**Chapter 6 Enzymes**

* A bag of sugar can remain on the shelf for years without any obvious conversion to CO2 and H2O. Although this chemical process is thermodynamically favorable, it is very slow.
* When sucrose is consumed by a human (or almost any other organism), it releases its chemical energy in seconds.
* The difference is catalysis.
* Without catalysis, chemical reactions such as sucrose oxidation could not occur on a useful time scale, and thus could not sustain life.
* The reaction catalysts of biological systems are called **enzymes**.

**6.1 An Introduction to Enzymes**

**Most Enzymes Are Proteins**

* With the exception of a small group of catalytic RNA molecules, all enzymes are proteins.
* Enzymes have molecular weights ranging from about 12,000 to more than 1 million.
* Some enzymes require no chemical groups for activity other than amino acid residues.
* Others require an additional chemical component called a **cofactor**
* inorganic ions (Fe2+, Mg2+, Zn2+) **(Table 6-1).**
* complex organic or metalloorganic molecule called a **coenzyme** (**Table 6-2).**
	+ most are derived from vitamins, organic nutrients required in small amounts in the diet.





* Some enzymes require both a coenzyme and one or more metal ions for activity.
* A cofactor is very tightly or covalently bound to the enzyme, it is called a **prosthetic group**.
* Enzyme with its cofactor is called a **holoenzyme**.
* Enzyme without its cofactor is called the **apoenzyme** or **apoprotein**.
* Some enzymes are modified covalently by phosphorylation, glycosylation, and other processes.

**Enzymes Are Classified by the Reactions They Catalyze**

* Many enzymes have been named by adding the suffix “-ase” to the name of their substrate or to a word or phrase describing their activity (urease catalyzes hydrolysis of urea).
* Other enzymes have names that do not denote their substrates or reactions (pepsin catalyzes digestion of foods).
* Sometimes the same enzyme has two or more names, or two different enzymes have the same name.
* Because of such ambiguities, a system for naming and classifying enzymes has been adopted by international agreement.
* This system divides enzymes into six classes, each with subclasses, based on the type of reaction catalyzed **(Table 6-3)**.



* Each enzyme is assigned a four-part classification number and systematic name.
* For example, ATP + Glucose ADP + Glucose 6-phosphate

ATP : glucose phosphotransferase

* + it catalyzes the transfer of a phosphoryl group from ATP to glucose.
	+ its Enzyme Commission number (E.C. number) is 2.7.1.1.
	+ (2) denotes the class name (transferase).
	+ (7) denotes the subclass (phosphotransferase).
	+ (1) denotes a phosphotransferase with a hydroxyl group as acceptor.
	+ (1) denotes glucose as the phosphoryl-group acceptor.
* For many enzymes, a trivial name is more commonly used (hexokinase).
	1. **How Enzyme Works**
* The enzymatic catalysis of reactions is essential to living systems.
* An enzyme-catalyzed reaction takes place within the confines of a pocket on the enzyme which is called the **active site**.
* amino acid residues at the active site are important.
* The molecule is bound in the active site and acted upon by the enzyme which is called the **substrate (Fig. 6-1).**





**Enzymes Affect Reaction Rates, Not Equilibria**

* A simple enzymatic reaction might be written

E + S ES EP E + P

* E, S and P represent the enzyme, substrate and product.
* ES and EP are transient complexes of the enzyme with the substrate and with the product.
* The function of a catalyst is to increase the rate of a reaction.
* Catalysts do not affect reaction equilibria.
* There is an energy barrier between S and P.
* The activation energy can be lowered by adding a catalyst.
* Enzymes increase reaction rates by lowering activation energies (**Fig. 6-3)**.





**A Few Principles Explain the Catalytic Power and Specificity of Enzymes**

* Covalent bonds and non-covalent interactions between enzyme and substrate occur during an enzyme-catalyzed reaction.
* Weak interactions between enzyme and substrate are optimized in the transition state.
* The weak interactions make the primary contribution to catalysis.
* There are two models for binding between S and E.
* the **lock and key** model
* the enzyme is completely complementary to its substrate.
* the **induced fit** model is used by almost every enzyme.
* the enzyme itself usually undergoes a change in conformation when the substrate binds.

**Specific Catalytic Groups Contribute to Catalysis**

* When a substrate is bound to an enzyme, catalytic functional groups aid in bond cleavage and formation by a variety of mechanisms.
* **General acid-base catalysis**, it uses only the H+ or OH- ions present in water, organic acids and organic bases.
* **Covalent catalysis**, a transient covalent bond is formed between the enzyme and the substrate.
* **Metal ion catalysis**, metals (bound to the enzyme or taken up from solution along with the substrate) can participate in catalysis.

