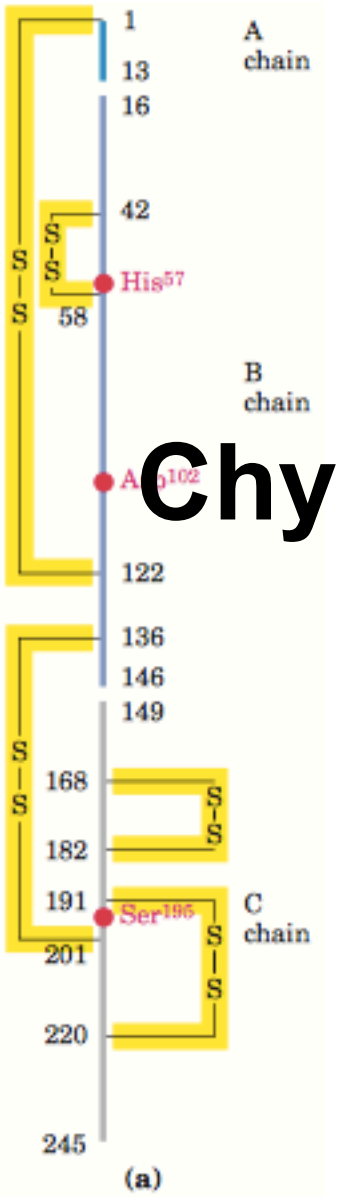
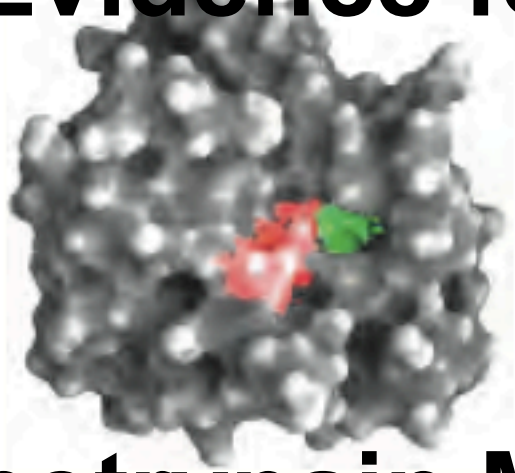
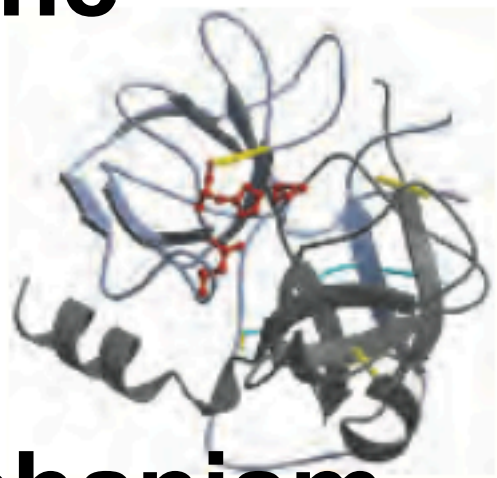
