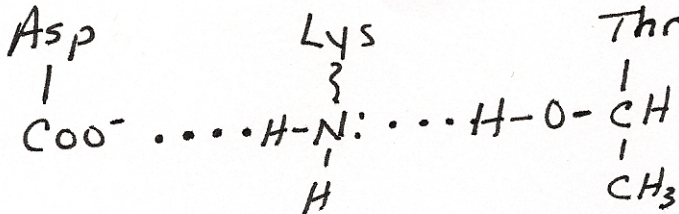
Middle His would act alternately as an acid and a base. Serine would act as nucleophile after losing its proton (to middle His,) and the other His (left) stabilizes the transition state via H-bonding in its unprotonated form.

3b) β -Lactamase: Glu, Lys, Ser



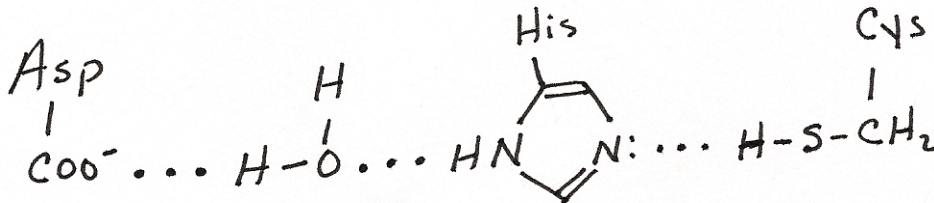
Lys would act alternately as an acid and a base. Serine would act as nucleophile after losing its proton (to Lys) and the deprotonated Glu stabilizes the transition state via H-bonding and electrostatic interactions with the positively charged Lys.

3c) Asparaginase: Asp, Lys, Thr



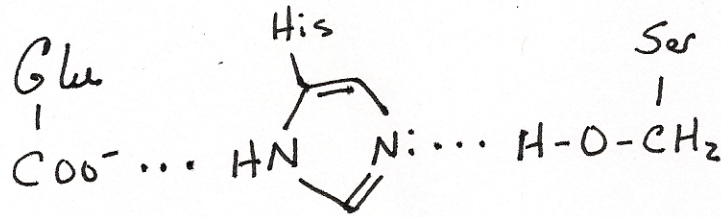
Lys would act alternately as an acid and a base. Threonine would act as nucleophile after losing its proton (to Lys) and the deprotonated Asp stabilizes the transition state via H-bonding and electrostatic interactions with the positively charged Lys.

