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INTRODUCTION TO THE CHEMISTRY OF ENZYMES

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PREFACE

There are available at the present time many excellent monographs and review articles which cover in a comprehensive manner the various aspects of enzyme chemistry. It is, however, somewhat difficult for one not already fairly well versed in the field to acquire a broad general knowledge of the subject, and the present book aims at helping the beginner to gain such a knowledge. It has been written with two types of reader primarily in mind: the biologist who does not require a detailed knowledge of enzymes but who wishes to learn something of the more important enzymes and to have some knowledge of the mechanism of enzyme action; and the physical chemist who is interested in applying his special techniques to the elucidation of the detailed nature of enzyme reactions.

In view of the types of reader for whom this book is intended, a detailed knowledge of organic chemistry and of biochemistry has not been assumed. In particular, an effort has been made to give the structures of the various compounds referred to, even though this involves a certain amount of repetition. Many readers will find certain parts of the book too elementary for them; this will be true, for example, with respect to much of the chapter on Kinetics. References to reviews are given in the Bibliography, but most other references have been omitted since they are readily found in the reviews; exceptions to this have been made where the original papers are particularly worthy of being consulted and in the case of very recent papers that have not been quoted in the reviews.

The author is grateful to the following who have read the whole or part of the manuscript and have made valuable sug-

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Keith J. Laidler

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CHAPTER 1

GENERAL CHARACTERISTICS OF ENZYMES

Practically all the chemical reactions that occur in biological systems are of such a nature that under ordinary laboratory conditions they do not occur at appreciable rates except at high temperatures or under the influence of high concentrations of acids or bases. Proteins and carbohydrates, for example, are hydrolyzed at reasonable rates only by boiling them with acids and bases; in the digestive system, however, reactions of this type occur rapidly. These and practically all other reactions which occur in living systems are brought about in these systems by substances known as enzymes, the biological catalysts.

It was first realized early in the nineteenth century that certain substances have the effect of speeding up chemical reactions, and in 1836 the German chemist Berzelius classified these substances under the general name of catalysts. Two years later Cagniard de la Tour and Schwann independently investigated the process of fermentation, which is the conversion of sugar into alcohol and carbon dioxide; they found that this process is brought about by minute yeast cells, the removal of which would cause the reactions to cease. Schwann further discovered that putrefaction is also brought about by cells. Later, about 1855, these conclusions were confirmed and extended by Pasteur, who demonstrated that bacteria were capable of catalyzing various types of chemical reactions.

Since these investigations, a great deal of study has been devoted to chemical reactions brought about by living cells. In 1878 Kühne gave to the biological catalysts the name *enzyme*, which comes from Greek words meaning "in yeast." Until 1897 the only work with these enzymes was done by using living cells such as bacteria and yeast, but in this year Büchner made an important advance by extracting enzymes from yeast cells; he

showed that his extract, which no longer consisted of living material, brought about the same fermentation as the yeast cells themselves. Since that time, much work has been done with enzymes extracted from living matter, and some has been done with highly purified materials.

Enzymes as Catalysts

A great many different types of catalysts are known, their essential property being that they enter into chemical reactions but remain intact during the process, their concentration in ideal cases remaining unchanged during the course of reaction. Hydrogen ions and hydroxide ions and various other ions are common catalysts in liquid systems, while solid surfaces frequently catalyze reactions in gaseous systems. The enzymes have many characteristics in common with these inorganic catalysts but are remarkable in certain respects; in particular, they are frequently much more effective, molecule for molecule, than the inorganic catalysts. A possible explanation of this superiority in the case of certain types of enzymes is considered in Chap. 9.

