

Chapter 6 "Mechanisms of Enzymes"

Reading Assignment: pp. 158-167, 171-176, 182-187.

Problem Assignment: 1, 3, and 4.

I. Introduction

The first objective of this chapter is to obtain a conceptual understanding of how enzymes are able to greatly increase the rates of chemical reactions. In brief, high rates of reactivity can be achieved because the active site provides a constellation of functional groups that surround and interact with the reactants bringing them together in the perfect orientation for the reaction to occur. Interactions between functional groups in the active site and the substrate also promote formation of the transition state structure which can break down to the product. In addition, enzymes also provide catalytic groups in the active site that can attack bonds within the substrate and carry out general acid-base or covalent catalysis needed to speed the reaction. The second objective of this chapter is to illustrate these general properties of enzymes using the digestive enzyme chymotrypsin as example. The mechanism of this reaction illustrates the way critically positioned functional groups within the 3D fold of an enzyme can carry out elaborate reaction schemes.

II. Examples of chemical mechanisms

The mechanism of a chemical reaction refers to the molecular and atomic events that occur during the course of the reaction, including the stereochemistry of the reaction. A few basic mechanisms are reviewed in the text (pp. 159-160), and one of these (nucleophilic substitution) is covered here as it is germane to our understanding of enzyme catalysis.

Many chemical reactions have ionic intermediates. There are two types of ionic intermediates: one species is electron-rich or nucleophilic, and the other species is electron-poor or electrophilic (see section 2.6)

Nucleophilic substitution reactions. These reactions involve the attack of a nucleophile on an electrophile. In some cases (depending on the structure of the substrate) the reaction proceeds via a short-lived, but detectable, tetrahedral intermediate as illustrated in **Reaction 6.1**. This is the mechanism used during the first step of the reaction catalyzed by chymotrypsin. In other cases, the reaction proceeds via a more unstable structure referred to as the transition

state, in which 5 groups are bonded to carbon (**Reaction 6.2**). The transition state has a structure between that of the reactant and the product. Note that the tetrahedral intermediate formed in Reaction 6.1 also is produced via a transition state structure (not shown). In this case, the structure of the transition state and the intermediate are thought to be very similar.

III. Binding modes of enzymatic catalysis: Enzymes accelerate reactions by properly orienting reactants and by stabilizing transition states

In all chemical reactions, regardless of whether they are uncatalyzed or catalyzed, the transition state is the shortest-lived structure found along the path from reactants to products. It is produced infrequently because it has the highest energy. The activation energy barrier (E_a) for a forward reaction is equal to the difference in free energy between the substrate (the ground state free energy) and the transition state structure. This can be illustrated with energy diagrams which plot the free energies of the different structures present during the course of the reaction (**Fig. 6.1**). (Note that the reaction coordinate is NOT time; it rather represents the progress of the reaction, beginning with the substance on the left and proceeding to the product on the right. In other words, the x-axis follows bond breaking and bond formation in a particular reaction). A reaction such as shown in **Reaction 6.2** would have an energy diagram like that shown in **Fig. 6.1**. In reactions such as **Reaction 6.1** where a relatively stable intermediate is formed, the energy diagram would resemble that shown in **Fig. 6.2**. To get to the intermediate, the reaction must pass through a transition state structure. In addition, a second transition state occurs between the intermediate and the final product. The height of the activation energy barrier ultimately sets the rate of the reaction, and the lower the E_a value, the faster the rate. It should be noted that the rate is exponentially related to the value of E_a .

In general a catalyst, such as an acid or base, provides a reaction pathway with a lower E_a . Enzymes also reduce the E_a for a reaction. As shown in **Fig. 6.3b**, the binding of a substrate to an enzyme properly positions it for the reaction, resulting in a large reduction in the height of E_a . This is the so-called proximity effect, and it causes a very large enhancement in reaction rate (see **Fig. 6.11** for an illustration of proximity effects in experiments comparing a non-enzymatic *biomolecular* reaction with a series of chemically similar *intramolecular* reactions).

The second major boost to the rate derives from the fact that the active site is closely complementary to the transition state structure. The enzyme stabilizes (and reduces the energy of) the transition state structure by establishing noncovalent bonds to it (**Fig. 6.3c**). The lowering of the E_a that occurs as a result of binding the transition state increases the likelihood that the transition state structure will form and convert to the product. Transition-state stabilization is considered the major factor in enzyme catalysis.

