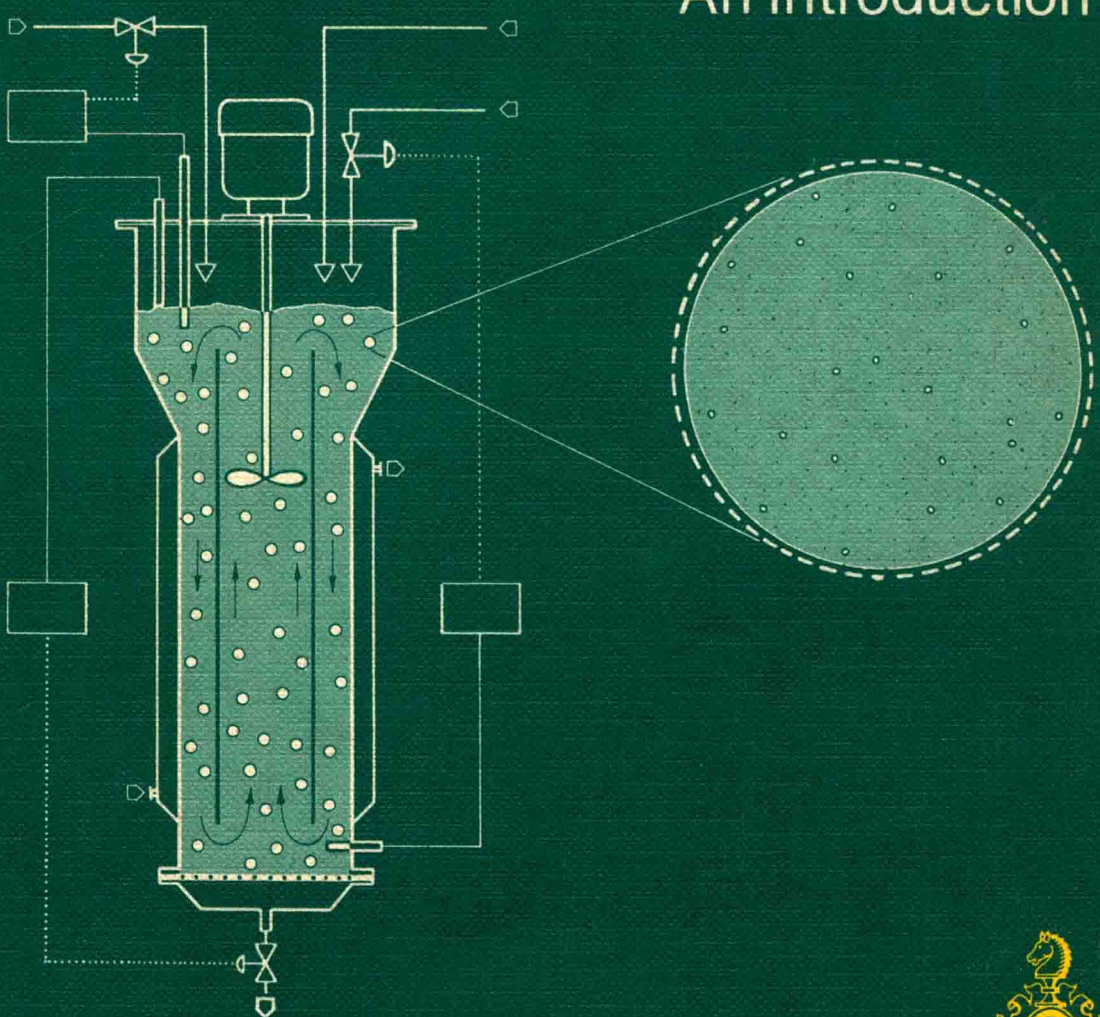
Winfried Hartmeier

Immobilized Biocatalysts

An Introduction



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Immobilized Biocatalysts

An Introduction

Translated by Joy Wieser

With 115 Figures

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Author's Preface to the English Edition

The appearance of an English translation one year after the publication of the first German edition is due to the conviction of publisher and author that, despite the wealth of Anglo-American literature concerning immobilized catalysts, there is a lack of inexpensive introductory books on the subject.

