CHEMICAL REACTIONS AND HOW ENZYME WORKS

The randomness or disorder of the components of a chemical system is expressed as **entropy**, $\Delta \boldsymbol{S}$. Any change in randomness of the system is expressed as entropy change, d*S*, which by convention has a positive value when randomness increases. J. Willard Gibbs, who developed the theory of energy changes during chemical reactions, showed that the **free-energy content**, ***G***, of any closed system can be defined in terms of three quantities: **enthalpy**, ***H***, reflecting the number and kinds of bonds; entropy, *S;* and the absolute temperature, *T* (in Kelvin). The definition of free energy is $\Delta G = \Delta H - T\Delta S$.

When a chemical reaction occurs at constant temperature, the **free energy change**, $\Delta \boldsymbol{G}$, is determined by the enthalpy change, ΔH, reflecting the kinds and numbers of chemical bonds and noncovalent interactions broken and formed, and the entropy change, ΔS, describing the change in the system's randomness: where, by definition, ΔH is negative for a reaction that releases heat, and ΔS is positive for a reaction that increases the system's randomness.

A process tends to occur spontaneously only if ΔG is negative (if free energy is *released* in the process). Yet cell function depends largely on molecules, such as proteins and nucleic acids, for which the free energy of formation is positive: the molecules are less stable and more highly ordered than a mixture of their monomeric components. To carry out these thermodynamically unfavorable, energy-requiring (**endergonic**) reactions, cells couple them to other reactions that liberate free energy (**exergonic** reactions), so that the overall process is exergonic: the *sum* of the free-energy changes is negative.

A+B = C+ D

When a system has reached equilibrium, then the equilibrium constant (K_{eq}) = $\frac{[C][D]}{[A][B]}$

Where, free energy change, $\Delta \boldsymbol{G} = \Delta \boldsymbol{G^0} + \boldsymbol{RTln}\ K_{eq}$ ----------(1)

$\Delta \boldsymbol{G^0}$**=standard free-energy change**,

R**=***R* is the gas constant;

T**=**the absolute temperature.

When a reaction has reached equilibrium, no driving force remains and it can do no work: _*G* _ 0. So, (1) will be $\Delta \boldsymbol{G^0} = -\boldsymbol{RTln}\ K_{eq}$

When *K*eq >>1, $\Delta \boldsymbol{G^0}$is large and negative; when *K*eq << 1, $\Delta \boldsymbol{G^0}$is large and positive.

Enzymes Promote Sequences of Chemical Reactions

All biological macromolecules are much less thermodynamically stable than their monomeric subunits, yet they are *kinetically stable*: their *uncatalyzed* breakdown occurs so slowly (over years rather than seconds) that, on a time scale that matters for the organism, these molecules are stable. Virtually every chemical reaction in a cell occurs at a significant rate only because of the presence of **enzymes**—biocatalysts that, like all other catalysts, greatly enhance the rate of specific chemical reactions without being consumed in the

process. The path from reactant(s) to product(s) almost invariably involves an energy barrier, called the activation barrier **(Fig. 1–27)**, that must be surmounted for any reaction to proceed. The breaking of existing bonds and formation of new ones generally requires, first, a distortion of the existing bonds to create a **transition state** of higher free energy than either reactant or product. The highest point in the reaction coordinate diagram represents the transition state, and

the difference in energy between the reactant in its ground state and in its transition state is the **activation energy**, _*G*‡. An enzyme
catalyzes a reaction by providing a more comfortable fit for the transition state: a surface that complements the transition state in stereochemistry, polarity, and charge. The binding of enzyme to the transition state is exergonic, and the energy released by this binding reduces the activation energy for the reaction and greatly increases the reaction rate. A further contribution to catalysis occurs when two or more reactants bind to the enzyme's surface close to each other and with stereospecific orientations that favor the reaction. This increases by orders of magnitude the probability of productive collisions between reactants. As a result of these factors and several others, discussed in Chapter 6, enzyme-catalyzed reactions commonly proceed at rates greater than 1012 times faster than the uncatalyzed reactions. (That is a *million million* times faster!)

