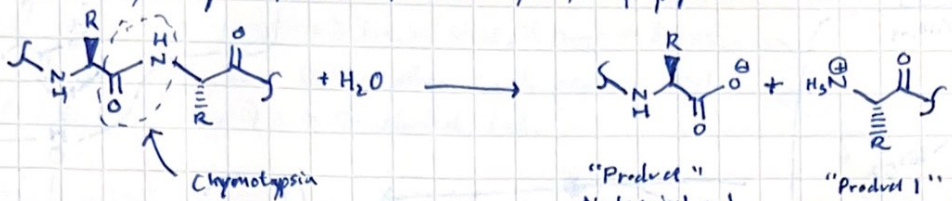
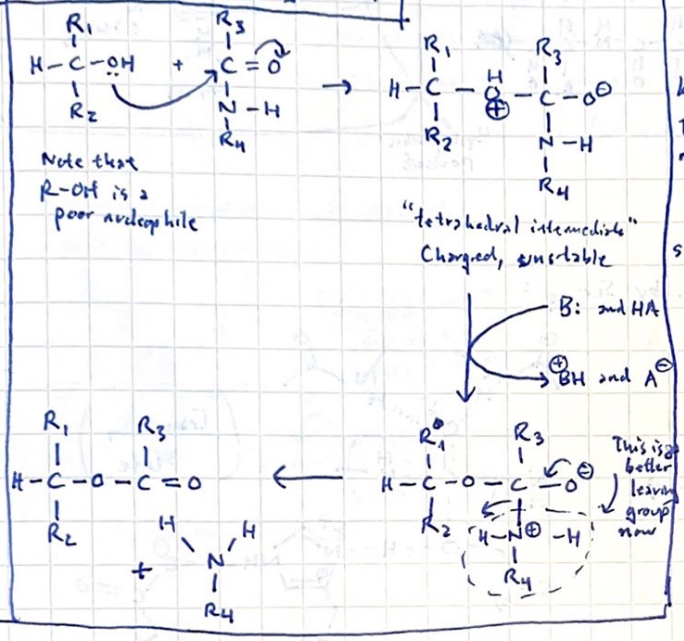
Chymotrypsin

→ Chymotrypsin is produced in the pancreas and is used to break down proteins in the small intestine from digested food. Therefore it is a protease.

– Specifically, chymotrypsin catalyzes the hydrolysis of peptide bonds:



This reaction is analogous to:



Note that at this step, the displaced electrons want to kick off a group so that the carbonyl can be re-formed. The issue is that the alcohol may be expelled or the nitrogen group, and $-\ddot{N}H$ (R) is a poor leaving group.

For enzymes to speed up the reaction, they must solve the following problems:

1) R-OH being a poor nucleophile
2) Stabilizing charged intermediates
3) $-\ddot{N}H$ (R) being a poor leaving group.

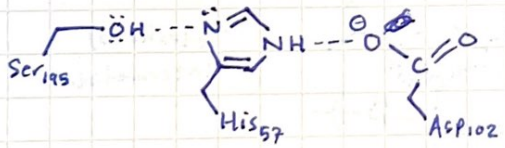
Chymotrypsin's active site contains a substrate binding pocket, which is largely non-polar and discriminates nonpolar side chains on ~~protein chains~~ polypeptide chains.

There are 3 fundamental amino acid residues in the binding site of ~~trypsin~~ chymotrypsin that form the "catalytic triad":

→ Ser-195
→ His-57
→ Asp-102

These interactions are displayed to the ~~side~~ left.

Chymotrypsin active site:



About this structure:

→ The R-OH group of serine is a poor nucleophile. However, $R-O^{\ominus}$ is a strong nucleophile. Serine acts as the base

→ His_{57} acts as a base at first, then as an acid.

→ Asp_{102} increases the basicity of His_{57} via the hydrogen bond. (Stabilizes a positively charged histidine)