Some slight extensions have been made to the original German text. In the practical section, two additional experiments introduce the student to recent techniques for using membrane reactors and for biocatalyst encapsulation using liquid membranes. Some of the most important publications that have appeared in the meantime have been taken into consideration and added to the list of recommended literature.

I am greatly indebted to the translator, Mrs. Joy Wieser, and to Springer-Verlag, especially to Dr. Dieter Czeschlik, who encouraged and supported me in many ways.

W. Hartmeier

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THEORETICAL SECTION

1 General Principles

1.1 Principles of Biocatalysis

Enzymes are the biocatalytically active entities upon which the metabolism of all living organisms is based. They speed up (bio)chemical reactions by lowering the energy of activation, without themselves appearing in the reaction products. In this, and in the fact that the catalyst itself is not used up, the action of enzymes resembles that of inorganic catalysts.

For the continued existence of organic compounds, and hence for the presence of life on Earth, the existence of an activation barrier is indispensable for preventing continual breakdown. At moderate temperatures many substances are metastable; in other words, they do not break down even though their energy content is considerably higher than that of their breakdown products. Not until an adequate stimulus is provided by the addition of energy, or the energy of activation is sufficiently lowered by a catalyst, are such substances transformed at a greater speed. Figure 1 and Table 1 illustrate the effect of enzymes and inorganic catalysts on the energy of activation and the speed of reaction. The breakdown of hydrogen peroxide into water and oxygen has been taken as an example.

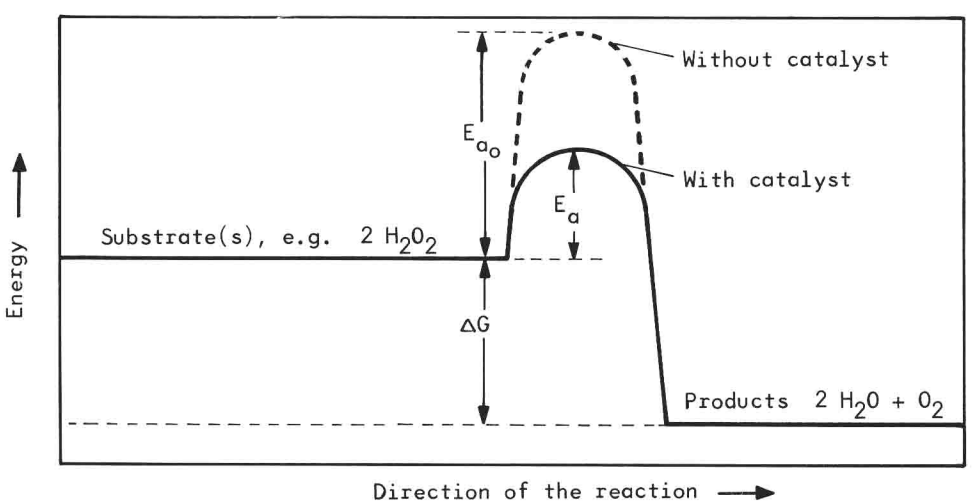


Fig. 1. Energy in a reaction with and without catalyst

Table 1. Breakdown of hydrogen peroxide with and without catalyst

Catalyst	Energy of activation kJ/mol	Relative reaction rate
Without catalyst	75.4	1
Platinum (inorganic)	50.2	$2 \cdot 10^4$
Catalase (enzyme)	8.4	$3 \cdot 10^{11}$

The catalytic action of enzymes and inorganic catalysts involves their ability to alter the distribution of charges on the compound to be converted, thus bringing about a lowering of the energy of activation, E_a (cf. Fig. 1). As a rule, enzymes are more efficient in this respect, i.e., they lead to a much greater decrease in the energy of activation than inorganic catalysts (cf. Table 1). This is why enzyme-catalyzed reactions usually proceed under mild conditions, i.e. at lower temperatures, atmospheric pressure and physiological pH values. Furthermore, in contrast to inorganic catalysts, they are highly specific; in other words, a particular enzyme usually catalyzes only one reaction. This means that, to a large extent, side reactions can be avoided by employing enzymatic breakdown.

