Principles of Enzyme Catalysis

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Review: Garrett and Grisham, "Enzyme Specificity and Regulation" (Chapt. 15) and "Mechanisms of Enzyme Action" (Chapt. 16), in *Biochemistry,* Second Edition, Saunders, Fort Worth, 1999.

Suggested reading: Benkovic, S.J. and Hammes-Schiffer, S. (2003) A perspective on enzyme catalysis *Science* **301**, 1196-1202; Garcia-Viloca, M., *et al.* (2004) How enzymes work: Analysis by modern rate theory and computer simulations. *Science* **303**, 186-195; Wolfenden, R. (2003) Thermodynamic and extrathermodynamic requirements of enzyme catalysis. *Biophys. Chem*. **105**, 559-572.

Enzymology, the study of enzymes, has evolved from a vitalistic view in which metabolic reactions occurred by special chemistry distinct from that of organic and inorganic chemistry to a precise understanding of their mechanisms at an atomic level. Enzymes operate by the same physical chemical properties that govern nonenzymatic reactions; however, the ability of the three dimensional structure of proteins to coordinate these physical properties in time and space allow enzymes to exploit these physical laws in ways not readily available otherwise. In these two lectures we shall examine the various factors contributing to the catalytic efficiency of enzymes.

I. The theory of catalysis

A. Catalysts rely on *kinetic rather than thermodynamic factors*. A **catalyst** acts by increasing the rate of a chemical reaction without being consumed in the process. Therefore, a single catalyst molecule can participate in multiple reaction cycles. The **rate**, or more correctly the **rate constant**, of the reaction is increased by stabilizing the **transition state** (*i.e*., lowering the activation energy $\Delta G^{\ddagger}$). Catalysts do not affect the thermodynamic stability of the reactant or product so they have no effect on the overall equilibrium constant. These concepts are illustrated best using a reaction coordinate diagram.

B. Properties of enzymes

1. **Rate accelerations** for different enzymes range from 10^6-10^{12} fold.

2. Enzymes are proteins having defined **active sites** capable of **substrate specificity**.

3. Enzymes also display **reaction path specificity** by guiding the reaction coordinate through a specific series of steps among multiple alternatives to a specific product.

4. Enzymes are subject to regulation by induced changes in protein conformation.

C. The physical chemistry of reactions.

1. The rate of chemical reactions is based on **collision theory**.

2. The reactants are present in a milieu of water molecules. Since chemical reactions involve reactive functional groups that are usually charged or polar, these groups are shielded by tightly bound **waters of hydration** in **hydration spheres**. For the reaction to occur, the molecules must diffuse together, the rate of which is a function of temperature and solvent viscosity.

3. As the reactants diffuse together, they enter into an **encounter complex** in which it becomes statistically more likely that they will collide with one another than to diffuse apart. This occurs because the water molecules effectively cage the reactants. At room temperature approximately 150 collisions can occur between two reactant molecules within such a collision complex before they are able to diffuse apart.

4. Within the encounter complex the reactants must collide with sufficient energy to exceed the **activation energy** ($\Delta G^{\ddagger}$) in order for the reaction to occur.

5. The geometry of the collision is critical since the reactants must also collide in the correct orientation for their respective reactive groups to interact. This frequently requires removing the waters of hydration (**desolvation**) from the reactive groups.

D. The irreversible thermodynamics of reactions

1. The rate constants for chemical reactions are formally described by **irreversible thermodynamics**. The activation energy ($\Delta G^{\ddagger}$) is the sum of the **enthalpy of activation** ($\Delta H^{\ddagger}$) and the **entropy of activation** ($\Delta S^{\ddagger}$).

2. These considerations suggest several means by which enzymes can stabilize the transition state (lower $\Delta G^{\ddagger}$) and increase the rate constant (and rate) of chemical reaction.

   a. By binding substrates in a Michaelis complex, enzymes increase the effect local concentration of reactants. Frequently the reactants can include amino acid groups of the protein active site (active site groups).

   b. Enzymes can also increase the effective local concentration of reacting species by directing reacting groups toward an optimal geometry in a process termed **orbital steering**.

