M.Sc. Botany

Semester-II (2018-20)

MBOTCC-7: Physiology & Biochemistry

Unit –IV
ENZYMOLOGY

Nitu Bharti
Assistant Professor
Department of Botany

ENZYMES

Frederick W. Kühne later gave the name **enzymes** (from the Greek *enzymos*, "leavened") to the molecules detected by Buchner.
Enzymes are biocatalysts that accelerate the rate of chemical reactions without permanent alteration of themselves,under milder reaction conditions in terms of temperature, pressure and pH.

Structure and Classification:

Virtually all enzymes are proteins or conjugated proteins, except some catalytically active RNAs (ribozyme), DNAs and antibodies (abzyme).

1. Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million.
2. Their catalytic activity depends on the integrity of their native protein conformation. The primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.
3. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component. A complete, catalytically active enzyme together with its bound chemical component is called a holoenzyme.

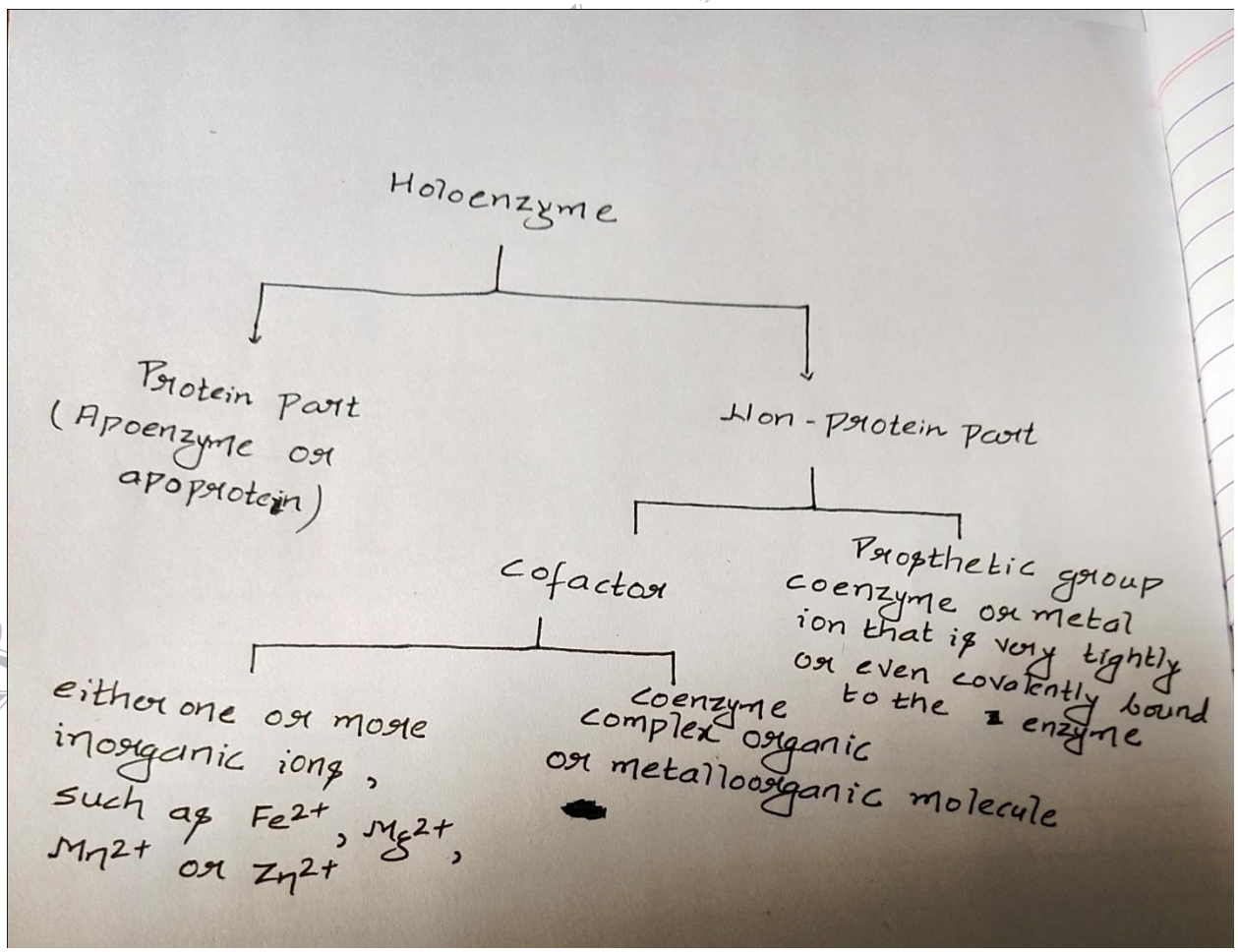

TABLE 6-1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

4. Coenzymes act as transient carriers of specific functional groups . Most are derived from vitamins, organic nutrients required in small amounts in the diet

TABLE 6-2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names. system divides enzymes into six classes, each with subclasses, based on the type of reaction catalyze..

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes. As an example, the formal systematic name of the enzyme catalyzing the reaction