Certain typical properties of catalysts, applicable also to enzymes, are worthy of mention at this stage. It is frequently found that the rate of a catalyzed reaction is directly proportional to the concentration of the catalyst, and this is almost universally the case with the enzymes. Most enzyme-catalyzed reactions do not occur at any appreciable rate in the complete absence of the enzyme, so that a plot of rate vs. enzyme concentration is simply a straight line passing through the origin. Another characteristic property of catalysts, including enzymes, is that they catalyze a reaction to the same degree in forward and reverse directions. An indication of why this is so is suggested by the following argument, which does not, however, constitute a rigorous proof. Consider the chemical equilibrium

$$A + B \rightleftharpoons C + D$$

The rate of this reaction from left to right may under certain conditions be given by

$$v_1 = k_1[A][B]$$

where the proportionality factor k_1 is known as a rate constant and $[A]$ and $[B]$ are the concentrations of the two reactants. The rate from right to left will be similarly

$$v_{-1} = k_{-1}[C][D]$$

At equilibrium these rates are equal, so that

$$k_1[A][B] = k_{-1}[C][D]$$

whence

$$\frac{[C][D]}{[A][B]} = \frac{k_1}{k_{-1}} = K$$

where K, equal to k_1/k_{-1}, is the equilibrium constant for the reaction. Now, a catalyst, present in small concentrations and not entering into the over-all reaction, cannot have any effect on the equilibrium constant K. If the addition of a catalyst increases the value of v_1 by a certain factor, it must do this by increasing k_1 by the same factor. However, if k_1 is increased by this factor, k_{-1} must also be increased by the same factor. In general, therefore, the effect of the catalyst must be the same on both k_1 and k_{-1} and the same on v_1 and v_{-1}.

Enzymes as Proteins

A number of enzymes have now been prepared in highly purified form and their chemical properties studied in some detail. A very important advance was made in 1926, when Sumner succeeded in crystallizing the enzyme urease. Since that time, several other enzymes have been obtained in crystalline, and therefore very pure, form. It has been found that the enzymes are soluble in water, in dilute salt solutions, and in dilute solutions of alcohol or glycerol in water. They are insoluble in water containing sufficiently high proportions of alcohol, are salted out of aqueous solution by neutral salts, and are precipitated by such substances as picric acid and phosphotungstic acid. These properties are all characteristic of the proteins, and the conclusion that enzymes are largely protein in nature is confirmed by direct chemical analysis of the pure substances. There is evidence, however, that some of the enzymes contain a nonprotein constituent in addition.

The general properties of the proteins cannot be discussed here

in any detail, but some of the more important features will be mentioned briefly. The essential structural unit occurring in a protein is a long chain arising from the condensation of a number of amino acid residues. If, for example, a protein molecule were formed by the condensation of a large number of molecules of glycine,

$$H_2NCH_2COOH$$

the resulting structure would be

$$—NH—CH_2—CO—NH—CH_2—CO—NH—CH_2—CO—NH—CH_2—$$

and there may be several hundreds or thousands of units in a protein molecule. The —CO—NH— linkage characteristic of proteins is known as the peptide linkage, and substances containing this linkage are known in general as peptides. Proteins are therefore peptides, as are also such simpler substances as glycylglycine,

$$H_2N—CH_2—CO—NH—CH_2—COOH$$

which is a dipeptide. The naturally occurring proteins largely consist of such chains made up of α-amino acids, which are derivatives of glycine of the general formula

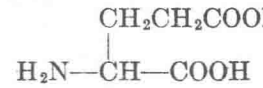
$$H_2N—\underset{\displaystyle R}{\underset{|}{C}}H—COOH$$

where R is one of a number of different groups. There are usually a considerable number of different amino acids within a single protein molecule, and these amino acids are almost invariably in the L configuration. The nature and proportions of the amino acids in a protein can be determined by hydrolyzing the protein (*e.g.*, by boiling with acids or bases) and analyzing for the amino acids. Little is known, however, about the order in which the acids are arranged in the protein molecule or about the configuration taken up by the chain; both of these important topics are being investigated at the present time.

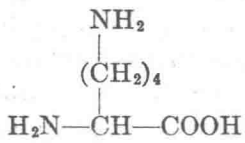
Many of the characteristic properties of the proteins and therefore of the enzymes are those which arise from the very large sizes of the molecules, *i.e.*, from their very high molecular weights. Large molecules diffuse slowly, so that the proteins in

biological systems do not readily move from one place to another. In particular, the proteins move with negligible speed through the usual types of membranes, such as the collodion sacs of dialysis experiments and the natural membranes of cells.