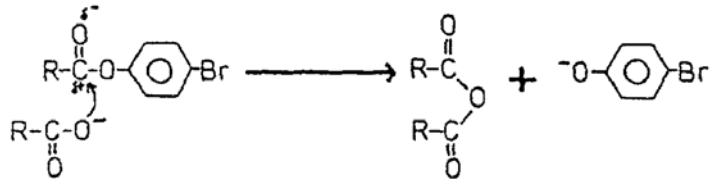
   c. Enzymes can promote desolvation.

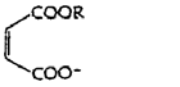
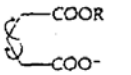
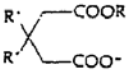
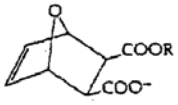
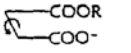
3. The reaction coordinate diagram for an enzyme catalyzed reaction contains an additional step representing substrate binding. Much of the unfavorable entropy of activation is removed from the activation step through compensation by the favorable enthalpy of activation (termed **entropy-enthalpy compensation**).

II. Contributions to enzyme catalysis

The rate accelerations exhibited by enzymes represent a composite of multiple quantifiable contributions. The identities of the contributions depend on the nature of the reaction catalyzed by the enzyme and the cellular environment (*e.g.*, pH, ionic strength, ion composition, and organelle among others) in which the reaction occurs.

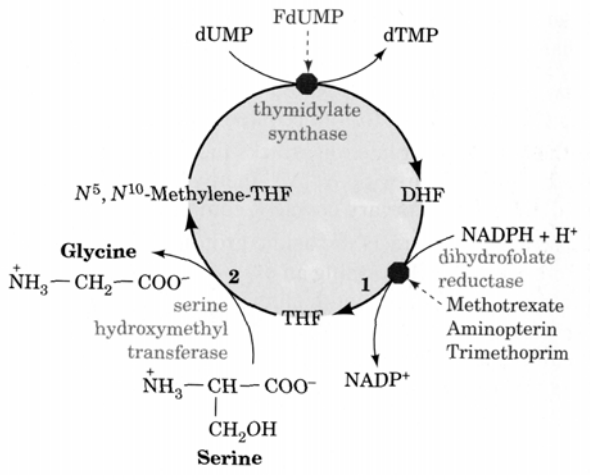
A. Since all enzyme-catalyzed reactions proceed through distinct enzyme-substrate Michaelis complexes, a major contribution to the catalytic efficiency of enzymes derives from **proximity effects** (also termed **anchimeric assistance**) which increase the effective local concentration of reactants within the active site. Such contributions are a direct consequence of collision theory and are predictable from kinetic equations for second and higher order reactions. The potential catalytic advantage of proximity effects were demonstrated in the late 70's through various studies of model intramolecular reactions. The most elegant examples of such studies are contained in a series of studies published by T.C. Bruice and colleagues at U.C. Santa Barbara.

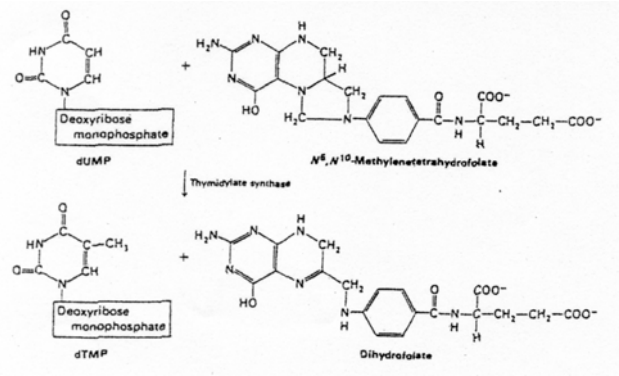


Structure*	Relative rate of hydrolysis	Structure	Relative rate of hydrolysis
$CH_3COO^- + CH_3COOR$	1.0		1×10^7
	$\sim 1 \times 10^3$		
	3×10^{3}† 1.3×10^6		$\sim 5 \times 10^7$
	$\sim 2.2 \times 10^4$		

From T.C. Bruice, *Ann. Rev. Biochem*. 49, 331 (1976)
R = *p*-Br-phenyl-
†Relative acceleration depends on nature of R groups.