$$\text{ATP} + \text{D-glucose} \rightarrow \text{ADP} + \text{D-glucose 6-phosphate}$$

is ATP:D-hexose 6-phosphotransferase, which indicates that it catalyzes the transfer of a phosphoryl group from ATP to glucose. Its Enzyme Commission number (E.C. number) is 2.7.1.1. The first number (2) denotes the class name (transferase); the second number (7), the subclass (phosphotransferase); the third number (1), a phosphotransferase with a hydroxyl group as acceptor; and the fourth number (1), D-glucose as the phosphoryl group acceptor.

Mechanism of enzyme action

- Enzymes are highly effective catalysts, commonly enhancing reaction rates by a factor of 10^5 to 10^{17}.

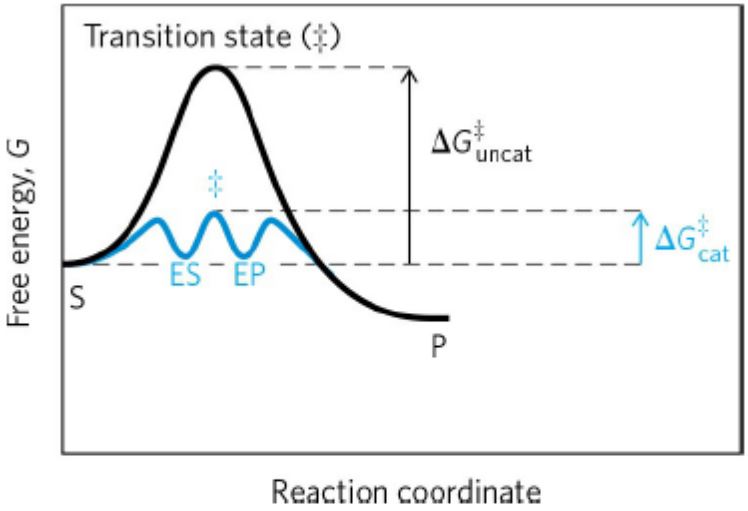

FIGURE 6-3 Reaction coordinate diagram comparing enzymecatalyzed and uncatalyzed reactions. In the reaction S → P, the ES and EP intermediates occupy minima in the energy progress curve of the enzymecatalyzed reaction. The terms $\Delta G^{\ddagger}_{uncat}$ and $\Delta G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction, respectively. The activation energy is lower when the enzyme catalyzes the reaction.

- A simple enzymatic reaction might be written as

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

where 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.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, $\Delta G^{\ddagger}$, for a reaction and thereby enhance the reaction rate. The equilibrium of a reaction is unaffected by the enzyme.

- The difference between the energy levels of the ground state and the transition state is the **activation energy, $\Delta G^{\ddagger}$**. The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction.