3d) Hepatitis A Protease

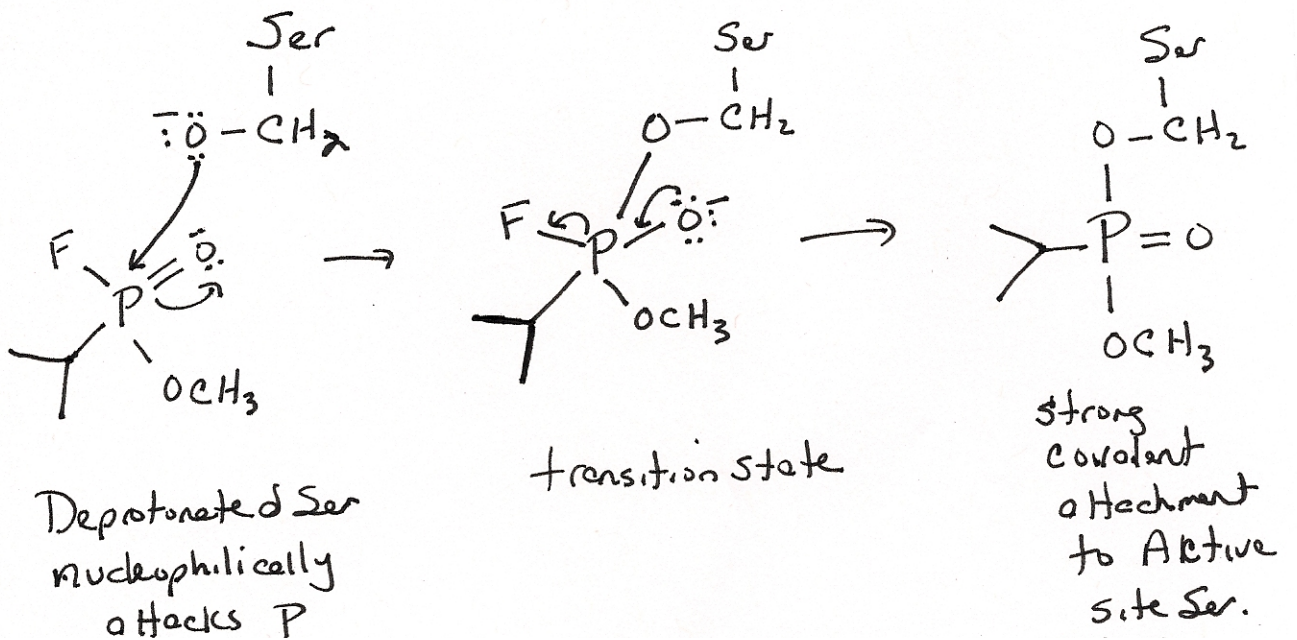


His would act alternately as an acid and a base. Cys would act as nucleophile after losing its proton (to His) and the deprotonated Asp stabilizes the transition state via H-bonding and electrostatic interactions with the positively charged His.

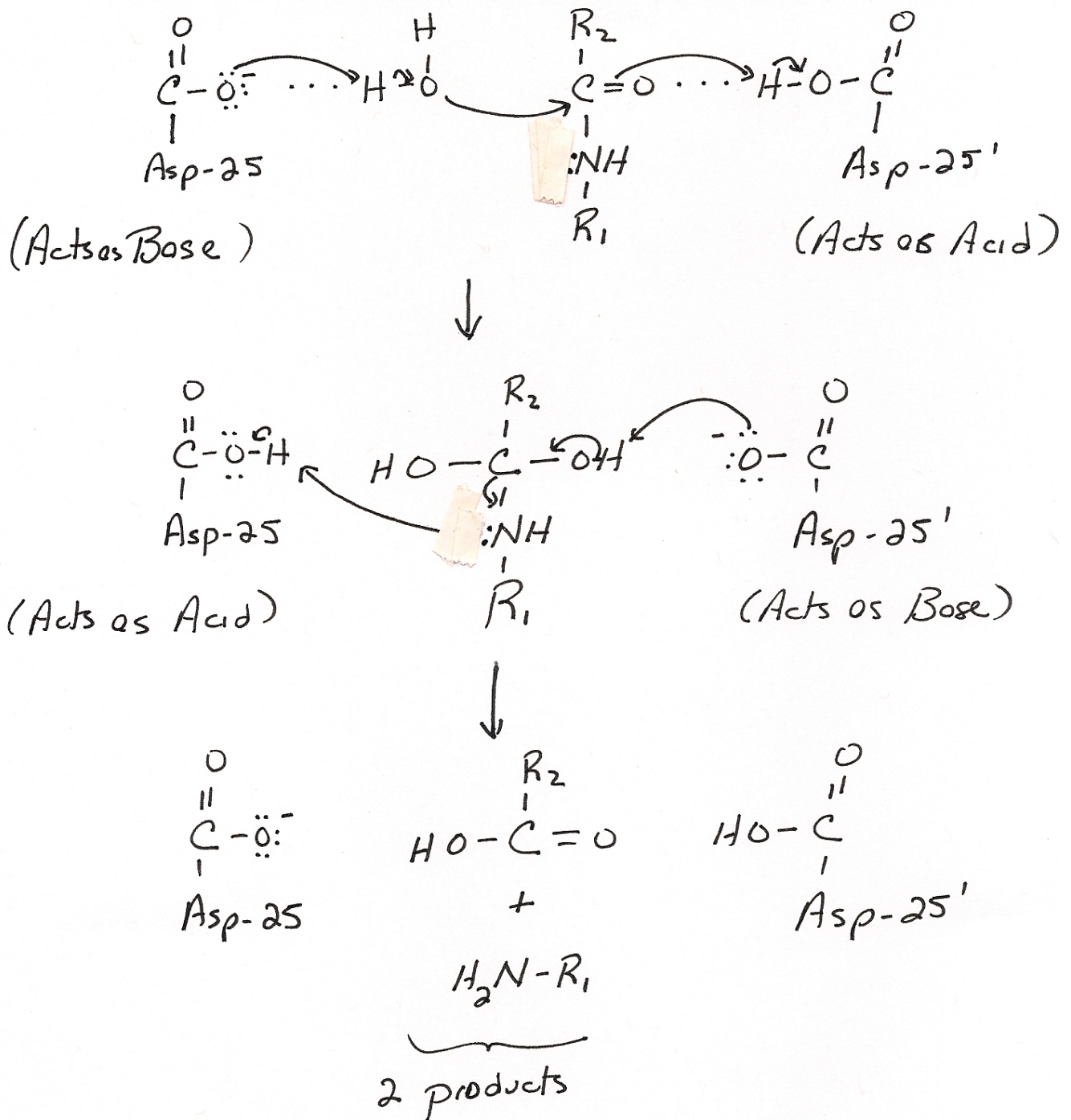
4) Acetylcholinesterase catalytic triad: Glu-His-Ser