The thermodynamic equilibrium of a reaction is in no way affected by the use of catalysts, whether inorganic or enzymic. In the presence of a catalyst, the state of equilibrium is simply reached sooner. In nature, however, for instance in the living cell, it is quite possible for reactions to take place even if the reaction products have considerably higher free energy (G) than the initial substrates. This is achieved by the coupling of such an energy-consuming (endergonic) reaction, whose $\triangle G$ value is positive, with an energy-liberating (exergonic) reaction, where the second reaction must be sufficiently exergonic (negative $\triangle G$) for the sum of the changes in free energy of the two reactions, $\triangle G$, to be zero or negative.

From what has been said so far it is clear that enzymes are specific biocatalysts. The term biocatalyst applies not only to single enzymes, but also includes chains of enzymes linked to form larger units. Even a cell, with its vast number of different enzymes, can be regarded as a complex biocatalyst capable, for example, of transforming sugar into ethanol and carbon dioxide, or of even more complicated biosynthetic feats.

1.2 Structure of Enzymes

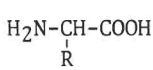
All enzymes, as far as we know, are proteins, although by no means every protein is an enzyme. Examples of catalytically inactive proteins are the antibodies, whose role is protective, or structural proteins which serve for support.

In order to develop catalytic properties some enzyme proteins require the cooperation of a low-molecular nonprotein substance or group. Such nonprotein accessory substances are called coenzymes or cofactors, unless they are tightly bound to the enzyme protein, in which case they are often termed prosthetic groups. The protein portion or moiety is known as the apoenzyme and the combination of enzyme protein and nonprotein active group is the holoenzyme.

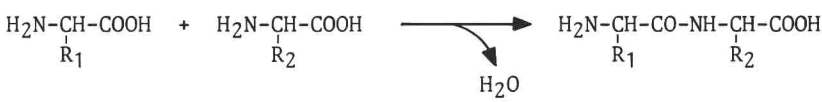
Not always does the nonprotein moiety of an enzyme play a direct role in biocatalysis. Many enzymes, especially those of technical importance, are glycoproteins, which means that they possess a carbohydrate moiety which, as a rule, is not involved in the catalytic action. Yeast invertase is an enzyme of this kind: about half of its molecular weight is due to the polysaccharide mannan.

Protein Moiety

The protein moiety of an enzyme consists of amino acids which, with the exception of proline and hydroxyproline (see Table 2), have the following general formula:

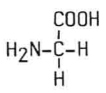
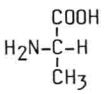
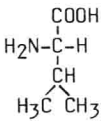
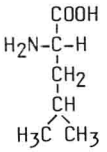
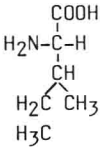
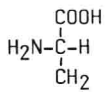
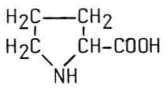
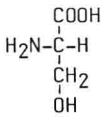
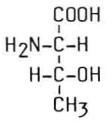
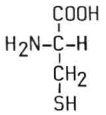
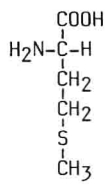
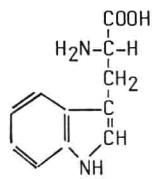
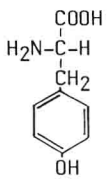
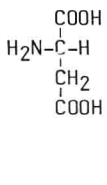
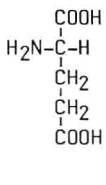
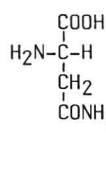
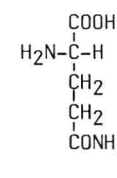
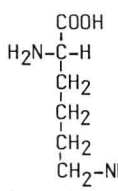
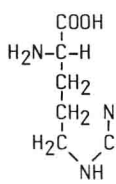
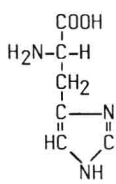


The splitting off of water between the α-carboxyl group of one amino acid and the α-amino group of another gives rise to a dipeptide.