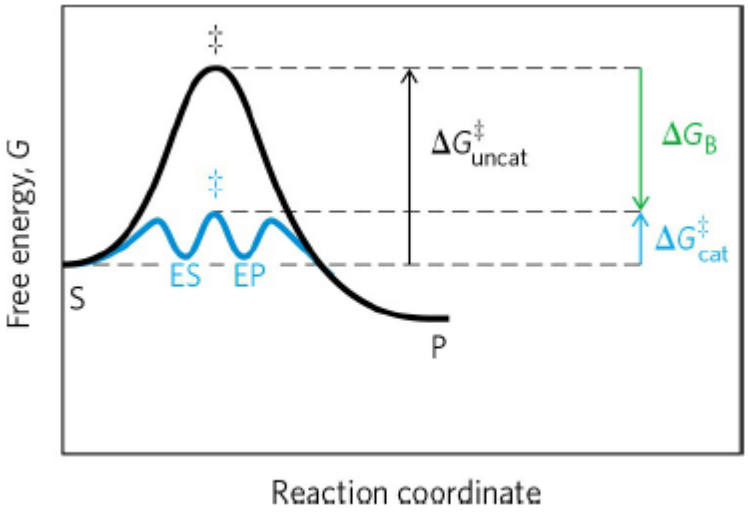

FIGURE 6-6 Role of binding energy in catalysis. To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $\Delta G^{\ddagger}$ is lowered. Much of this energy comes from binding energy, ΔG_B, contributed by formation of weak noncovalent 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.

- A significant part of the energy used for enzymatic rate enhancements is derived from weak interactions (hydrogen bonds, aggregation due to the hydrophobic effect, and ionic interactions) between substrate and enzyme.The enzyme active site is structured so that some of these weak interactions occur preferentially in the reaction transition state, thus stabilizing the transition state.
- The need for multiple interactions is one reason for the large size of enzymes. The binding energy, ΔG_B, is used to offset the energy required for activation, $\Delta G^{\ddagger}$, in several ways—for example, lowering substrate entropy, causing substrate desolvation, or causing a conformational change in the enzyme (induced fit). Binding energy also accounts for the exquisite specificity of enzymes for their substrates.
- Additional catalytic mechanisms employed by enzymes include general acid-base catalysis, covalent catalysis, and metal ion catalysis. Catalysis often involves transient covalent interactions between the substrate and the enzyme, or group transfers to and from the enzyme, to provide a new, lower energy reaction path. In all cases, the enzyme reverts to the unbound state once the reaction is complete.

Isozymes

The term 'iszoyme' was first defined by Markert and Moller in 1959.

- Isozymes are defined as multiple molecular forms of an enzyme catalyzing the same reaction.
- The different forms (isoforms) of the enzyme generally differ in kinetic or regulatory properties, in the cofactor they use (NADH or NADPH for dehydrogenase isozymes, for example), or in their subcellular distribution (soluble or membrane-bound).
- They are physically distinct and differ in electrophoretic mobility and liability to inhibitors.
- Isozymes may have similar, but not identical, amino acid sequences, and in many cases they clearly share a common evolutionary origin.
- Three major categories of isozymes are tabulated as:

(a) Isozymes resulting from multiple gene loci coding distinctly different polypeptide chains;
(b) Isozymes arising as a result of multiple alleles at a single locus;

(c) Isozymes which are formed as a result of secondary modifications (covalent modifications, conformational isomers, and the formation of monomers, dimmers, trimers etc;) of a basic subunit which may be composed of more than one polypeptide chain.

- Many enzymes are present in isozyme form:

1. Lactate dehydrogenase
2. Creatine kinase
3. Acid phosphatase
4. Alkaline phosphatase

- One of the first enzymes found to have isozymes was lactate dehydrogenase , which in vertebrate tissues exists as at least five different isozymes separable by electrophoresis. All LDH isozymes contain four polypeptide chains (each of Mr 33,500), each type containing a different ratio of two kinds of polypeptides. The M (for muscle) chain and the H (for heart) chain are encoded by two different genes.
- In skeletal muscle the predominant isozyme contains four M chains, and in heart the predominant isozyme contains four H chains. Other tissues have some combination of the five possible types of LDH isozymes:

Type	Composition	Location
LDH_1	HHHH	Heart and erythrocyte
LDH_2	HHHM	Heart and erythrocyte
LDH_3	HHMM	Brain and kidney
LDH_4	HMMM	Skeletal muscle and liver

Multienzyme Complexes

Multienzyme complexes are discrete and stable structures composed of enzymes associated noncovalently that catalyze two or more sequential steps of a metabolic pathway.