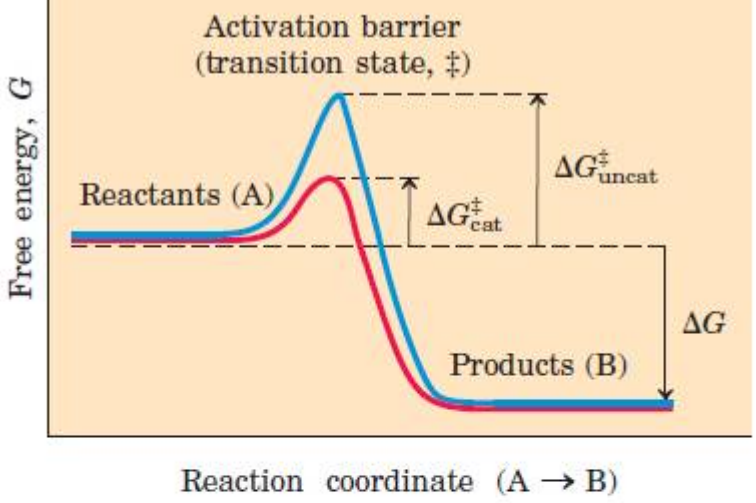


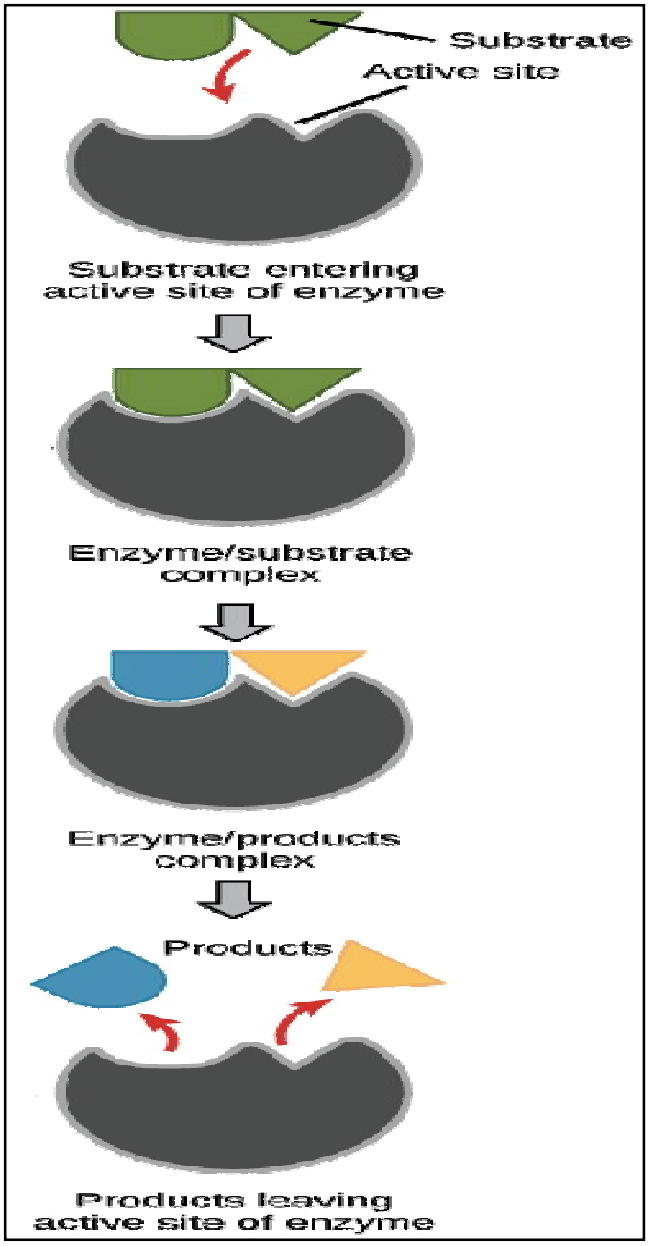
FIGURE 1–27 Energy changes during a chemical reaction. An activation barrier, representing the transition state (see Chapter 6), must be overcome in the conversion of reactants (A) into products (B), even though the products are more stable than the reactants, as indicated by a large, negative free-energy change (ΔG). The energy required to overcome the activation barrier is the activation energy ($\Delta G^{\ddagger}$). Enzymes catalyze reactions by lowering the activation barrier. They bind the transition-state intermediates tightly, and the binding energy of this interaction effectively reduces the activation energy from $\Delta G^{\ddagger}_{uncat}$ (blue curve) to $\Delta G^{\ddagger}_{cat}$ (red curve). (Note that activation energy is *not* related to free-energy change, ΔG.)

