BIOCATALYSTS IN ORGANIC SYNTHESIS

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Introduction

Modern society is dependent on chemistry to a great extent. The number of synthetic chemicals currently used by man is approximately 65,000. Many of them, originally considered as harmless or only slightly harmful, had, in fact, very negative side effects on the environment and human health and, thus, it was necessary to stop their use. They are frequently replaced by other chemical substances. However, these are supposed to adhere to very strict criteria concerning their safety. Ariëns [1] characterized this situation very ingeniously as a domestication of chemistry and chemical compounds. By the use of an analogy of the definition of the word domestication he proposes "to adapt chemistry and chemicals to intimate association with and to the advantage of man". It is clear that not only chemists, but also all users of chemicals have to bring a positive contribution to this domestication. The task of chemistry, and mainly of synthetic organic chemistry, is to offer new compounds characterized by high activities in the desired directions with as few undesirable side effects as possible. In the preparation of the technology of producing these chemicals, it is also necessary to adhere to ecological criteria.

Chemists ever more frequently consider highly efficient natural substances as positive examples when searching for these compounds. It is possible to mention prostanoids (prostaglandins, prostacyclins, thromboxanes), natural substances of plant origin for pharmaceutical use, or pheromones and pyrethroids helping in the fight against insects. These substances are, however, usually characterized by a complex structure and their syntheses by common methods may afford many positional isomers, geometric isomers, enantiomers and diastereoisomers. From such a mixture only one isomer usually exhibits the desired properties.

Special attention should be paid to the relationship between stereoisomerism and biological activity. It is known, for example, that higher organisms can utilize only the D-form of sugars and the L-form of amino acids. It would seem, that the opposite forms, L-sugars and D-amino acids, are passive in metabolism and that they are simply excreted unaltered. This is, however, not always the case. The differences in physiological effects can be very dramatic. Phenylalanine, for example, which is an essential amino acid for human nutrition in its L-form, is in its D-form a highly efficient analgesic — it inhibits specific enzymes which decompose endogenous substances moderating pain, i.e., endorphins. In

this way, endorphins can be accumulated in the body, and can even result in dangerous levels of anaesthesia with elimination of pain, for example, in severe mechanical or thermal damage to tissue.

There are also considerable differences in biological activities of optical isomers of synthetic pharmaceuticals. The (*S*)-enantiomer of propranolol, an efficient drug used in the treatment of hypertension and similar diseases, has about 100 times higher β-antiadrenergic efficiency in comparison with the (*R*)-enantiomer. Thus, the more active (*S*)-propranolol can be administered in smaller doses. In this way, the danger of side effects occurring after administering the racemate is reduced. Tragic consequences of the administration of thalidoimide (Kontergan) are well known. The pure enantiomer (*R*)-thalidoimide has no detrimental side effects, however, (*S*)-thalidoimide is an unusually strong teratogenic agent and its low admixture was sufficient to make the drug highly dangerous.

Chemicals for use in agriculture are currently also produced as pure optical isomers for analogous reasons. The above-mentioned insecticides, pyretroids, are a typical example. Some herbicides are also being produced as pure or partially pure enantiomeric forms (suffix BW, carbetamide).

Thus, in the future, organic chemists would prefer synthetic methods making possible the production of pure enantiomeric forms, not only in the laboratory, but also on the industrial scale. Optically active substances can be only rarely obtained by spontaneous crystallization from the racemate. The synthesized racemic mixtures are most typically resolved to enantiomers through diastereoisomers with the help of chiral auxiliary substances. It is, however, more advantageous to use a method of synthesis leading to the optical isomer desired and not to the racemate. In this procedure, it is possible to use as raw materials pure natural enantiomers, e.g., amino acids, sugars, terpenes, etc. (the chiral pool), or to accomplish a stereospecific reaction at a certain stage of the synthesis when using optically inactive substances at the beginning. In the latter case, an auxiliary chiral substance or chiral catalyst are usually used. Biocatalysts, in addition to complexes of transition metals with chiral ligands, fall into this group of chiral catalysts.

