ENZYMOLOGY

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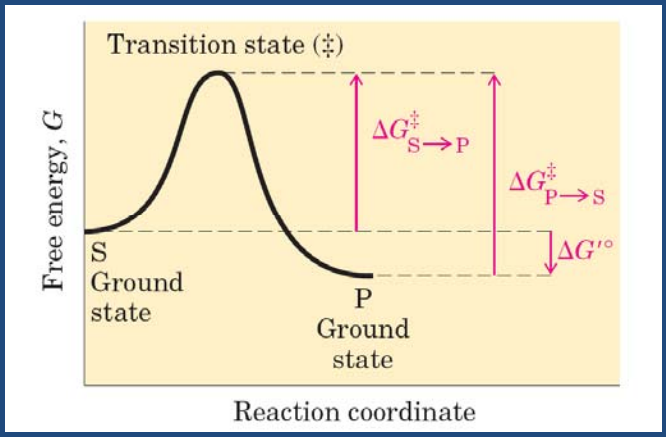
How Enzymes Work

- Enzymes are highly effective catalysts, commonly enhancing **reaction rates** by a factor of 105 to 1017.
- Enzyme-catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (an ES complex).
- Substrate binding occurs in a pocket on the enzyme called the **active site**.
- The function of enzymes and other catalysts is to *lower the activation energy*, $\Delta G‡$, *for* a reaction and thereby enhance the reaction rate.

Enzymes Affect Reaction Rates, Not Equilibria

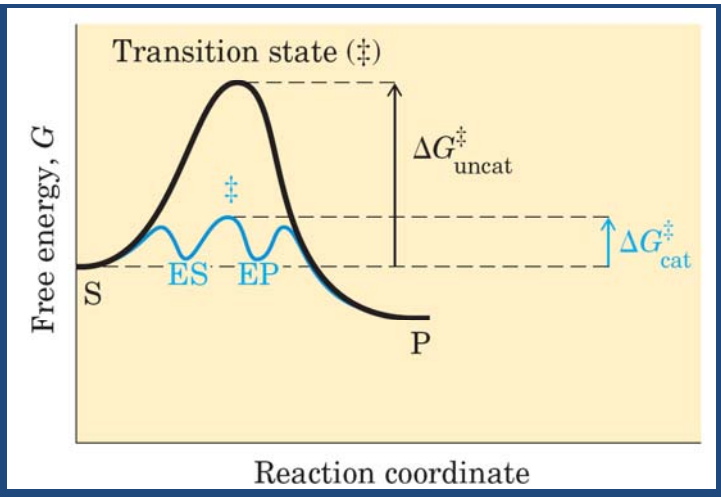
$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$



- temperature 298 K;
- partial pressure of each gas 1 atm, or 101.3 kPa;
- concentration of each solute 1 M

= **ΔG°, *the standard free energy* change.**

- Because biochemical systems commonly involve H concentrations far below 1 M, biochemists define a **biochemical standard free-energy change, $\Delta G'^{\circ}$**
- The difference between the energy levels of the ground state and the transition state is the **activation energy, $\Delta G^{\ddagger}$.**



‡rate-limiting step

$$K'_{eq} = \frac{[P]}{[S]}$$

$$\Delta G'^{\circ} = -RT \ln K'_{eq}$$

R is the gas constant, 8.315 J/mol K
T is the absolute temperature, 298 K (25 °C).

$$V = k[S]$$

TABLE 6-4 Relationship between K'_{eq} and $\Delta G'^{\circ}$

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^{1}	−5.7
10^{2}	−11.4
10^{3}	−17.1

Note: The relationship is calculated from $\Delta G'^{\circ} = -RT \ln K'_{eq}$ (Eqn 6-3).

$$V = k[S_1][S_2]$$

$$k = \frac{\mathbf{k}T}{h} e^{-\Delta G^{\ddagger}/RT}$$

k is the Boltzmann constant
h is Planck's constant

TABLE 6-5 Some Rate Enhancements Produced by Enzymes

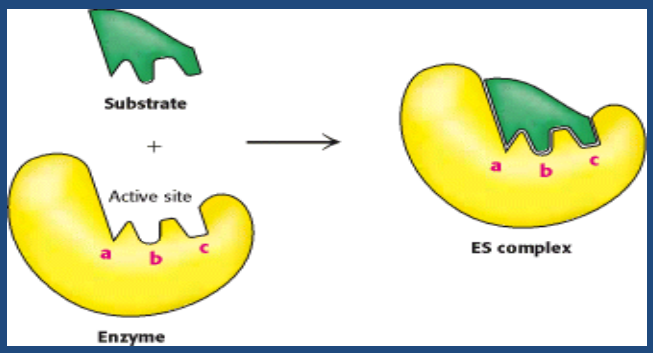
Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

- Two fundamental and interrelated principles provide a general explanation for how enzymes use noncovalent binding energy:

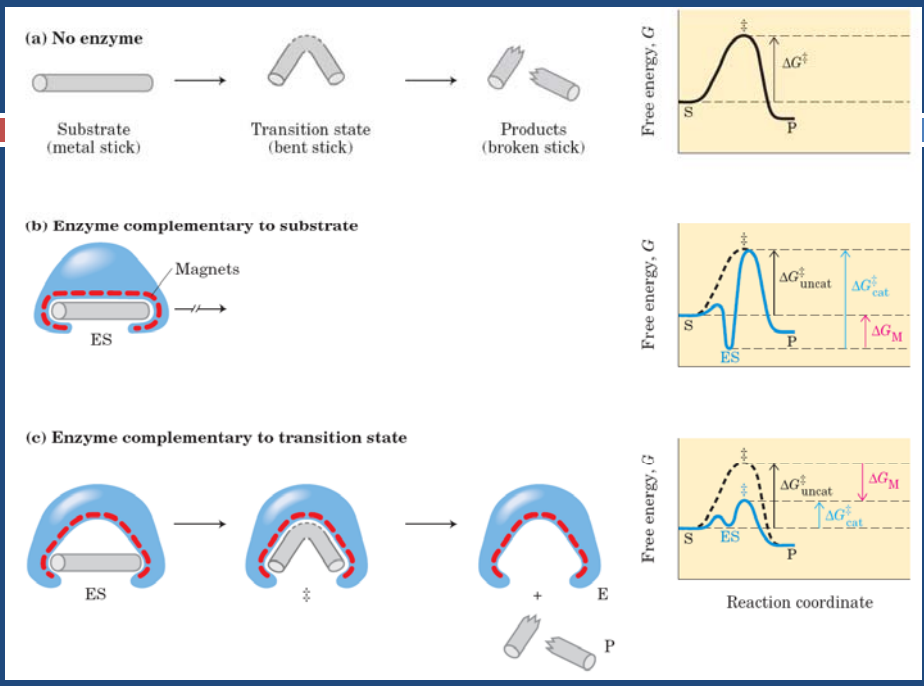
- 1. Much of the catalytic power of enzymes is ultimately derived from the free energy released in forming many weak bonds and interactions between an enzyme and its substrate. This binding energy contributes to specificity as well as to catalysis.

- 2. Weak interactions are optimized in the reaction transition state; enzyme active sites are complementary not to the substrates per se but to the transition states through which substrates pass as they are converted to products during an enzymatic reaction.

- **"lock and key"** (Emil Fischer., 1894)



- **"Induced fit"** (Michael Polanyi., 1921 and Haldane., 1930, Linus Pauling., 1946)



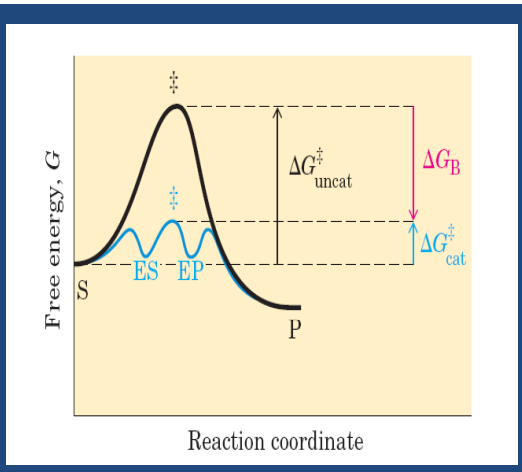


FIGURE 6-6 Role of binding energy in catalysis. To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $\Delta G^{\ddagger}$ is lowered. Much of this energy comes from binding energy (ΔG_B) contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state. The role of ΔG_B is analogous to that of ΔG_M in Figure 6–5.

Prominent physical and thermodynamic factors contributing to $\Delta G^{\ddagger}$, *the barrier to reaction*, might include…

- (1) a reduction in entropy, in the form of decreased freedom of motion of two molecules in solution; **entropy reduction**
- (2) the solvation shell of hydrogen-bonded water that surrounds and helps to stabilize most biomolecules in aqueous solution; **desolvation**
- (3) the distortion of substrates that must occur in many reactions;
- (4) the need for proper alignment of catalytic functional groups on the enzyme.
- *Binding energy can be used to overcome all these barriers.*

Specific Catalytic Groups Contribute to Catalysis

- general acid-base catalysis,
- covalent catalysis,
- metal ion catalysis

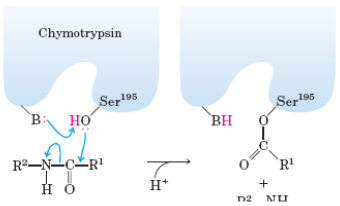


FIGURE 6–10 **Covalent and general acid-base catalysis.** The first step in the reaction catalyzed by chymotrypsin is the acylation step. The hydroxyl group of Ser^{195} is the nucleophile in a reaction aided by general base catalysis (the base is the side chain of His^{57}). This provides a new pathway for the hydrolytic cleavage of a peptide bond. Catalysis occurs only if each step in the new pathway is faster than the uncatalyzed reaction. The chymotrypsin reaction is described in more detail in Figure 6–21.

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—$\overset{H}{\overset{+}{N}}$H (H)	R—$\ddot{N}H_2$
Cys	R—SH	R—S⁻
His	R—C=CH, HN, $\overset{+}{N}$H, C, H	R—C=CH, HN, N:, C, H
Ser	R—OH	R—O⁻
Tyr	R—⟨⟩—OH	R—⟨⟩—O⁻

FIGURE 6–9 **Amino acids in general acid-base catalysis.** Many organic reactions are promoted by proton donors (general acids) or proton acceptors (general bases). The active sites of some enzymes contain amino acid functional groups, such as those shown here, that can participate in the catalytic process as proton donors or proton acceptors.