The linkage between the amino acids is termed an amide link or a peptide bond, and the amino acids thus linked are known as residues. By means of further condensation, i.e., formation of a chain accompanied by the elimination of water, large molecules known as polypetides are formed. It seems that a chain length of about 50 amino acids is necessary for enzyme activity, although the number of residues is usually much higher. Up to several thousands of amino acid units are often found in enzyme proteins, giving a molecular weight of 5000 to several million. Table 2 shows the 20 amino acids generally involved in the structure of enzymes.

Table 2. The most important amino acids

 Glycine (Gly)	 L-Alanine (Ala)	 L-Valine (Val)	 L-Leucine (Leu)	 L-Isoleucine (Ile)
 L-Phenylalanine (Phe)	 L-Proline (Pro)	 L-Serine (Ser)	 L-Threonine (Thr)	 L-Cysteine (Cys)
 L-Methionine (Met)	 L-Tryptophane (Trp)	 L-Tyrosine (Tyr)	 L-Aspartic acid (Asp)	 L-Glutamic acid (Glu)
 L-Asparagine (Asn)	 L-Glutamine (Gln)	 L-Lysine (Lys)	 L-Arginine (Arg)	 L-Histidine (His)

The sequence in which the amino acids of an enzyme protein are linked to one another is termed its primary structure. In describing the makeup of a polypeptide the convention is to begin with the N-terminal, i.e., the amino acid whose amino group is unbound, and to employ the abbreviations shown in brackets in Table 2. The amino acid sequence of many enzymes important in industry has already been elucidated. Figure 2 shows as an example the amino acid sequence of papain, a plant protease used in stabilizing beer (chill-proofing) and in tenderizing meat.