As proof that the active site is most complementary to the transition state structure, chemicals known as transition-state analogs, which resemble the structure of the transition state, have been shown to bind to enzymes with higher affinity than substrates. Due to tight binding, many of these molecules are good inhibitors of enzymes. For example, the antibiotic, penicillin, inhibits the transpeptidase enzyme that catalyzes cross-linking of bacterial cell wall because it resembles the transition state for this reaction. It makes sense that enzymes are more complementary to the transition state than to their substrates. If they were most complementary to the substrate, they might bind them so tightly that the reaction would not be able to proceed.

Induced fit (Dan Koshland)

An enzyme is most effective if it is in the active form initially (so no energy is consumed in converting it to an active conformation). In some cases the enzymes are in an inactive conformation; when substrate molecules bind, the enzymes undergo major conformational transitions. This enzyme activation is called induced fit; it is not directly affecting the catalysis, but is primarily a substrate-specificity effect.

Koshland performed his experiments on an enzyme called hexokinase (catalyses the phosphorylation of glucose by ATP). Structural data show that hexokinase exists in two conformations: open, with no glucose bound, and closed, with glucose bound (**Fig. 6.13**). This sugar-induced closure of the active site prevents wasteful hydrolysis of ATP by water (water resembles the alcoholic group at C-6 of glucose and thus should be a good substrate, leading to hydrolysis of ATP; it is though not large enough to induce the closure of the enzyme). Since some of the binding energy is consumed in changing the conformation of the enzyme, it cannot be used for catalysis: thus induced-fit enzymes are less effective in catalysis.

IV. Chemical modes of enzymatic catalysis

Another way in which enzymes reduce the E_a for a reaction and increase reaction rates is by providing functional groups that directly react with the substrates or donate/accept protons used/generated

during the reaction. The first mode of catalysis is known as covalent catalysis, whereas the second is known as general acid-base catalysis. Both of these features of enzymes depend on the presence of polar amino acids within the active site.

A. Polar amino acid residues in active sites

The active site cavity of an enzyme is generally lined with hydrophobic aa residues. The side chains of the few polar amino acids located in the enzyme active site often participate directly in catalysis. As a result of the 3D fold of the enzyme, catalytic groups are ideally positioned to attack the appropriate bonds, transfer leaving groups to products, and bind to and stabilize the transition state structure. Some functions of the side-chains of ionizable amino acids in enzyme catalysis are summarized in **Table 6.1**. The typical pK_a values of these side-chains in enzymes are listed in **Table 6.2**. It is important to note that the local microenvironment of the enzyme active site often can significantly change pK_a s, as will be illustrated for the reactive serine side-chain in chymotrypsin.

B. General acid-base catalysis

In acid-base catalysis, the reaction rate is accelerated via proton transfers to and from the reactants. Enzymes can carry out general acid-base catalysis using side-chains which donate and accept protons under the nearly neutral conditions inside cells. As illustrated in **Reactions 6.6 & 6.7**, a basic group (proton acceptor) can assist in a reaction by removing a proton from the substrate leading to cleavage of that bond. It can also generate a potent nucleophile like OH^- by removing a proton from a water molecule residing within the active site. Acid groups (proton donors), on the other hand, can donate a proton to a leaving group in a chemical reaction promoting cleavage of a bond (**Reaction 6.8**). Because the side-chain of histidine has a pK_a near neutrality, it is an ideal group for proton transfer reactions at neutral pH values. Acid-base catalysis occurs during the mechanism of the chymotrypsin reaction.

C. Covalent catalysis

In covalent catalysis, a portion of the substrate is covalently bound to a functional group within the active site during the reaction. This is a transient event which is followed by a second reaction, in which a portion of the substrate is transferred to a second substrate. The general scheme is illustrated in **Reactions 6.9 & 6.10**. Most often

a nucleophilic side-chain attacks the substrate, attaching all or part of it to the enzyme. 20% of the enzymes employ covalent catalysis. A proof of covalent catalysis relies on isolation/identification of intermediates (which are sufficiently reactive). Again, covalent catalysis is observed in the mechanism of the chymotrypsin reaction.

D. pH affects enzymatic rates

The solution pH affects the ionization states of amino acids within proteins and enzymes (just as it affects ionizable groups in free amino acids); when the affected aa are involved in catalysis, the activity of the enzyme is affected. Enzymes are sensitive to pH because at pH extremes the ionization states of key side-chains in the active site may be incorrect for catalysis. Most enzymes display optimum activity across a relatively narrow range of pH, as illustrated in **Fig. 6.4** for the enzyme papain, which is used in meat tenderization.