Chemical conversions of substances by the action of microorganisms have long been studied with the aim of using them for specific purposes. The microbial oxidation of ethanol and glucose were studied by Pasteur and other scientists as long ago as in the 19th century. About 1910 Lintner and von Liebig observed that baker's yeast was able to reduce furfural. At approximately the same time, the hydrolytic activity of a biopreparation obtained by homogenization of pancreas was discovered. Many interesting facts were revealed by Neuberg and co-workers who studied microbial transformations of different organic compounds. From the thirties these methods have been used in the chemistry of steroids and many successful syntheses have been accomplished. In

the fifties and sixties, in addition to investigations of microorganisms, the catalytic properties of pure enzymes and their applicability in organic syntheses were also studied. A great contribution was made by a group led by Professor Prelog (ETH Zürich) [2]. Volume X (two parts) of the series Techniques in Chemistry [3] about the use of biosystems in organic chemistry, and the book by Kieslich [4] dealing with microbial transformations are extensive monographs published in 1976. Further books about biocatalysis in organic synthesis [5–10], chapters in more general works [11, 12] and many reviews in journals [13–24] indicate the growing interest in these methods of organic synthesis. In spite of this, the use of biocatalysts is still not common in organic laboratories. There are several reasons for this, however, many of them result from the fact that organic chemists do not know the characteristics of enzymes and microorganisms, their availability and the methods of working with them. They often do not realize that it is not necessary to use different methods or different laboratory equipment when working with some enzymatic preparations or cells.

The purpose of this book is not to provide a detailed explanation of enzymology or a summarization of all the known cases of the use of biocatalysts in organic synthesis. Basic characteristics of biocatalysts, important from the point of view of their use in organic synthesis, will be described only briefly. The main purpose of this book is rather to bring a review of particular types of reactions and, thus, to inspire the reader to search for possibilities of applying biocatalysts in his field of syntheses, in addition to classical methods. This was also the reason for the classification of reactions in a manner common in organic chemistry (substitution, elimination, addition, oxidation, reduction) and not according to the biochemical classification of the enzymes used.

Most original papers cited in the present book have been published from 1985 onwards. They can be used as a source of more detailed information as well as references to the earlier literature.

Abbreviations

AAO	amino acid oxidase
AD	alcohol dehydrogenase
AldD	aldehyde dehydrogenase
ANL	lipase from *Aspergillus niger*
ATP	adenosine triphosphate
CCL	lipase from *Candida cylindracea*
CFAD	alcohol dehydrogenase from *Curvularia falcata*
CIP	Cahn–Ingold–Prelog convention for designating the absolute configuration
CM-	carboxymethyl-
α-CT	α-chymotrypsin
CVL	lipase from *Chromobacterium viscosum*
CXG	cyclohexanone oxygenase
DEAE-	diethylaminoethyl
DHAP	dihydroxyacetone phosphate
DOPA	3,4-dihydroxyphenylalanine
e.e.	enantiomeric excess
FD	formate dehydrogenase
FDPA	fructose-1,6-diphosphate aldolase
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
GCL	lipase from *Geotrichum candidum*
GDH	glutamate dehydrogenase
GGDH	glycerol dehydrogenase from *G. candidum*
GlyDH	glycerol dehydrogenase
HICD	α-hydroxyisocaproate dehydrogenase
HLAD	horse liver alcohol dehydrogenase
HLE	horse liver esterase
HSD	3α,20β-hydroxysteroid dehydrogenase
LDH	lactate hydrogenase
MCPBA	*m*-chloroperbenzoic acid
MDH	mandelate dehydrogenase
MJAD	alcohol dehydrogenase from *Mucor javanicus*

MML	lipase from *Mucor miehei*
NAD(P)	nicotinamide adenine dinucleotide(phosphate)
PFL	lipase from *Pseudomonas fluorescens*
PLAD	pig liver alcohol dehydrogenase
PLE	pig liver esterase
PLM	pig liver monooxygenase
PPL	pig pancreas lipase
PRA	pig renal aminoacylase
PSL	lipase from *Pseudomonas* sp.
TBAD	alcohol dehydrogenase from *Thermoanaerobium brockii*
YAD	yeast alcohol dehydrogenase