Other characteristic properties of enzymes arise from their electrical nature. Proteins bear a number of positive and negative charges, these arising from the presence of carboxyl (—COOH), amino (—NH_2), and other groups in the molecule. The peptide chain has two ends, one of which will be a free —COOH group and the other a free —NH_2 group. More important, however, is the fact that the side chains in proteins frequently contain —COOH, —NH_2, and other ionizable groups. For example, the amino acid glutamic acid is



so that even when this is involved in peptide formation, there is still a free —COOH group. The amino acid lysine is



so that this retains a free —NH_2 group after peptide formation. Under suitable conditions of acidity these groups ionize to give the ions —COO^- and —NH_3^+,

$$—COOH + H_2O \rightleftharpoons —COO^- + H_3O^+$$

$$—NH_2 + H_2O \rightleftharpoons —NH_3^+ + OH^-$$

In fairly neutral solutions, such as usually exist in physiological conditions, the majority of these groups are in the ionized state, so that as a result protein molecules may contain a considerable number of positive and negative charges. These charges tend to bind ions that are added to a solution of the protein, and enzymatic action is frequently related to such binding of ions. Moreover, as will be discussed in the next chapter, the ionizations of the various charged groups have much to do with the marked effect of acidity on the activities of enzymes.

One result of this type of ionization is that proteins in solution may migrate in an electrical field; this phenomenon is known as *electrophoresis*. In sufficiently acid solution the carboxyl groups in the protein will be in the undissociated —COOH form, whereas the amino groups will be in the charged $—NH_3^+$ form; the molecule will therefore have a net positive charge and will migrate toward the negative electrode (cathode) if a potential is applied. Conversely, at low acidity (high pH) the groups will be present as $—COO^-$ and $—NH_2$, and the molecule, having a net negative charge, will migrate toward the anode. At some intermediate pH the number of positive groups will be equal to the number of negative groups; the molecule will then have no net charge, and there will be no movement in a field. This pH at which there is no migration is known as the *isoelectric point* and is a characteristic property of a protein.

Prosthetic Groups

Certain enzymes have been shown on detailed study to be pure enzymes; the digestive enzymes pepsin and trypsin are of this type. Others, however, have been found to consist of a nonprotein part in addition to the protein and are therefore conjugated proteins. In such cases the protein part of the enzyme is known as the *apoenzyme*, and the nonprotein part as the *prosthetic group*. The enzyme as a whole, consisting of both the apoenzyme and the prosthetic group, is known as the *holoenzyme*. In some cases the prosthetic group is readily separated from the apoenzyme, in which case it is generally known as a *coenzyme;* thus the enzyme lactic dehydrogenase, which is concerned in the oxidation of lactic acid to pyruvic acid, is readily split into its apoenzyme and a substance known as Coenzyme I, which is a fairly complex organic substance (page 82). In the case of other enzymes it is found that ions are attached to the protein and that if these are removed, the enzyme loses its activity; such ions are usually known not as coenzymes but as *activators*. In the above cases the prosthetic group is usually removed from the apoenzyme by dialysis, the prosthetic group passing readily through the membrane, the apoenzyme being unable to pass through.

In other cases the apoenzyme is firmly bound to the prosthetic group and cannot be separated from it by dialysis or by any other means. This is so with the enzyme catalase, which consists of an apoenzyme very firmly bound to hematin, its prosthetic group. In other enzymes the apoenzyme is very firmly bound to metal, which cannot be removed by dialysis. It appears that the distinction between the coenzymes and the firmly bound prosthetic groups is simply one of degree, as the function of the prosthetic group does not seem to be related to the strength of its binding to the apoenzyme. There exists, in fact, a similar variation in the firmness of the attachment of the substrates to the enzymes.