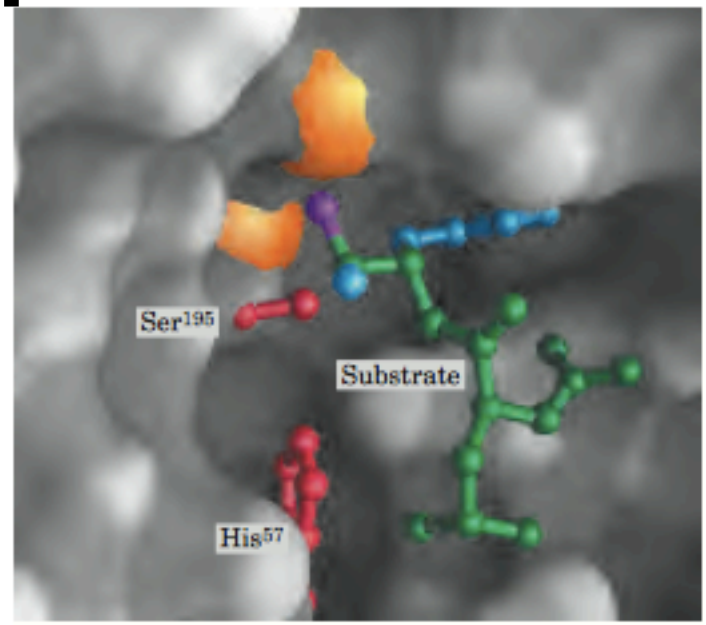
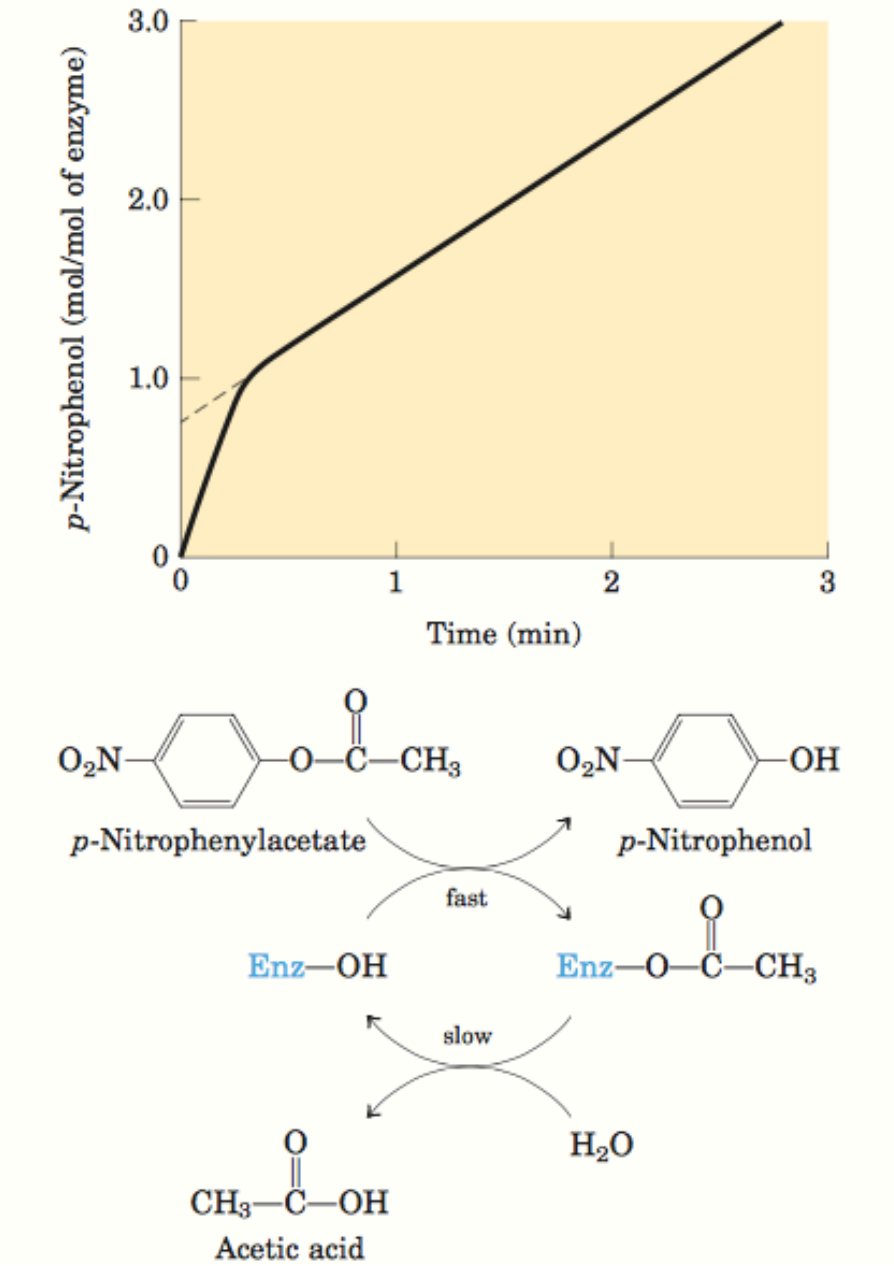
Evidence for the Chymotrypsin Mechanism