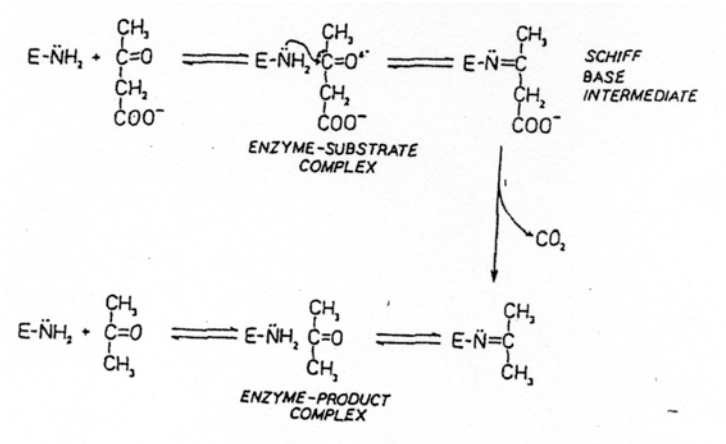
B. Based on thermodynamic arguments, Wolfenden demonstrated that all enzymes obligatorily must bind their respective transition states more tightly than either their substrate(s) or product(s). Tighter binding (lower K_{diss}) of the transition state by the enzyme requires the active site to physically distort the substrate/product structure to a configuration resembling the transitions state (**induced fit model**).



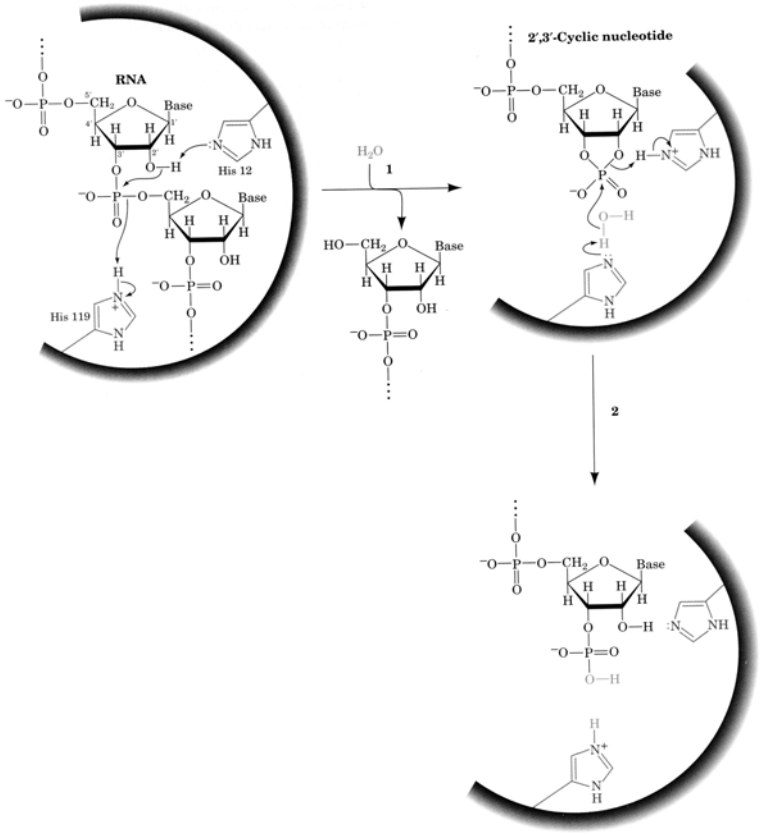


C. Desolvation of substrate and active site groups within the ES contact face produces a unique **microenvironment** of lower dielectric constant than bulk solution. This effect can be further enhanced by substrate binding induced conformational changes in the protein to engulf the bound substrate. The resulting **hydrophobic microenvironment** enhances the reactivity of nucleophiles and electrophiles, alters pK_a values, and stabilizes certain transition states. Strategically placed polar and charged groups can add to these effects.