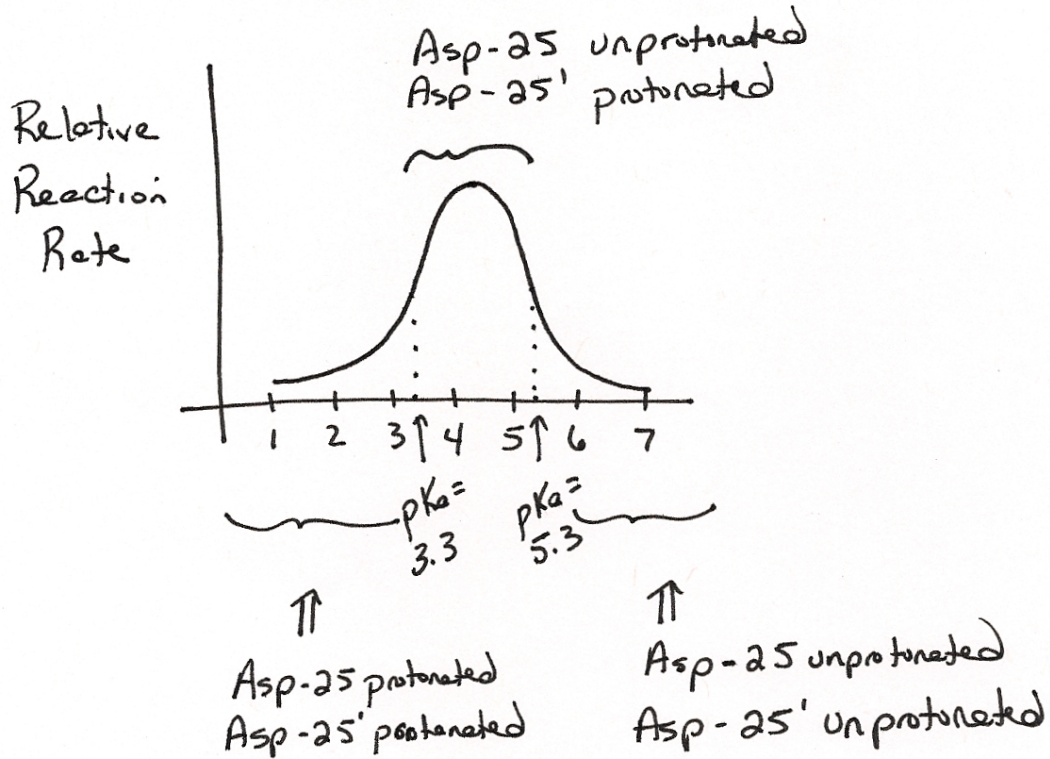
5) Serine loses a proton to the His becoming a good nucleophile. Deprotonated serine attacks Phosphorous in Sarin ($\text{P}=\text{O}$ is similar in electrophilicity to $\text{C}=\text{O}$). Fluorine is good leaving group and leaves. Sarin is covalently bound to the serine strongly and is unable to be hydrolyzed by water.