**6.3 Enzyme Kinetics as an Approach to Understand Mechanism**

* Central approach to studying the mechanism of an enzyme-catalyzed reaction is to determine the **rate** of a reaction.
* How is the rate changed in experimental parameters, a discipline known as **enzyme kinetics**.

**Substrate Concentration Affects the Rate of Enzyme-Catalyzed Reaction**

* The effect on V0 of varying [S] when the enzyme concentration is held constant is shown in **(Fig. 6-11)**.



*k1*

*k2*

E + S ES E + P

*k-2*

*k-1*

* At low [S],
* V0 increases linearly with an increase in [S].
* most of the E is in the uncombined form E.
* [S] at which V0 is half maximal is **Km**, the **Michaelis constant**.
* At higher [S],
* V0 increases by smaller with an increase in [S].
* most of the E is in form ES.
* The ES complex is the key to understanding this kinetic behavior.
* At the highest [S],
* V0 increases by vanishingly small as [S] increases.
* all of the E is in form ES. E is saturated with its S. Further increases in [S] have no effect on rate.
* V0 is close to the **maximum velocity**, **Vmax**.

**The Relationship between Substrate Concentration and Reaction Rate Can Be Expressed Quantitatively**

* *k-2* can be ignored.
* ( *k2 + k-1* )/ *k1* = **Km**, **Michaelis constant**
* It can be expressed by the **Michaelis-Menten equation**

 Vmax [S]

V0 =

 Km + [S]

 Vmax [S]

V0 =

 KM + [S]

**Kinetic Parameters Are Used to Compare Enzyme Activities**

* Both the magnitude and the real meaning of Vmax and Km can differ from one enzyme to the next.
* The Km can vary greatly from enzyme to enzyme and even for different substrates of the same enzyme **(Table 6–6)**.



* It is useful to define a more general rate constant, **kca**t, to describe the limiting rate of any enzyme-catalyzed reaction at saturation.
* When the single enzyme molecule is saturated with substrate, the number of substrate molecule is converted to product in a second. It is called **turnover number** **(Table 6-7)**.



* The Michaelis-Menten equation can be transformed into **Lineweaver-Burk equation**



* is more useful in plotting experimental data **(Lineweaver-Burk plot)**.



* is more useful in practical accurate determination Km and of Vmax.
* is very useful in distinguishing between certain types of enzymatic reaction mechanisms.
* is very useful in analyzing enzyme inhibition.

**Enzymes Are Subject to Reversible or Irreversible Inhibition**

* Enzyme inhibitors are molecular agents.
* They interfere with catalysis, slowing or halting enzymatic reactions.
* There are two classes of enzyme inhibitors: **reversible** and **irreversible**.
* Reversible inhibition can be **competitive**, **uncompetitive** or **mixed** **(Fig. 6-15)**.
* A **competitive inhibitor** competes with the substrate for the active site of an enzyme.
* An **uncompetitive inhibitor** binds at a site distinct from the substrate active site, binds only the ES complex.
* A **mixed inhibitor** also binds at a site distinct from the substrate active site, but it binds to either E or ES.





* Lineweaver-Burk plot offers an easy way of determining whether an enzyme inhibitor is competitive, uncompetitive or mixed. **(Fig. 1, 2, 3) (Table 6-9).**



 [E] [I]

KI =

 [EI]

 [I]

 = 1 +

 KI

* Competitive inhibition
* Vmax is unchanged
* Km is increased by the factor
* Uncompetitive inhibition

 [ES] [I]

KI’  =

 [ESI]

 [I]

' = 1 +

 KI’

* Vmax is decreased by the factor '
* Km is decreased by the factor'
* Mixed inhibition
* Vmax is decreased by the factor '
* Km is increased by the factorand decreased by the factor '
* The special case of = ', rarely, classically has been defined as **noncompetitive inhibition**. Vmax is decreased by the factorand Km is unchanged.



 Noncompetitive Vmax/’ KM

* **Irreversible inhibitors** bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme’s activity, or form a particularly stable noncovalent association.

**Enzyme Activity Depends on pH**

* Enzymes have an optimum pH (or pH range) at which their activity is maximal (**Fig. 6-18)**;at higher or lower pH, activity decreases.





* Amino acid side chains in the active site may act as weak acids and bases with critical functions.