Enzyme Specificity

The enzymes are remarkable not only for their very high effectiveness as catalysts, but also for the fact that they exhibit specificity with respect to their substrates (the substances the reactions of which they will catalyze). A wide range of specificity has been observed, and the following special cases may be distinguished in particular:

1. *Absolute specificity.* When an enzyme will bring about reaction in only a single substrate, it is said to exhibit absolute specificity. Urease, for example, catalyzes the hydrolysis of urea, H_2NCONH_2, to ammonia and carbon dioxide but is ineffective with any other substance; even such a closely analogous compound as methylurea, $CH_3NHCONH_2$, is not acted upon. Similarly, succinic dehydrogenase is absolutely specific with respect to succinic acid, $HOOC(CH_2)_2COOH$; it has no action on the analogous malonic acid, $HOOCCH_2COOH$, which in fact inhibits the action of the enzyme on succinic acid.

2. *Group specificity.* A lower degree of specificity is shown by certain enzymes which act upon a series of substrates but which have definite requirements with regard to the types of groupings that must be present in the molecules on which they act. The enzyme pepsin, for example, will hydrolyze certain peptide linkages, but only provided that, among other things, an aromatic group is present in a certain position with respect to the peptide linkage; the precise requirements are considered in detail in

Chap. 3. Similar group specificity is shown by other proteolytic enzymes, as will be discussed later.

3. *Reaction, or linkage, specificity.* The lowest degree of specificity is shown by certain enzymes which catalyze a certain type of reaction, *i.e.*, which act upon a certain kind of linkage, irrespective of what groups are present in the vicinity of the linkage. Thus there are a number of enzymes known as lipases, any one of which will act upon any organic ester, hydrolyzing it into the acid and the alcohol. There are usually differences in rates for the different substrates for enzymes of this type; thus the lipases found in the liver act more rapidly on the esters of short-chain acids than on those of long-chain acids.

4. *Stereochemical specificity* is said to occur when the enzyme acts upon only one stereochemical form. This type of specificity is extremely common; for example, the digestive enzymes usually act only upon peptides which are made up of amino acids in their L forms. Another example is arginase, which acts upon L-arginine but not on the D form; arginase also shows absolute specificity, L-arginine being the only substrate for it. Similarly, lactic dehydrogenase catalyzes the oxidation of L-lactic acid, but not of the D form.

Classification of Enzymes

Since there are a great many different enzymes, it is essential to discuss them with reference to some type of classification. The most useful methods of classifying them are with respect to their compositions (*e.g.*, of the metals they contain) and with respect to the types of reactions they catalyze. The latter procedure will be most useful for present purposes. It is not possible to devise a completely logical system, since certain reactions fall into more than one class, but the following classification is found to be very satisfactory for purposes of discussion of the properties of the enzymes.

1. The *hydrolytic enzymes*, which catalyze reactions of type

$$AB + H_2O \rightleftharpoons AOH + HB$$

There are four main classes of hydrolytic enzymes, as follows:

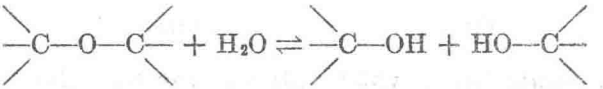
a. The *proteolytic enzymes*, which catalyze the hydrolysis of the peptide linkage,

$$—CO—NH— + H_2O \rightleftharpoons —COOH + —NH_2$$

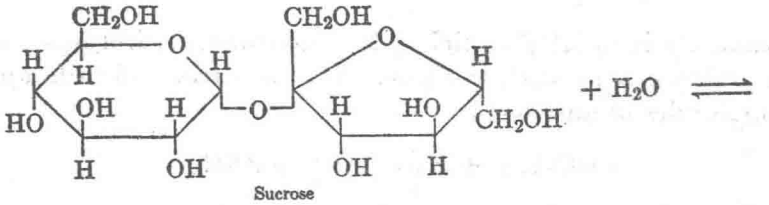
Examples are pepsin and trypsin, which act upon native proteins and certain synthetic peptides, and dipeptidase, which splits dipeptides into two amino acids,

$$\underset{\text{Glycylglycine}}{H_2NCH_2CO—NHCH_2COOH} + H_2O \rightleftharpoons 2\underset{\text{Glycine}}{H_2NCH_2COOH}$$