- They can be considered a step forward in the evolution of catalytic efficiency as they provide advantages that individual enzymes, even those that have achieved catalytic perfection, would not have alone.
- The proximity of the different types of enzymes increases the efficiency of the pathway: the overall reaction rate is increased with respect to catalysis by unassociated units, in several ways, briefly describe below.
    1. The diffusion of substrates and products in the bulk solvent is minimized This leads to the production of high local concentrations, even when their intracellular concentration is low. In turn this leads to an increase in the frequency of enzyme –substrate collisions.
    2. The time required by substrates to diffuse between successive active sites is minimized.
    3. The probability of side reactions is minimized.
    4. Chemically labile intermediates are protected from degradation by the solvent.

Examples of multienzyme complexes

A classical example of multienzyme complexes are

a. Pyruvate dehydrogenase complex (PDC)
b. Branched-chain α-keto acid dehydrogenase complex
c. α-ketoglutarate dehydrogenase complex**,** also called 2-oxoglutarate dehydrogenase complex

These three complexes belongs to the **2-ketoacid dehydrogenase family**, also called 2-oxoacid dehydrogenase family.r

d. Carbamoyl phosphate synthetase complex

Pyruvate Dehydrogenase Complex

This multienzyme complex catalyses the conversion of pyruvate and coenzyme A to acetyl CoA

Pyruvate + CoA + NAD+ ⟶ acetyl CoA+ carbon dioxide + NADH

There are four stages in this pathway, which are catalyzed by three enzymes:

"E1"- pyruvate dehydrogenase

This enzyme catalyzes the decarboxylation of pyruvate. This involves the prosthetic group **thiamine pyrophosphate (TPP)**

"E2" – dihydrolipoyl transacetylase

Two steps of the pathway are catalyzed by this enzyme:

- Oxidation of the 2-carbon (acetyl) unit, which is transferred to the **lipoamide** prosthetic group of the enzyme, giving an **acetyllipoamide** group
- Transfer of the acetyl group from the lipoamide to CoA, giving acetyl CoA.

"E3"- dihydrolipoyl dehydrogenase

Finally, this enzyme regenerates the oxidized form of of lipoamide. This involves the **FAD** prosthetic group

- TPP, lipoamide and FAD are **catalytic cofactors** which remain unaltered by the net reaction, whereas CoA and NAD+ are **stoichiometric cofactors.**

Allosteric enzymes (Greek 'allo' means 'other')

Allosteric enzymes are enzymes which have additional site/s, called allosteric site/s called allosteric modulators or allosteric effectors
as well as the active site.

- Allosteric enzymes function through reversible, noncovalent binding of regulatory compounds called allosteric modulators or allosteric effectors,which are generally small metabolites or cofactors.
- Allosteric enzymes are larger and more complex than non-allosteric enzymes and often have many subunits
- Enzymes with more than one effector have different and specific binding sites for each one.
- The substrate binding site is on the **catalytic subunit**-often referred to as the **C subunit**. The effector binding site is on the **regulatory subunit** – often referred to as the **R subunit**.
- The activity of the enzyme is increased when a positive allosteric effector binds to the allosteric site and decreased when a negative allosteric effector binds to the allosteric site.
- Conformational changes induced by one or more modulators interconvert more-active and less-active forms of the enzyme.
- **Homotropic Regulation and Heterotropic regulation**

In homotropic enzymes, the active site and regulatory site are the same; eg; oxygen (O_2) acts as a homotropic allosteric effector of haemoglobin. Homotropic effectors are generally activators of the enzyme.