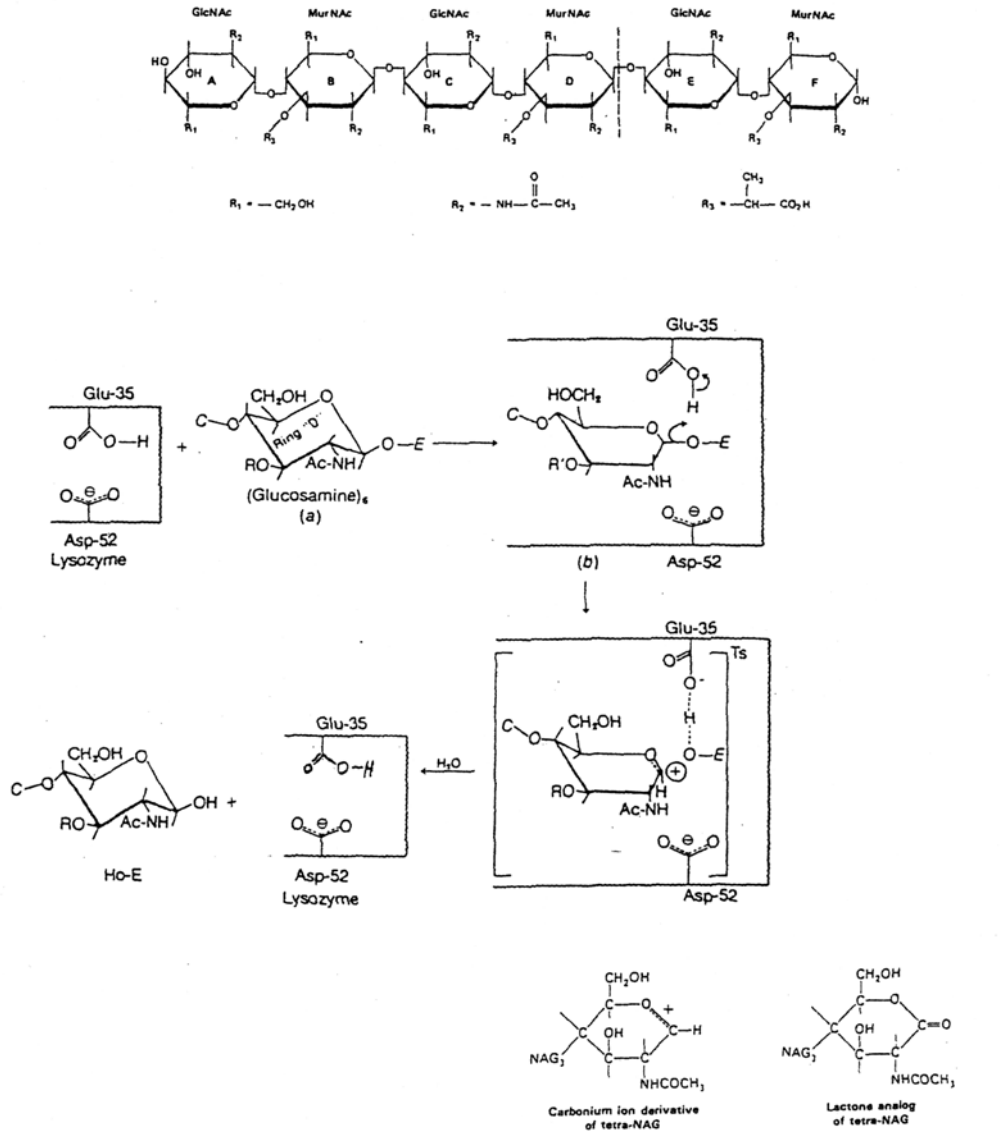
Charged or polar amino acids near reactive functional groups can alter pKa vales or reactivity. An extreme example of pKa alteration by a **charged microenvironment** is illustrated by **acetoacetate decarboxylase**.



C. Most chemical reactions require the abstraction and donation of H^+. **General acid-base catalysis** results if a suitable acid or base group is present to donate or abstract H^+. If the acid is **hydronium ion** (H_3O^+) or the base is hydroxide (OH^-) then this is termed **specific acid-base catalysis**. Enzymes enhance this effect by using proximity effects to precisely position the acid-base groups. The mechanism of **ribonuclease** serves as a good example of acid-base catalysis in a simple hydrolytic reaction involving RNA.