Active sites and substrate specificity

To catalyze a reaction, an enzyme will grab on (bind) to one or more reactant molecules. These molecules are the enzyme's **substrates**.

In some reactions, one substrate is broken down into multiple products. In others, two substrates come together to create one larger molecule or to swap pieces. In fact, whatever type of biological reaction you can think of, there is probably an enzyme to speed it up!

The part of the enzyme where the substrate binds is called the **active site** (since that’s where the catalytic “action” happens).



A substrate enters the active site of the enzyme. This forms the enzyme-substrate complex.The reaction then occurs, converting the substrate into products and forming an enzyme products complex. The products then leave the active site of the enzyme.

Proteins are made of units called amino acids, and in enzymes that are proteins, the active site gets its properties from the amino acids it's built out of. These amino acids may have side chains that are large or small, acidic or basic, hydrophilic or hydrophobic.

The set of amino acids found in the active site, along with their positions in 3D space, give the active site a very specific size, shape, and chemical behavior. Thanks to these amino acids, an enzyme's active site is uniquely suited to bind to a particular target—the enzyme's substrate or substrates—and help them undergo a chemical reaction.

Environmental effects on enzyme function

Because active sites are finely tuned to help a chemical reaction happen, they can be very sensitive to changes in the enzyme's environment. Factors that may affect the active site and enzyme function include:

- **Temperature.** A higher temperature generally makes for higher rates of reaction, enzyme-catalyzed or otherwise. However, either increasing or decreasing the temperature outside of a tolerable range can affect chemical bonds in the active site, making them less well-suited to bind substrates.

pH. pH can also affect enzyme function. Active site amino acid residues often have acidic or basic properties that are important for catalysis. Changes in pH can affect these residues and make it hard for substrates to bind. Enzymes work best within a certain pH range, and, as with temperature, extreme pH values (acidic or basic) can make enzymes denature.

Michaelis-Menten

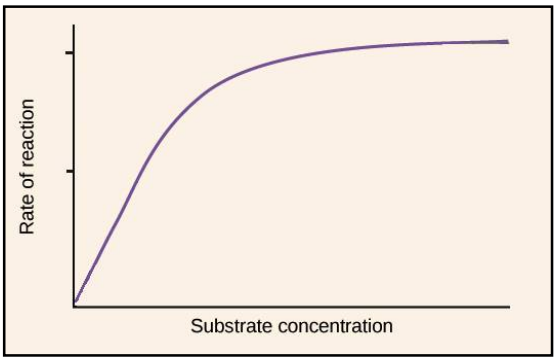
Many enzymes act similarly to the hypothetical enzyme in the example above, producing parabolic curves when reaction rate is graphed as a function of substrate concentration. Enzymes that display this behavior can often be described by an equation relating substrate concentration, initial velocity, K_m and V_{max}, known as the Michaelis-Menten equation . Enzymes whose kinetics obey this equation are called Michaelis-Menten enzymes.