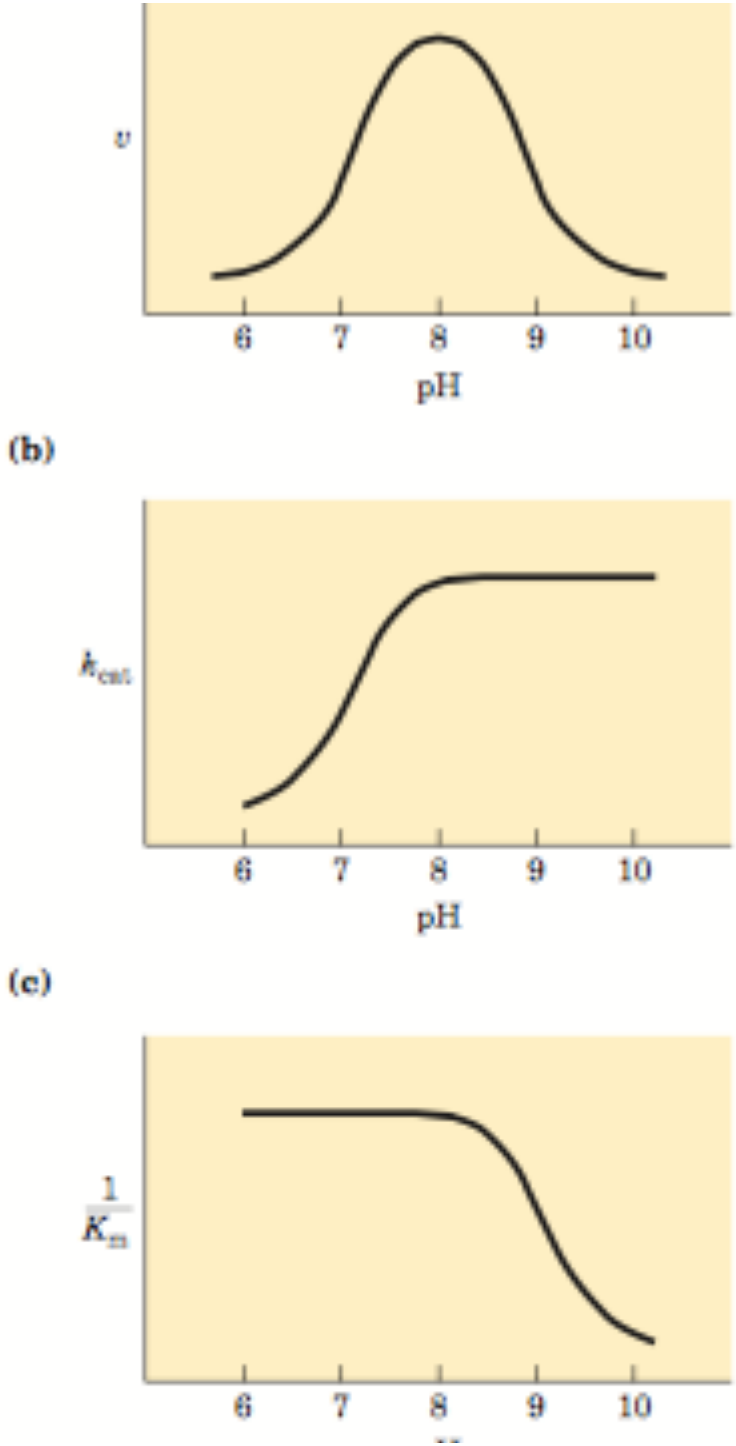


- The initial velocity is very fast.
- Then it becomes slower (after about 20 sec).
- This is evidence that there is a intermediate that is covalently bound to the enzyme.
- Also, the slow step is removing the substrate from the enzyme.

1. Why was p-nitrophenylacetate used as a substrate?

2. How was the rate of the reaction measured?

Lehninger's Biochemistry, 4th edition by Nelson and Cox, 2002.



Graph (a) shows the velocity of the reaction as a function of pH. The velocity is comparable to k_{cat}/K_m

Graph (b) shows the relationship to the overall rate constant kcat vs pH. The rate of the reaction increases sharply from pH7 to the maximum at pH 8. This is evidence that His^{57} must not be protonated. It has to be in the deprotonated form so it can abstract a proton from Ser^{195}.

Graph (c) shows that pH is increased above pH 8, the Km increases. A large Km means less substrate binding. Above pH 8, the amino terminus of Ile^{16} is not protonated. (Recall that chymotrypsin is cut into three pieces to be activated.) The protonated Ile16 is needed to form a salt bridge with Asp194. This salt bridge forms the hydrophobic pocket and confers substrate specificity to the enzyme.