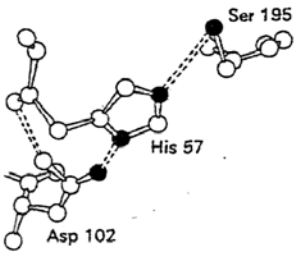


D. All of these factors are represented in the reaction of the well-characterized enzyme **egg white lysozyme**.

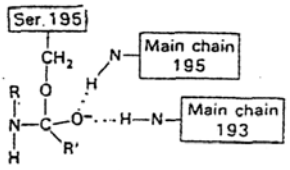


The lactone analog of tetra-NAG resembles the transition-state intermediate in the reaction catalyzed by lysozyme because its D ring has a conformation like that of a half-chair form.

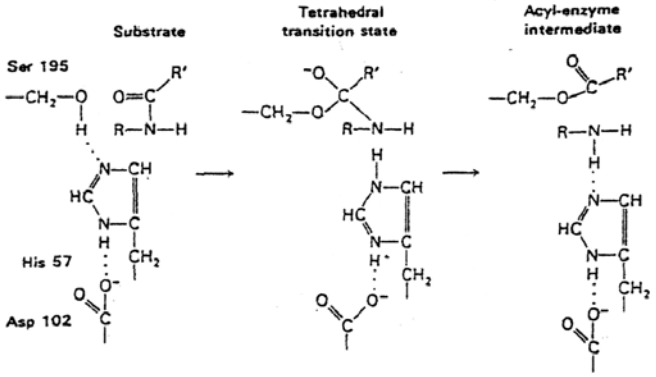
E. Enzymes can also exploit new mechanisms by **covalent catalysis**. Covalent catalysis results when a covalent enzyme-substrate intermediate is formed during the reaction cycle. Rate accelerations arise when the covalent intermediate is more reactive toward the final acceptor molecule than was the original substrate. This additional catalytic factor is exemplified by the superfamily of **serine proteases** which promote peptide bond hydrolysis.



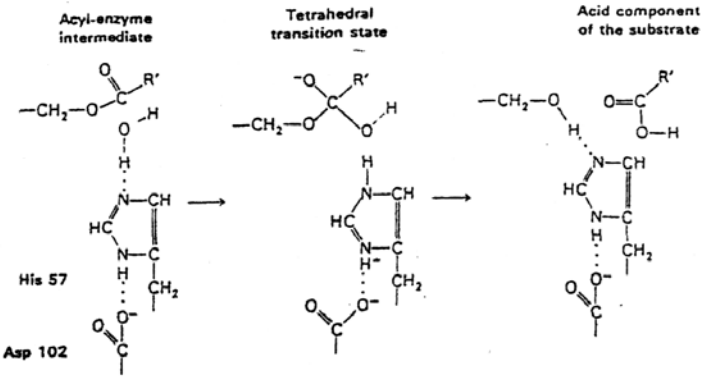
Conformation of the serine-histidine-aspartate catalytic triad in chymotrypsin. [After D. M. Blow and T. A. Steitz. X-ray diffraction studies of enzymes. *Ann. Rev. Biochem.* 39(1970):86. Copyright © 1970 by Annual Reviews Inc. All rights reserved.]



The tetrahedral transition state in the acylation reaction of chymotrypsin. The hydrogen bonds formed by two NH groups from the main chain of the enzyme are critical in stabilizing this species. This site is called the *oxyanion hole*.



First stage in the hydrolysis of a peptide by chymotrypsin: *acylation*. A tetrahedral transition state is formed, in which the peptide bond is cleaved. The amine component then rapidly diffuses away, leaving an acyl-enzyme intermediate.



Second stage in the hydrolysis of a peptide by chymotrypsin: *deacylation*. The acyl-enzyme intermediate is hydrolyzed by water. Note that deacylation is essentially the reverse of acylation, with water in the role of the amine component of the original substrate.