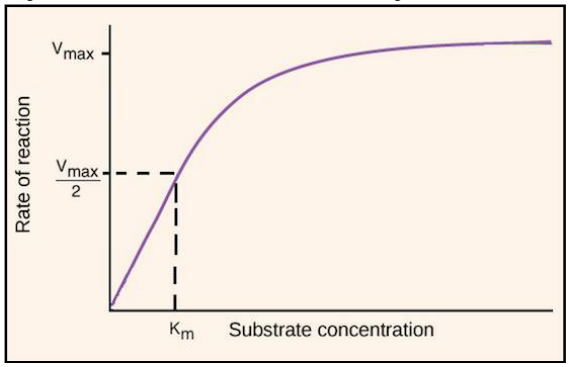
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

V0 = Initial velocity (moles/times)
[S] = substrate concentration (molar)
V_{max}= maximum velocity
K_m = substrate concentration at half V_{max}

- **Basic enzyme kinetics graphs**

- Graphs like the one shown below (graphing reaction rate as a function of substrate concentration) are often used to display information about enzyme kinetics.





- Enzyme kinetics graph showing rate of reaction as a function of substrate concentration.
- Imagine that you have your favorite enzyme in a test tube, and you want to know more about how it behaves under different conditions. So, you run a series of trials in which you take different concentrations of substrate - say, 0 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M - and find the rate of reaction (that is, how fast your substrate is turned into product) when you add enzyme in each case. Of course, you have to be careful to add the same concentration of enzyme to each reaction, so that you are comparing apples to apples.
- How do you determine the rate of reaction? Well, what you actually want is the initial rate of reaction, when you've just combined the enzyme and substrate and the enzyme is catalyzing the reaction as fast as it can at that particular substrate concentration (because the reaction rate will eventually slow to zero as the substrate is used up). So, you would measure the amount of product made per unit time right at the beginning of the reaction, when the product concentration is increasing linearly. This value, the amount of product produced per unit time at the start of the reaction, is called the **initial velocity**, or V_0,

- Enzyme kinetics graph showing rate of reaction as a function of substrate concentration, with Vmax (maximum velocity) and Km (substrate concentration giving reaction rate of 1/2 Vmax) marked.
- This plateau occurs because the enzyme is **saturated**, meaning that all available enzyme molecules are already tied up processing substrates. Any additional substrate molecules will simply have to wait around until another enzyme becomes available, so the rate of reaction (amount of product produced per unit time) is limited by the concentration of enzyme.

EC 6 Ligases joining to molecule together at the expense of an high-energy (th

Ligases catalyze the formation of C–C, C–S, C–O, and C–N bonds. The energy for these reactions is always supplied by ATP hydrolysis. Other common names for ligases include *synthetases*, because they are used to synthesize new molecules.

$$A + B + ATP \longrightarrow A-B + ADP$$

Example

Carboxylases — Use CO_2 as a substrate.

Biochemical nomenclature distinguishes synthetases from synthases. *Synthases* catalyze condensation reactions in which no nucleoside triphosphate (ATP and GTP) is required as an energy source. *Synthetases* catalyze condensations that *do* use nucleoside triphosphate as a source of energy for the synthetic reaction. A synthase is a lyase and does not require any energy, whereas a synthetase is a ligase and thus requires energy.

1.12.2 What enzyme does?

A chemical reaction between two substances occurs only when an atom, ion, or molecule of one collides with an atom, ion, or molecule of the other. Only a fraction of the total collisions result in a reaction, because usually only a small percentage of the molecules interacting have the minimum amount of kinetic energy that a molecule must possess for it to react. When the reactants collide, they may form an intermediate product whose chemical energy is higher than the combined chemical energy of the reactants. In order for this transition state in the reaction to be achieved, some energy must enter into the reaction other than the chemical energy of the reactants. The transition state is the one with the highest free energy. The difference in free energy between the transition state and the reactants is called the *Gibbs free energy of activation* or simply the *activation energy*.