6) In the first step Asp-25 acts as a base and asp-25' acts as an acid. in the second step, the roles of the two asp are reversed, leading to the formation of the acid and amine products without an intermediate covalent enzyme substrate intermediate.

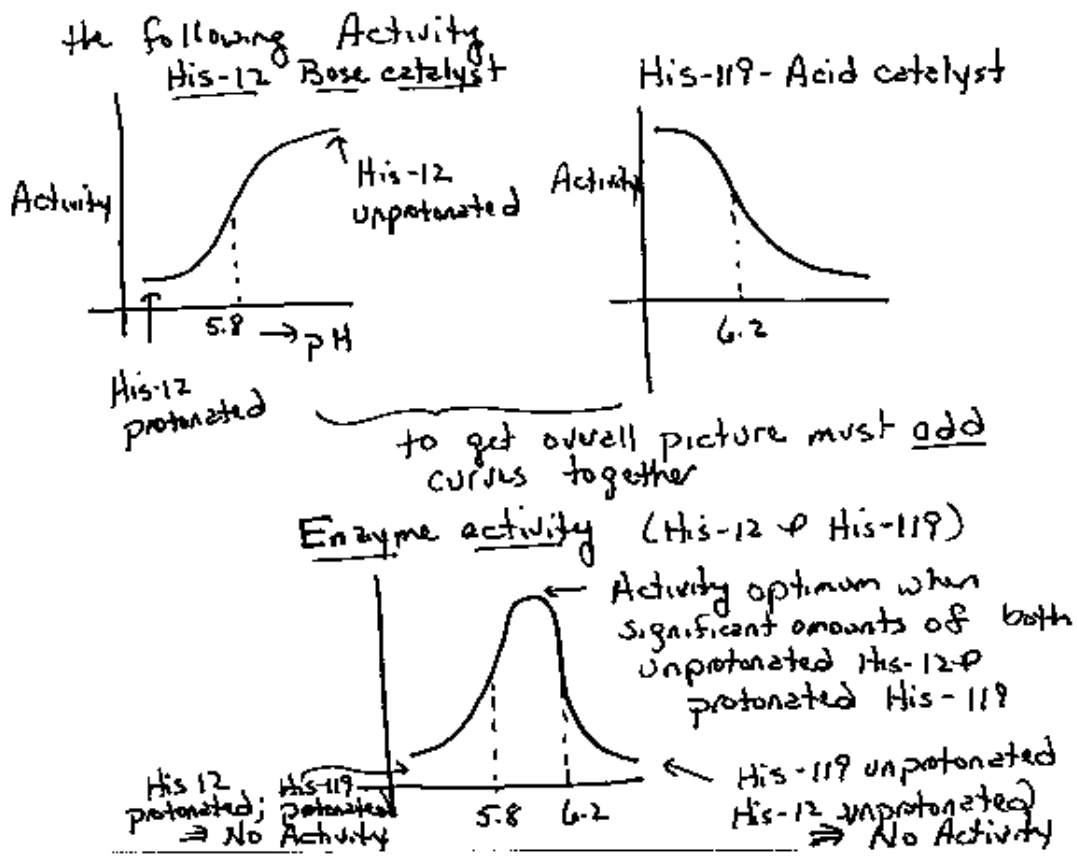


7) Asp-25 ($pK_a=3.3$) must be unprotonated ($RCOO^-$) to act as a base, while Asp-25' ($pK_a=5.30$) must be protonated ($RCOOH$) to donate a proton to the peptide carbonyl group. The aspartate residues exist predominantly in the correct forms in the pH region between the 2 pK_a 's.