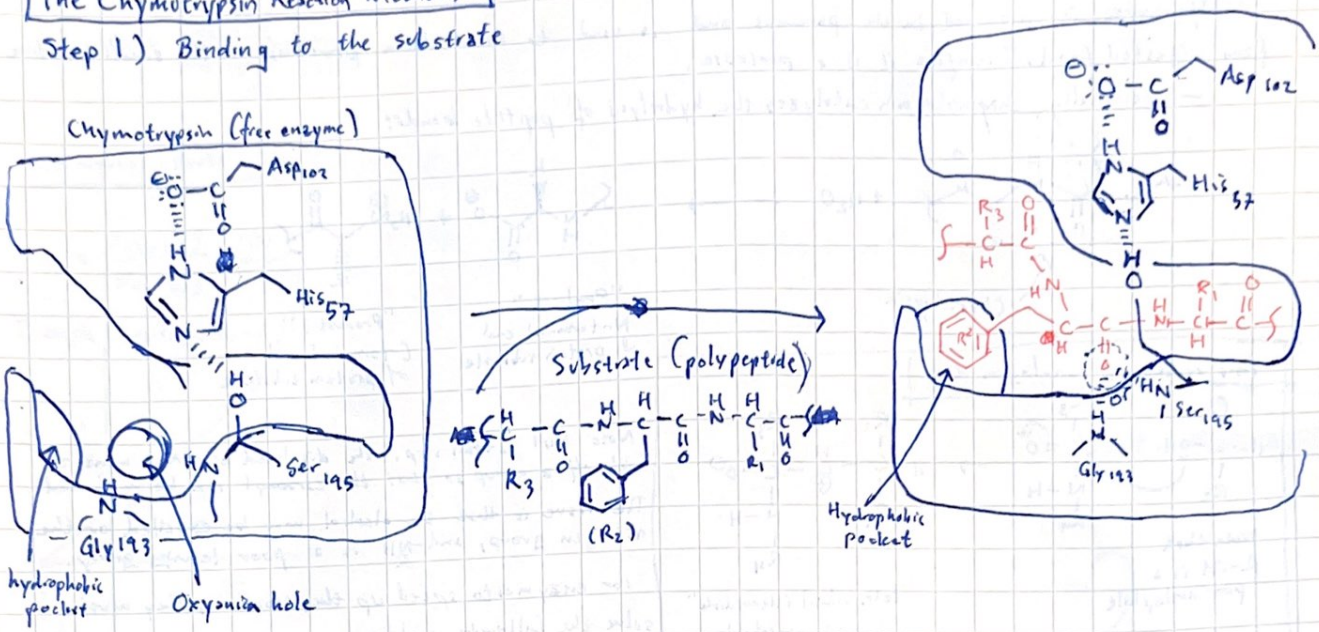
Additionally, an "oxyanion hole" (in the two main chain N-H groups) also contribute to stabilizing the structure of a tetrahedral intermediate:

These are the main players for the chymotrypsin reaction mechanism.

The detailed steps are shown in the following page.