An enzyme lowers the activation energy of a reaction, thereby increasing the fraction of molecules that have enough energy to attain the transition state and making the reaction go faster in both directions. However, the catalyst does not change the relative energies of the initial and final states. The free energy of reaction, $\Delta G°$, remains unchanged in the presence of a catalyst, so the relative amounts of reactants and products at equilibrium are unchanged. In other words, a catalyst does not influence the position of equilibrium. It only increases the rate of a reaction by lowering the activation energy.

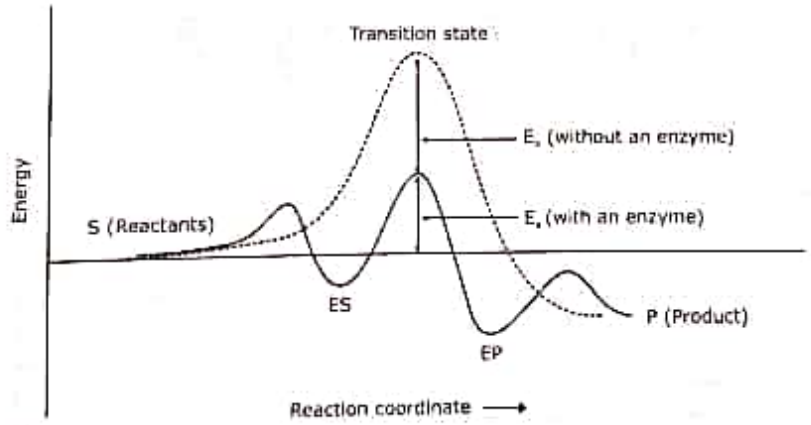
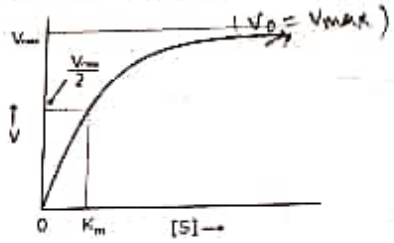


Figure 1.88 Energy profile of a simple enzyme-catalyzed reaction. The non-enzyme catalyzed reaction proceeds via a higher energy transition state and hence the reaction has a higher activation energy than the enzyme catalyzed reaction.

In this equation k_1 is the rate constant for the formation of the [illegible] E, and the substrate, S; k_{-1} is the rate constant for the reverse reaction, dissociation of the ES complex to free enzyme and substrate; and k_2 is the rate constant for the conversion of the ES complex to product P and the subsequent release of product from the enzyme.

According to Michaelis-Menten approach, when the rate (also called the velocity) of an enzyme catalyzed reaction is measured at varying substrate concentrations, the rate depends on the substrate concentrations [S]. At a relatively low concentration of substrate, initial velocity (V) increases almost linearly with an increase in a substrate concentration. The reaction reaches a maximum velocity (V_{max}) with an increase in substrate concentration and it doesn't increase any further by increasing the concentration of substrate.



The hyperbolic relationship between initial velocity (V) and substrate concentration [S] of an enzyme catalyzed reaction.

Concentration of substrate at which reaction velocity reaches half its maximum velocity is called **Michaelis constant** (K_m). It is the ratio of constants $(k_{-1} + k_2)/k_1$. The K_m is expressed as mole of substrate per litre. Lower value of K_m describes the greater affinity of the enzymes for the substrate.

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

Michaelis and Menten put forward a mathematical equation to establish the mathematical relationship among the quantities [E], [S], V_{max} and K_m.

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

1. When $[S] << K_m$, then $V \propto [S]$
2. When $[S] >> K_m$, then $V = V_{max}$
3. When $[S] = K_m$, then $V = \frac{V_{max}}{2}$