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Contents

1 Enzymes — catalysts of life

Enzymes are simple or complex proteins and their catalytic activity is essential in the chemical reactions (metabolism) of living organisms. When an enzyme contains, in addition to a protein component (apoenzyme), a non-protein component the latter is referred to as a prosthetic group or (particularly in the case of a reversibly bound component) as a coenzyme. The protein part forms the active site of the enzyme by the spatial arrangement of its polypeptide chain, where the amino acid units provide by their functional groups suitable spatial and binding conditions for the formation of an enzyme–substrate complex facilitating the catalytic conversion of the bound substrate, i.e., formation of an enzyme–product complex. In other words, the protein part of the enzyme is responsible for the substrate specificity and, in a cooperation with the coenzyme, also for the specificity of the effect. The coenzyme is a donor or possibly an acceptor of particles, that are bound to or released from the substrate in the reaction. Thus, it acts as an agent; it is consumed in an equimolar ratio to the substrate. It is usually present at low concentrations and, thus, a system should be available to facilitate coenzyme recovery when the reaction is run with larger amounts of the substrate.

The presence of metal ions (Mg^{2+}, Zn^{2+}, Mn^{2+}, etc.) can also frequently play an important role to provide a necessary spatial structure of the apoenzyme for the formation of the enzyme–substrate complex as well as for its conversion.

Enzymes are very efficient catalysts. Under identical conditions, the rate of an enzymatic reaction may be higher by a factor of one million or many millions than the rate of the reaction in the absence of the catalyst. An example of a very efficient enzyme is urease, which increases the rate of urea hydrolysis by a factor of about 10^{14} at pH 8.0 and 20°C.

Enzymes differ from classical chemical catalysts particularly in two characteristics, stability and selectivity. Enzymes are stable only in a relatively narrow range of physical and chemical parameters (see Section 1.3), whereas chemical catalysts are much more stable. Activity is closely related to stability. Enzymes are frequently more advantageous than chemical catalysts when comparing the conditions necessary for the highest activity. For example, a temperature of 25 to 30°C, suitable for an enzyme process, is more economic than a high or very low temperature necessary for many classical chemical processes.

As far as the second characteristic is concerned, the selectivity, enzymes are unambiguously superior to chemical catalysts. This is the decisive reason why different forms of biocatalysts find ever more frequent use in preparative organic reactions (see Chapter 3).

1.1 Classification of enzymes

International rules for the enzyme nomenclature and classification [25] consider 6 main classes of enzymes according to the type of the chemical conversion catalyzed (Table 1). In each main class, the enzymes are subdivided according to further criteria. Each enzyme falls into a certain class, subclass and subsubclass, and depending on this and on its order in the subsubclass, a four-figure classification code (the EC number) is attributed to each particular enzyme.

Table 1. Classification of enzymes: main classes and subclasses

1. Oxidoreductases — acting on: 1.1 bond CH—OH 1.2 bond C=O 1.3 bond C=CH 1.4 bond CH—NH_2 1.5 bond CH—NH 1.6 NAD(P)H	4. Lyases — facilitating elimination forming double bond or addition of groups by means of the following atoms: 4.1 carbon 4.2 oxygen 4.3 nitrogen 4.4 sulphur 4.5 halogen
2. Transferases — transferring groups: 2.1 one-carbon 2.2 equivalents of aldehydes and ketones 2.3 acyl 2.4 glycosyl 2.5 alkyl (except for Me) and aryl 2.6 nitrogen 2.7 phosphate	5. Isomerases 5.1 racemases and epimerases 5.2 *cis-trans*-isomerases 5.3 intramolecular oxidoreductases 5.4 intramolecular transferases 5.5 intramolecular lyases
3. Hydrolases — hydrolyzing: 3.1 esters 3.2 glycosyl compounds 3.3 ethers and thioethers 3.4 peptide bonds 3.5 C—N bonds except for peptides	6. Ligases (synthetases): forming the following bonds with simultaneous splitting of ATP: 6.1 C—O 6.2 C—S 6.3 C—N 6.4 C—C

Table 1 can partially help an organic chemist with respect to the large number of enzymes known and their nomenclature. When selecting a suitable enzyme for its use in an organic synthesis, it is, however, useful to establish its class out of the following four groups [15]:

(1) Enzymes without coenzymes, simple hydrolases, lyases, isomerases.

(2) Enzymes with no requirement for the addition of coenzyme (they contain a firmly bound prosthetic group, e.g., of a flavin or pyridoxal type), transaminases, peroxidases, etc.
(3) Enzymes that require the addition and regeneration of coenzyme, most frequently ATP or NAD(P)H, e.g., kinases, most oxidoreductases.
(4) Enzymes occurring exclusively in multienzymatic systems.