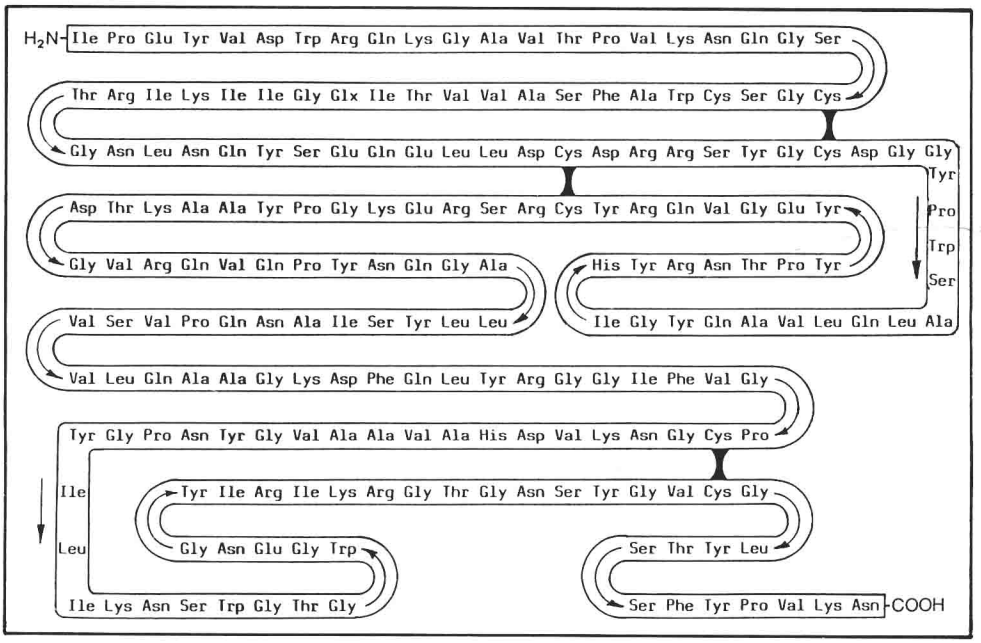


Fig. 2. Sequence of amino acids of papain

Conformation and Active Center

As flexible structures, polypeptide chains have a tendency to adopt characteristic spatial conformations due to the steric properties and charge distribution of their constituent amino acids, as well as to their ability to form hydrogen bonds between the carbonyl-oxygen and amide-nitrogen atoms. A common arrangement of this kind is the α-helix, a corkscrew structure comprising 18 residues per 5 turns. Another arrangement, less often found in enzymes, is the ß-structure or ß-sheet. α-helix and ß-sheet structure are also known as the secondary structure of a protein.

In addition to α-helix and ß-sheet regions there are always some seemingly random structural conformations in the polypeptide chain of an enzyme molecule. In order for a protein to exert a specific enzymatic action at least some regions in each polypetide chain must have a certain typical spatial conformation. This confers upon the enzyme its own characteristic form and activity. The spatial structure assumed by an enzyme as a result of interaction between the amino acid side chains is termed the tertiary structure: it is stabilized partly by disulfide bridges that form between the cysteine residues of near-lying portions of the chain (cf. Fig. 2). To a very considerable extent the conformation assumed by an enzyme protein and the stabilization of its structure is due to ionic bonds, hydrophobic interaction, hydrogen bonds and van der Waals forces.

Some enzymes consist of several, often two or four, identical or different polypeptide subchains spatially arranged to give a specific, so-

called quaternary structure. Such a complex structure is usually held together by noncovalent bonds, particularly by ionic bridges, hydrogen bonds or hydrophobic interactions. The quaternary structure is very important in enzymes playing a regulatory role in metabolism (allosteric enzymes), but is seldom present in enzymes of industrial importance.

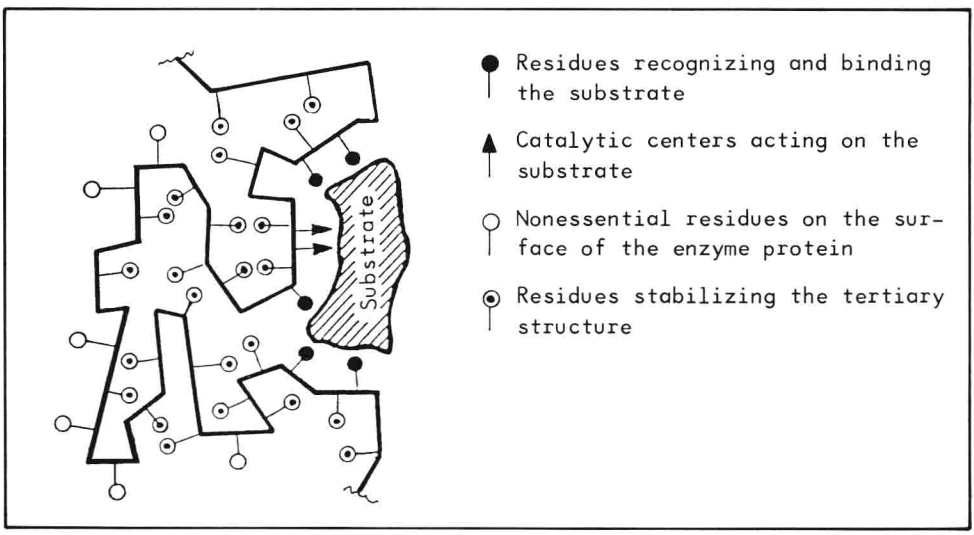


Fig. 3. Scheme of an enzyme protein with its active site

In a certain region of every enzyme the amino acids are arranged in a specific sequence and spatial conformation that are responsible for the catalytic function. This region is known as the active site or catalytic center. It is here that the substrate is bound to the enzyme and broken down into the reaction products. Only a very few amino acid residues participate directly in the catalytic action although most of the other amino acid side chains are also important and their indirect effect is in many cases even essential for biocatalysis to take place. Some of them are involved in the recognition and binding of the substrate molecule to the active site, others participate in the formation and stabilization of the three-dimensional conformation of the enzyme molecule. Figure 3 shows a schematized section of an amino acid chain with its active site, and amino acid side chains with their various functions.