In heterotropic enzymes, the active site and regulatory site are different; eg: carbon dioxide (CO_2) acts as an effector of haemoglobin but is not the enzyme's substrate.

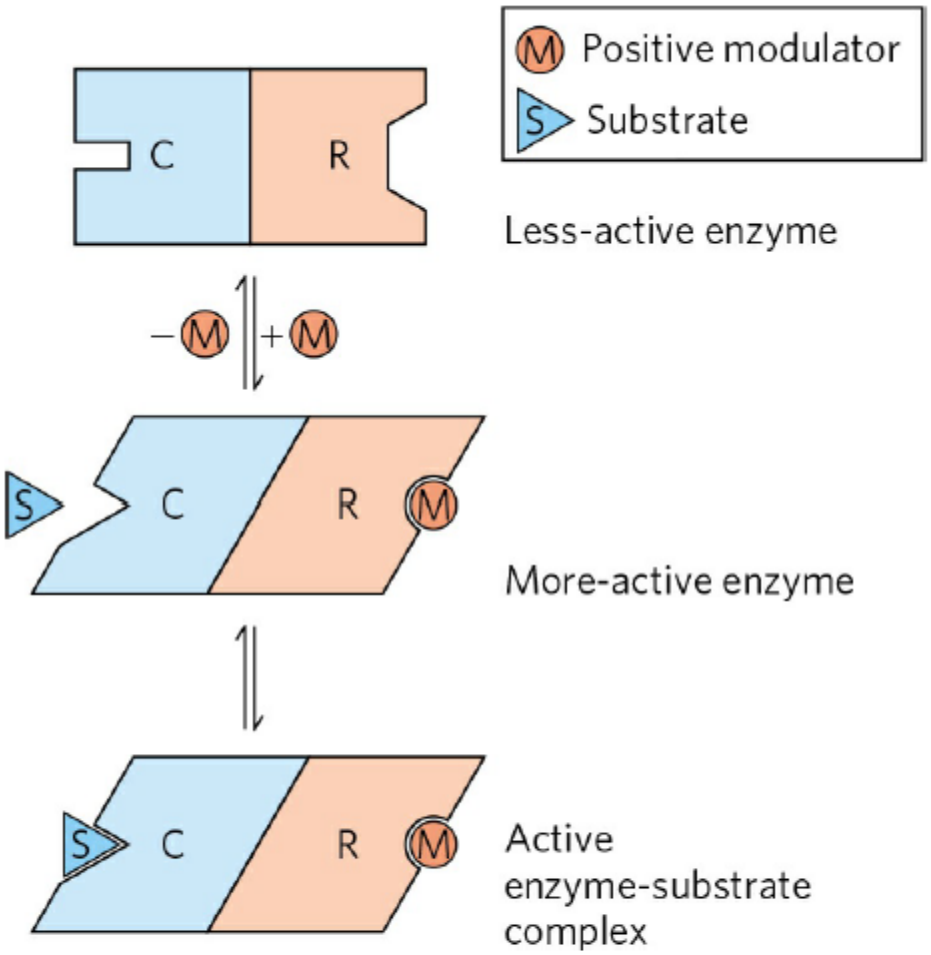

FIGURE 6-33 Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activators. In many allosteric enzymes, the substrate-binding site and the modulator-binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively. Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.

- The Kinetic Properties of Allosteric Enzymes diverge from Michaelis-Menten Behavior. for allosteric enzymes, plots of V_0 versus [S] (usually produce a sigmoid saturation curve, rather than the hyperbolic curve typical of nonregulatory enzymes.

Sigmoid kinetic behavior generally reflects cooperative interactions between multiple protein subunits; i.e;changes in the structure of one subunit are translated into structural changes in adjacent subunits, an

effect mediated by noncovalent interactions at the interface between subunits.