The enzymes of the first group are currently being used most extensively, frequently also on the industrial scale (production of L-amino acids, 6-aminopenicillanic acid, isomerization of glucose to fructose, etc.). Further groups of enzymes have more requirements for their use and, consequently, they have found applications only in the laboratory for small scale synthesis. Their use on the industrial scale may be considered in the future, however, there is a possibility of more extensive developments of microbial transformations, where certain problems, for example, these connected with providing and recovering the coenzyme, are solved by the cells of the microorganisms used.

1.2 Principles of the kinetics of enzymatic reactions

Kinetic parameters of enzymatic reactions are not as important for organic chemists as for biochemical studies of the mechanism of the enzyme action, however, in spite of this, some of them may be very useful for selecting the conditions of a preparative reaction. Their experimental determination is relatively easy (see, e.g., Ref. [26]).

The main factors affecting the rate of enzymatic reactions are as follows: the enzyme concentration, substrate concentration, presence of inhibitors, activators or organic solvents, pH, temperature and ionic strength of the medium.

The Michaelis–Menten theory of enzyme kinetics assumes that the enzymatic reaction occurs in two steps. In the first step, a complex ES is formed by rapid reaction of the enzyme E and substrate S (k_1 and k_{-1} are rate constants of the reaction, and of the reverse reaction, respectively)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

In the second step, the complex ES dissociates forming the enzyme and product P at a reaction rate that is essentially lower than the rate of the reverse reaction ($k_2 \ll k_{-1}$):

$$ES \xrightarrow{k_2} E + P$$

From this it follows that the total reaction rate is proportional to the concentra-

tion of the enzyme–substrate complex [ES]. As the amount of enzyme remains unaltered and the concentration increases, the concentration of the complex ES also increases. At the end of this process all the enzyme is present in the form of the complex ES and the reaction rate achieves its maximum value V_{max} (Fig. 1). The substrate concentration corresponding to a maximum saturation

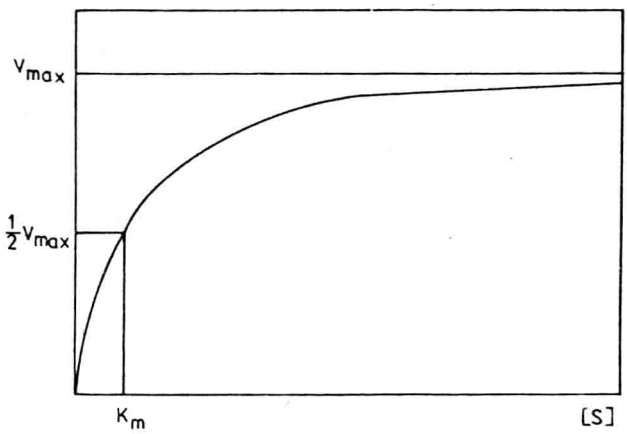

Fig. 1 Effect of substrate concentration on the rate of an enzymatic reaction (K_m — Michaelis constant).

of the enzyme with the substrate could be a useful quantity to know, however, its value cannot be precisely established from the curve in Fig. 1. For a reaction rate of one half of V_{max}, it is possible to read off precisely the relevant substrate concentration and this value is termed the Michaelis constant K_m. At the rate of $1/2V_{max}$, one half of the total amount of the enzyme is present in the form of ES, the second half being free so that [ES] = [E]. As mentioned above $k_2 \ll k_{-1}$ and thus, the Michaelis constant K_m equals the dissociation constant of the complex ES (substrate constant K_s) defined according to the Guldberg–Waage law:

$$K_s = \frac{[E][S]}{[ES]} = K_m$$

Thus, the Michaelis constant expresses the affinity of the enzyme to the substrate. The lower its value (the enzyme half-saturation achieved at lower concentration of the substrate), the higher is the enzyme affinity. For most typical biochemical substrates the values of K_m range between 10^{-2} and 10^{-5} mol dm^{-3}.

The experimental determination of the values of k_1, k_{-1} and k_2 is rather complicated. The rate constant of the whole enzymatic reaction, denoted by k_{cat}

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