Coenzymes

Coenzymes or cofactors are low-molecular, nonprotein molecules whose presence is essential for many enzyme reactions. There are two types of coenzymes: those that dissociate from their apoenzymes after completion of

the reaction and those that remain associated. In cases where a cofactor is relatively tightly bound to its apoenzyme it can be termed a prosthetic group. Unfortunately, it is not always easy to make a distinction between the two types of coenzyme since transitional degrees of binding and dissociation are also encountered. For the purposes of immobilization, however, permanence of association between co- and apoenzyme is of crucial importance because this determines whether or not special procedures have to be adopted for coenzyme immobilization (cf. Sect. 2.8). In Table 3 some examples of freely dissociating (above broken line) and tightly bound cofactors (below broken line) are listed.

Table 3. Examples of coenzymes

Abbreviation and full name		Function	Corresponding apoenzymes
NAD	Nicotinamide-adenine dinucleotide	Transfer of hydrogen	Dehydrogenases
NADP	Nicotinamide-adenine dinucleotide phosphate	Transfer of hydrogen	Dehydrogenases
ATP	Adenosine-triphosphate	Phosphorylations, transphosphorylations	Kinases, transferases, synthetases
CoA	Coenzyme A	Transfer of acyl residues	Acyl transferases, thiol ligases
FAD	Flavin-adenine dinucleotide	Transfer of hydrogen	Oxidases
PAL	Pyridoxal phosphate	Transamination, decarboxylation	Transaminases, decarboxylases
TPP	Thiamine pyrophosphate	Decarboxylation	Decarboxylases
---	Heme coenzymes	Electron transport	Monooxygenases, mutases peroxidases
---	Biotin	Transfer of CO_2	Carboxylases

Most of the coenzymes listed in Table 3 are synthesized from vitamins. For example NAD contains nicotinic acid and the major part of FAD consists of riboflavin (vitamin B_2).

Metals are often involved in the catalytic function as inorganic complements of enzyme reactions. These metals are not termed coenzymes, but rather cofactors. Thus, the term cofactor is used in a more general and wider sense than coenzyme.

1.3 Classification and Nomenclature of Enzymes

In the early days of enzyme research the name given to an enzyme was largely a matter of chance or a whim of its discoverer. Inevitably, the result was a bewildering variety of names, sometimes even for one and the same enzyme. In 1956 the International Union of Biochemistry (IUB) called into being an Enzyme Commission (EC), followed by a Nomenclature Commission (NC), whose recommendations on nomenclature and classification of enzymes are recognized and adhered to throughout the scientific world. A few of the criteria involved are outlined below; further details can always be found in the most recent edition of ***Enzyme Nomenclature*** (International Union of Biochemistry, 1984).

Systematic Names

The principle employed is that the systematic name of an enzyme should include the substrate, the type of reaction to be catalyzed and the suffix "-ase." Thus, an enzyme catalyzing a simple monosubstrate reaction is named according to the following scheme:

substrate - type of reaction catalyzed - ase.

If the enzyme acts with two substrates both substrate names are included, separated by a colon. The general form of the name is then:

substrate A : substrate B - type of reaction - ase.

Abbreviations commonly used in biochemistry, such as NAD or ADP, are also used in enzyme names. For substrates that usually occur in the ionized form the name of the corresponding salt is used (e.g. pyruvate or succinate). Within the various classes of enzymes the rules governing the systematic names are more narrowly defined with respect to the particular type of reaction. In redox reactions (first main class) for example, the enzyme substrates are electron donors and acceptors, which results in the following systematic names:

electron donor : electron acceptor - oxidoreductase.

This can be shown more explicitly for alcohol dehydrogenase, which catalyzes the oxidation of alcohol to aldehyde, in the course of which