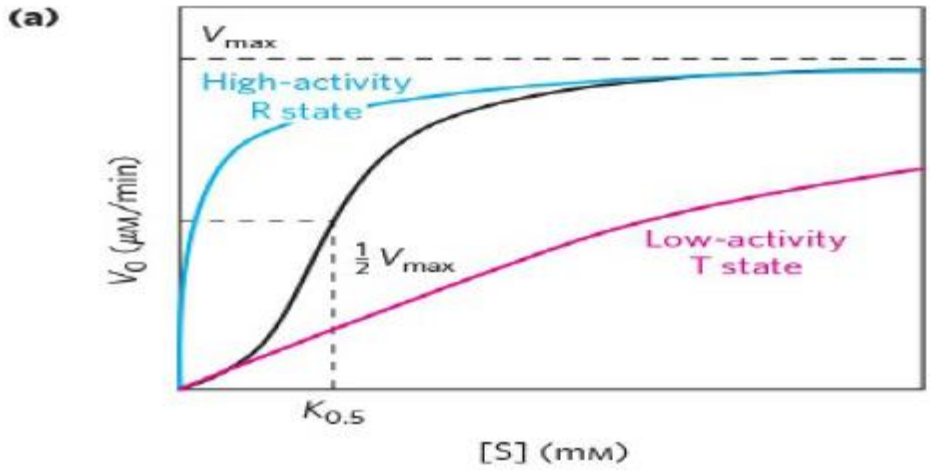

Fig: The sigmoid curve of a homotropic enzyme, in which the substrate also serves as a positive (stimulatory) modulator, or activator.

- This interaction between all of the subunits can be expressed using the **Hill coefficient n_H** (measure of the degree of cooperativity).

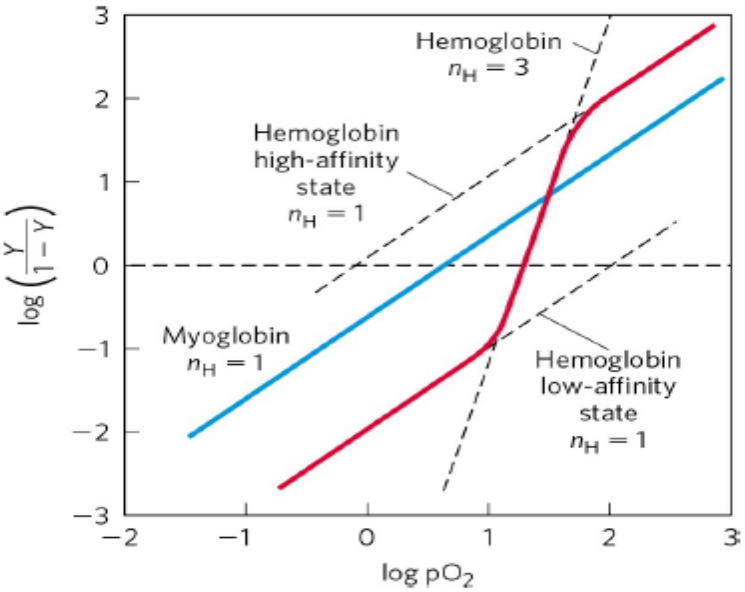

FIGURE 5-14 Hill plots for oxygen binding to myoglobin and hemoglobin. When $n_H = 1$, there is no evident cooperativity. The maximum degree of cooperativity observed for hemoglobin corresponds approximately to $n_H = 3$. Note that while this indicates a high level of cooperativity, n_H is less than n, the number of O_2-binding sites in hemoglobin. This is normal for a protein that exhibits allosteric binding behavior.

- ✓ If n_H equals 1, ligand binding is not cooperative, a situation that can arise even in a multisubunit protein if the subunits do not communicate.
- ✓ An n_H of greater than 1 indicates positive cooperativity in ligand binding. This is the situation observed in hemoglobin,in which the binding of one molecule of ligand facilitates the binding of others.
- ✓ The theoretical upper limit for n_H is reached when $n_H = n$. In this case the binding would be completely cooperative. This limit is never reached in practice.
- ✓ An n_H of less than 1 indicates negative cooperativity, in which the binding of one molecule of ligand impedes the binding of others. Well documented cases of negative cooperativity are rare.