The effect of small molecules and ions as competitive and noncompetitive inhibitors has contributed to an understanding of the catalytic properties of enzymes.

The specificity of an enzyme is associated with a binding site that is complementary to the substrate.

An enzyme such as chymotrypsin which has a specificity for peptide bonds involving aromatic amino acids is due to a substrate binding site that is nonpolar leading to the interaction of the substrate with the enzyme by hydrophobic interactions as in Fig 9-35.

The bond breaking/making activity of enzymes is associated with a catalytic site such as the triad of amino acids in chymotrypsin.

The substrate binding-site and the catalytic site together form what is called the <u>active site</u> of the enzyme.

Consider the catalytic properties of regulatory enzymes. Regulatory enzymes are enzymes whose activity is controlled by interactions with other molecules.

Regulation is achieved in four basic ways.

1. Enzyme activity can be controlled by small molecules that act as allosteric effectors; binding of a small molecule to the enzyme changes it's structure resulting in a more or less active enzyme.

This type of behavior is most typical of enzymes that have quaternary structure.

Allosteric enzymes do not follow M-M kinetics but instead show a sigmoidal dependence of velocity on substrate concentration as shown in Fig 8-22.

Fig 8-23 shows two models for the allosteric effect of substrate binding on enzyme activity.

A hypothetical enzyme is considered to have two identical subunits each containing an active site.

The subunits each have a tensed (T) low substrate affinity and relaxed (R) high substrate affinity conformation.

Binding of substrate to one subunit contributes to a change from the T to R conformation of the other subunit that increases the affinity for substrate binding and thus the velocity.

Allosteric effector molecules other than the substrate can also bind to the enzyme and either inhibit or activate the enzyme by stabilizing the T or R conformation as shown in Fig 8-24.

Aspartate transcarboxylase is an example of such an enzyme.

Aspartate transcarboxylase catalyzes the the formation of N-carbamoylaspartate from aspartate and carbamoyl phosphate as shown below

This reaction is the first step in a sequence of reactions which leads to biosynthesis of the pyrimidine nucleotide UMP as shown in the handout.

UMP can be converted to UTP by ATP and then be aminated by glutamine to give CTP as indicated below

In bacteria ATP acts as an activator while CTP acts as an inhibitor of aspartate transcarboxylase as shown in Fig 10-2.

The regulation by CTP is an example of feedback inhibition in which the final product of a sequence of reactions (pyrimidine) inhibits the synthesis of further product.

2. Enzyme activity can be controlled by other proteins that can bind to the enzyme and activate or inhibit the enzyme by changing its conformation.

The activity of the Ca^{2+}-ATPase is regulated by the protein calmodulin.

Ca^{2+}-ATPase catalyzes the transport of Ca^{2+} across cell membranes coupled to the hydrolysis of ATP as shown in Fig18-20.

Calmodulin binds Ca^{2+} that leads to a change in its structure. Ca^{2+}-calmodulin activates the Ca^{2+}-ATPase by binding to the enzyme that leads to a change in its structure.

3. Enzyme activity can be regulated by covalent modification.

Phosphorylation of a particular ser or thr residue of an enzyme by a protein kinase can change the tertiary structure of the enzyme from an active to an inactive or an inactive to an active form.

4. Zymogens or proenzymes are proteins that are converted into active enzymes by hydrolysis of one or more peptide bonds.

Chymotrypsinogen is the inactive precursor of chymotrypsin.

Hydrolysis of the peptide bond between Arg 15 and Ile 16 by trypsin converts chymotrypsinogen into π-chymotrypsin as shown in Fig10-19.