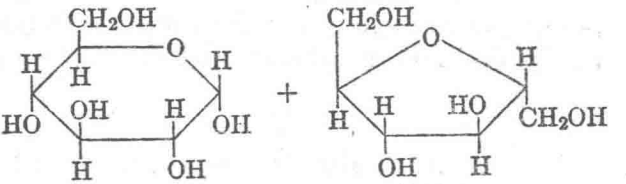
b. The *carbohydrases*, which catalyze the hydrolysis of the glycosidic linkage, which occurs in the di- and polysaccharides and in certain other substances,



Examples are amylase, which catalyzes the hydrolysis of starch to maltose, and sucrase, which catalyzes the hydrolysis of sucrose to fructose and glucose,

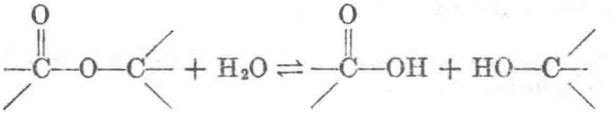


Sucrose



Glucose Fructose

c. The *esterases*, which hydrolyze esters to the acid and alcohol,



Examples are the *lipases*, which hydrolyze fats to fatty acids and glycerol, *e.g.*,

$$\begin{array}{l} CH_2OCOC_{17}H_{35} \\ | \\ CHOCOC_{17}H_{35} \\ | \\ CH_2OCOC_{17}H_{35} \\ \text{Tristearin} \end{array} + 3H_2O \rightleftharpoons \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array} + 3C_{17}H_{35}COOH \quad \text{Stearic acid}$$

and the *phosphatases*, which hydrolyze phosphate esters to phosphoric acid and the alcohol,

$$HO{-}\overset{\displaystyle O}{\overset{\uparrow}{\underset{|}{\overset{|}{P}}}}{-}OR + H_2O \rightleftharpoons HO{-}\overset{\displaystyle O}{\overset{\uparrow}{\underset{|}{\overset{|}{P}}}}{-}OH + HOR$$
$$\qquad\qquad\quad OH \qquad\qquad\qquad\quad OH$$

d. The *deaminases*, which catalyze the hydrolysis of amines, with the liberation of ammonia,

$$\diagdown\!\!\diagup C{-}NH_2 + H_2O \rightleftharpoons \diagdown\!\!\diagup C{-}OH + NH_3$$

An example is urease,† which acts upon urea, hydrolyzing off both amino groups with the production of carbon dioxide and two molecules of ammonia,

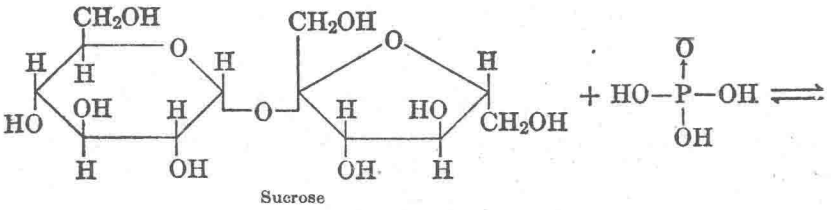
$$CO(NH_2)_2 + H_2O \rightleftharpoons CO_2 + 2NH_3$$

2. The *phosphorylytic enzymes*, or *phosphorylases*, are the phosphate analogues of the hydrolytic enzymes: a molecule of phosphoric acid, instead of a molecule of water, is added,