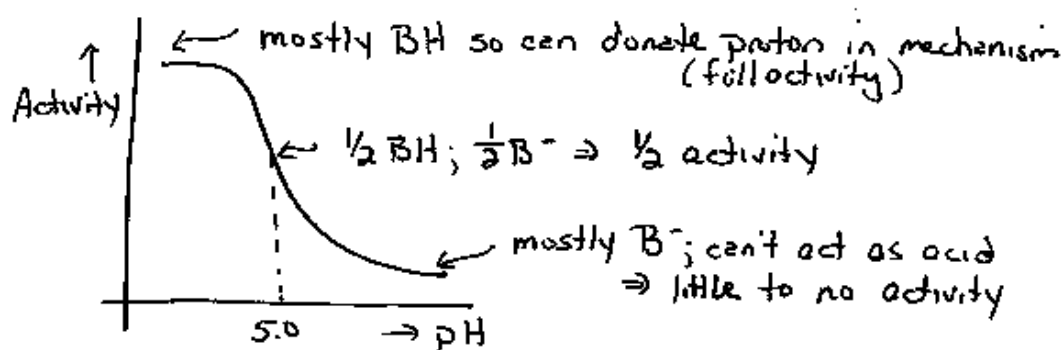
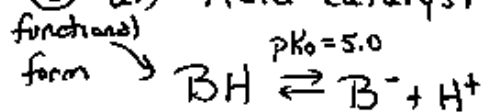
8)

⑧ In order for ribonuclease to function according to the mechanism shown, His-12 must be in the unprotonated form to serve as a base catalyst and His-119 must be protonated to serve as an acid catalyst. The apparent pKa values corresponding to the inflection points are approx. 5.8 (His-12) & 6.2 (His-119). Independently each His would have

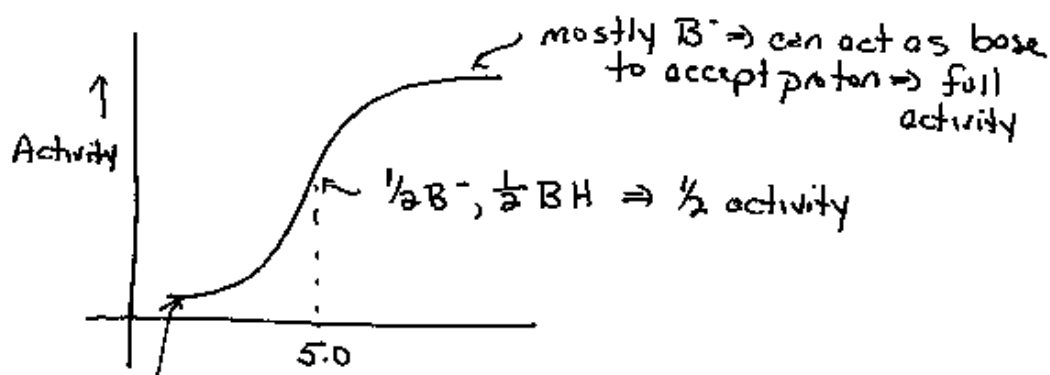
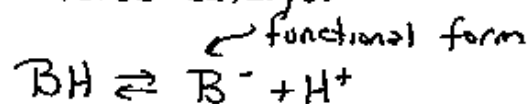


9)

⑥ a.) Acid catalyst



b.) Base catalyst



mostly BH,
can't act as base
to accept proton \Rightarrow little to no activity