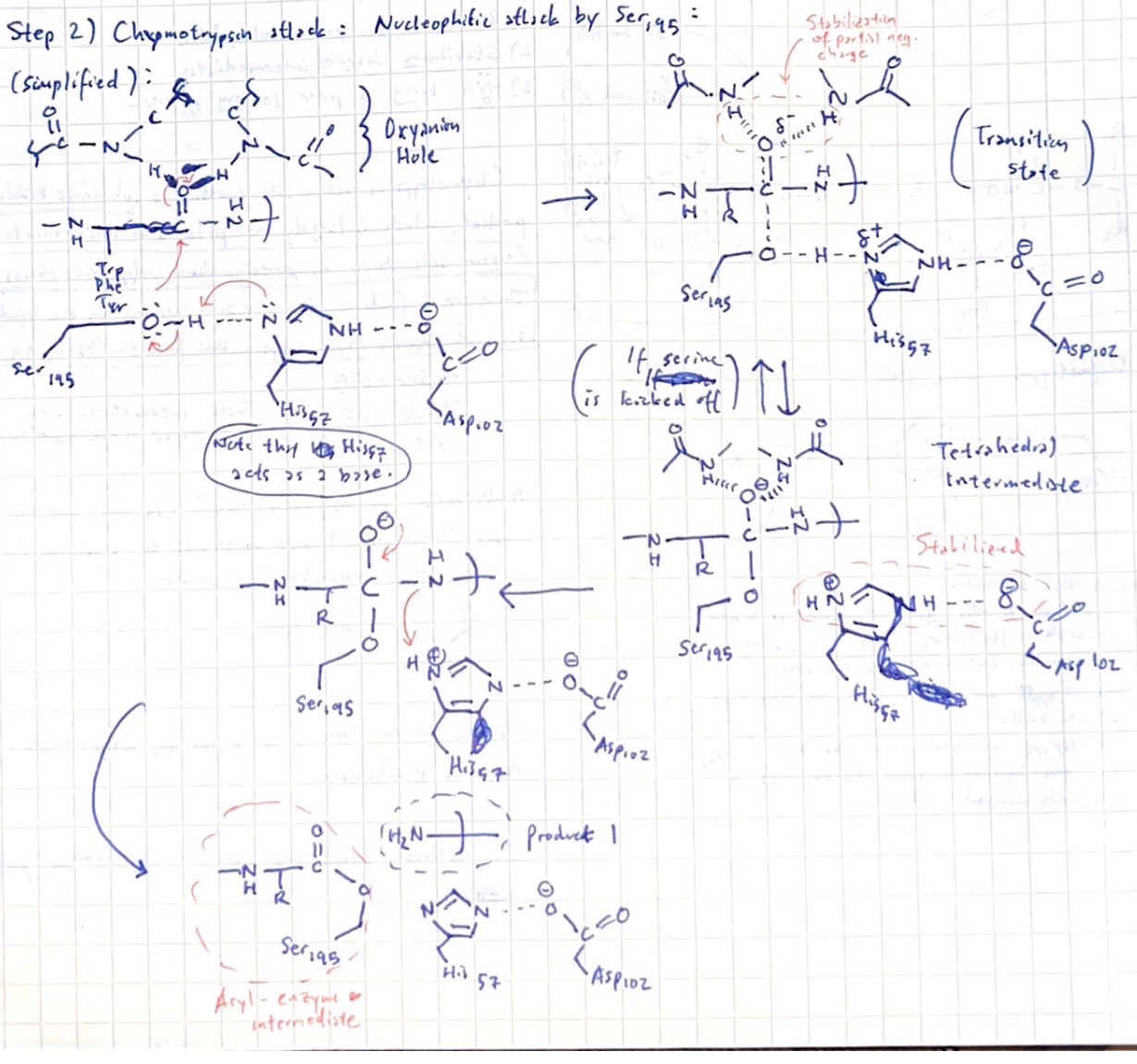
The Chymotrypsin Reaction Mechanism

Step 1) Binding to the substrate



Step 2) Chymotrypsin attack: Nucleophilic attack by Ser_{195}:

(simplified):

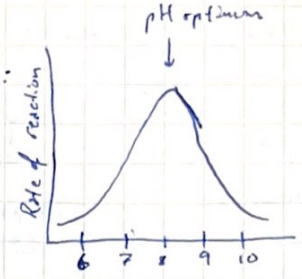


pH-dependence of enzyme activity (tool to study reaction mechanisms)

Experiment:

→ Measure rate (v_0) of enzyme-catalyzed reaction as a function of pH:

- Some enzymes have different v_0 s than others. For example, g6pase (glucose-6-phosphatase) has an ideal pH range ~ 8, as it is located in the liver. On the other hand, pepsin's ideal pH range ~ 2 as it is in the stomach acid.



- Note that the rate decreases → lower pH as a group in the active site must be unprotonated for catalytic activity.
- Similarly, at high pH the rate decreases, so a group in the active site must be protonated for catalytic activity.

OR:

→ Determine Michaelis-Menten parameters (K_m, V_{max}, k_{cat}), and V_{max}/K_m ($\frac{k_{cat}}{K_m}$), as a function of pH.

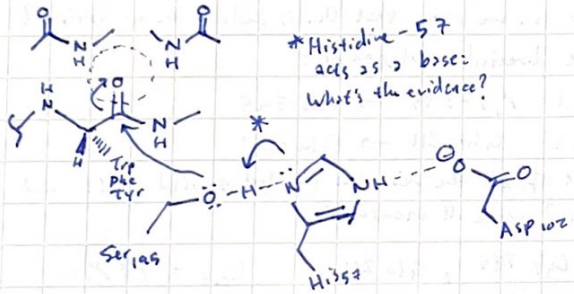
- recall V_{max} - The rate at very high [S]

k_{cat} - The rate constant, for reaction at very high [S]

k_{cat}/K_m - The rate constant, for reaction at very low [S].

Using chymotrypsin as an example:

Recall the reaction step:



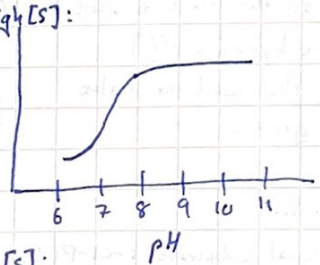
$E\text{-}His_{57} \cdot H^+ \rightleftharpoons E\text{-}His_{57} + H^+$

"Inactive" "Active"

Evidence for the chymotrypsin mechanism:

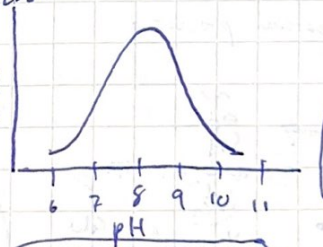
pH-dependence of k_{cat} (high [S]):

At high [S]:



- This curve is the same curve for the fraction dissociated of an acid!
- As pH decreases, the protonation of a group with $pK_a \sim 7$ causes the k_{cat} to decrease.
∴ We can estimate that this group is histidine.

At low [S]:



Why are the two graphs of k_{cat} vs pH and $\frac{k_{cat}}{K_m}$ vs pH so different?

But why only at low [S]?

S binds preferentially to $E\text{-}NH_3^+$, so high [S] pulls the equilibrium to the $E\text{-}(NH_3^+)\cdot S$ to overcome the deprotonation.

A: Preferential binding.

Consider the process:

$E\text{-}NH_3^+ + S \rightleftharpoons E\text{-}NH_3^+ \cdot S \rightarrow E\text{-}P$

$\downarrow\uparrow$

$E\text{-}NH_2 + H^+$ (inactive, can't bind S)

At high [S], ~~the substrate and proton compete to bind to the enzyme, and since~~ [S] is large, [S] would ~~overcome [H+],~~ produce more $E\text{-}NH_3^+ \cdot S$ over $E\text{-}NH_2 + H^+$

- At a low pH, the rate is reduced at a high [S] (k_{cat}) and at a low [S] ($\frac{k_{cat}}{K_m}$) because of the protonation of the histidine.
- At a high pH, however, the rate is only reduced at low [S] concentrations.
- For this particular example (chymotrypsin) it is because N-term-NH_3^+ (active) $\rightleftharpoons$ N-term-NH_2 + H^+ (inactive)

pH ≳ 9

The deprotonated form of the N-terminus is inactive.