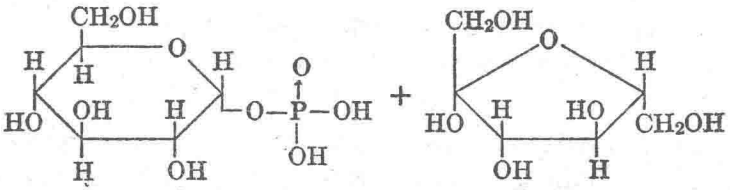
$$AB + HO{-}\overset{\displaystyle O}{\overset{\uparrow}{\underset{|}{\overset{|}{P}}}}{-}OH \rightleftharpoons AO{-}\overset{\displaystyle O}{\overset{\uparrow}{\underset{|}{\overset{|}{P}}}}{-}OH + HB$$
$$\qquad\qquad\quad OH \qquad\qquad\qquad\quad OH$$

For example, sucrose phosphorylase is involved in the phosphorolysis of sucrose,

† Urease may also be considered to be a *deamidase*, since it hydrolyzes a $-CO-NH_2$ linkage.



Sucrose



Glucose-1-phosphate Fructose

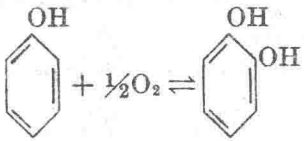
3. The *oxidative enzymes* are concerned with oxidative processes of various types.

a. The *dehydrogenases* catalyze the removal of two hydrogen atoms from a substrate molecule, these atoms being transferred to the coenzyme. Thus lactic dehydrogenase brings about the oxidation of lactic acid to pyruvic acid, the hydrogen atoms being taken up by Coenzyme I, which must be present in order for the enzyme to function,

$$CH_3CHOHCOOH + Co\text{-}I \rightleftharpoons CH_3COCOOH + H_2Co\text{-}I$$

By definition, the dehydrogenases cannot transfer hydrogen atoms to molecular oxygen.

b. The *oxidases* bring about oxidation processes in which hydrogen is transferred directly to molecular oxygen. An example is tyrosinase, which among other things catalyzes the oxidation of monophenols to *o*-dihydric ones,



Phenol Catechol

c. The *oxidative deaminases* act upon amino compounds, bringing about oxidation together with the elimination of a molecule of ammonia; examples are the amino acid oxidases, which cat-

alyze reactions of the type

$$\begin{matrix} & R \\ & | \\ H_2NCHCOOH &+ O_2 + H_2O \rightleftharpoons RCOCOOH + NH_3 + H_2O_2 \end{matrix}$$

4. The *adding enzymes* catalyze simple addition reactions of the type

$$A + B \rightarrow AB$$

Since enzymes catalyze reactions equally in both directions, the adding enzymes also catalyze the reverse reactions,

$$AB \rightarrow A + B$$

and consequently may also be known as *splitting enzymes*. An example is fumarase, which catalyzes the interconversion of fumaric and malic acids,

$$\begin{matrix} COOH \\ | \\ CH \\ \| \\ CH \\ | \\ COOH \\ \text{Fumaric acid} \end{matrix} + H_2O \rightleftharpoons \begin{matrix} COOH \\ | \\ CH_2 \\ | \\ CHOH \\ | \\ COOH \\ \text{Malic acid} \end{matrix}$$

Carbonic anhydrase, which catalyzes the forward and reverse reactions,

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

is also of this class.

5. The *transferring enzymes* catalyze reactions in which groups are interchanged between two molecules,

$$AB + CD \rightleftharpoons AC + BD$$

Two important types of enzymes belonging to this class are:

a. The *transaminases*, which bring about the exchange of amino and keto groups; for example, glutamic transaminase catalyzes reactions such as

$$\begin{matrix} COOH \\ | \\ (CH_2)_2 \\ | \\ CHNH_2 \\ | \\ COOH \\ \text{Glutamic acid} \end{matrix} + \begin{matrix} COOH \\ | \\ CH_2 \\ | \\ CO \\ | \\ COOH \\ \text{Oxalacetic acid} \end{matrix} \rightleftharpoons \begin{matrix} COOH \\ | \\ (CH_2)_2 \\ | \\ CO \\ | \\ COOH \\ \alpha\text{-Ketoglutaric acid} \end{matrix} + \begin{matrix} COOH \\ | \\ CH_2 \\ | \\ CHNH_2 \\ | \\ COOH \\ \text{Aspartic acid} \end{matrix}$$