The pHs at which the inflection points occur on the ascending and descending arms of the papain titration curve provide insight into the pK_a s of the functional groups within the active site. These inflection points occur at pH 4.2 and pH 8.2. The functional groups responsible for these pK_a s have been traced to a cysteine and a histidine within the active site. Note that the pK_a s of these residues differ considerably from the pK_a s of these two free amino acids due to the local microenvironment of the active site (compare with **Table 3.2**).

V. Properties of serine proteases

Serine proteases are enzymes that contain a reactive serine residue within the active site. There are a wide variety of these enzymes found in mammals, and they participate in diverse reactions such as acetylcholine cleavage (acetylcholinesterase), blood coagulation (factor XII protease and others), and digestion of dietary protein (chymotrypsin, trypsin, and elastase). The biology and mechanism of action of chymotrypsin is discussed here.

A. Zymogens are inactive enzyme precursors

Chymotrypsin is synthesized by the pancreas and is secreted into the small intestine where the final stages of protein digestion occur. The enzyme actually is synthesized in an inactive form (i.e., a zymogen) which is called chymotrypsinogen. It is converted to its active form by a proteolytic processing event catalyzed by trypsin and molecules of active chymotrypsin produced by earlier trypsin cleavage

(**Fig. 6.21**). (Trypsin itself is produced from cleavage of the zymogen trypsinogen by enteropeptidase which is secreted by the duodenum.) Chymotrypsinogen is cleaved at 4 locations, resulting in the release of two dipeptides. The cleaved form of the enzyme remains folded and is stabilized in part by 5 disulfide bonds. Cleavage creates a functional active site that is not present in chymotrypsinogen (**Fig. 6.22**).

B. Substrate specificity of serine proteases

The tertiary structures of chymotrypsin, trypsin, and elastase are all highly similar. In fact, the 3D folds of these enzymes are nearly superimposable (**Fig. 6.23**). All three enzymes contain the Ser-His-Asp catalytic triad (see below), and their mechanisms of catalysis are identical. However, their cleavage site specificities differ due to the structure of the binding pocket which interacts with the side-chain of the amino acid immediately on the C-terminal side of the cleavage site (**Fig. 6.24**). Chymotrypsin has a nonpolar pocket that can bind the R-groups of the aromatic amino acids. This explains why it is specific for cleaving after phenylalanine, tyrosine, and tryptophan. Trypsin has a negatively-charged pocket which is able to bind R-groups of the basic amino acids, arginine and lysine. Elastase has a very small binding pocket due to the projection of nearby valine and threonine side-chains into the active site. Therefore, elastase only can cleave after glycine and alanine whose side-chains are small and uncharged. An illustration of how chymotrypsin and trypsin will cleave a peptide is given in **Fig. 6.27** (please do not memorize, just look carefully and try to follow).

C. Overview of the chymotrypsin reaction mechanism

Chymotrypsin is called a serine protease because it contains a serine residue in its active site which is essential for activity. Early experiments showed that chymotrypsin can be irreversibly inactivated by reaction with the reagent diisopropyl fluorophosphate (DFP). Peptide mapping experiments indicated that DFP modifies Ser-195. Other serines in chymotrypsin are unreactive, suggesting Ser-195 is located in the active site. Ser-195 is the only reactive serine in the enzyme because the catalytic triad makes it a stronger nucleophile (has a lower pK_a) than the other serines. Other experimental data and x-ray crystallography ultimately revealed that Ser-195, His-57, and Asp-102 are located near one another in the active site. The three residues form what is called the "catalytic triad" (**Figs. 6.25 & 6.26**) which is important for carrying out catalysis.

During the chymotrypsin reaction, both covalent catalysis and general acid-base catalysis take place. Ser-195 functions as a nucleophile by attacking the peptide bond on the C-terminal side of aromatic residues. It transiently becomes covalently linked to the substrate. His-57 facilitates Ser-195 attack by acting as an acceptor (general base) of the serine hydroxyl group proton during the first stage of the reaction. It then facilitates release of the peptide leaving group by acting as a proton donor (general acid) during the second stage of the overall reaction. Asp-102 functions by stabilizing the positive charge that appears on His-57 during the catalytic cycle. A nonpolar binding "pocket" also is present in the active site. The aromatic side-chains of phe, tyr, or trp residues must be positioned in the pocket to align the substrate for cleavage. Lastly, the tetrahedral transition state that forms twice during the mechanism is stabilized by the enzyme. The combined modes of catalysis greatly increase the rate of the reaction above the uncatalyzed rate.