1
Introduction

Enzymes are the most important catalysts and regulators indispensably involved in each process in living organisms. Any investigation of the cell metabolism requires a thorough understanding of enzyme action. Enzymes are very sensitive markers for correct function and, consequently, also for dysfunction of the metabolism, serving as indicators both for health and manifestation of diseases. Accordingly, they are used as invaluable tools in medical diagnostics. Beyond that, enzymes are applied in many technical operations. They play an essential role in the environmental processes in the microbial world in waters, rivers, lakes, and soil, and are important for filter plants as well as for fermentation procedures in dairies and breweries.

According to current estimates, about 25 000 enzymes are expected to exist in the living world, where more than 3000 are described in detail, and some hundreds are commercially available. Enzymes are extremely efficient catalysts, enhancing the turnover rates of spontaneous reactions by factors between 10^8 and 10^{10}, sometimes even up to 10^{12} (Menger, 1993). Orotidine-5′-phosphate decarboxylase is a striking example: the spontaneous reaction proceeds with a half-life of 78 million years and the enzyme increases the velocity by a factor of 10^{17} (Radzicka and Wolfenden, 1995). Triosephosphate isomerase accelerates the enolization of dihydroxyacetone phosphate by more than 10^9 (Alberty and Knowles, 1976).

Even reactions spontaneously proceeding with a considerable rate, such as the formation of water from hydrogen and oxygen in the respiratory chain, are subject to enzyme catalysis: each reaction step in metabolism is controlled by a special enzyme. Thus the role of enzymes in the metabolism is broader than to act only as biocatalysts. The peculiarity of catalysis is not only restricted to the acceleration of spontaneous reactions, but it also allows controlling reactions. Spontaneous reactions, after initiating, run off to the end and cannot be stopped. Catalyzed reactions, in contrast, proceed only in the presence of the catalyst; its activity and amount determines the reaction rate. Consequently, tuning the activity of an enzyme from the outside by activating or inhibiting mediates an exact control of the velocity. In the living cell, a strictly coordinate network of regulation exists, comprising enzymes whose activity is controlled by the concentration levels of metabolites, hormones, and transmitter substances. The precise interaction of all these components is a prerequisite of life.

Practical Enzymology, Second Edition. Hans Bisswanger.

The protein nature of enzymes is excellently suited for this dual function as catalyst and regulator; it supplies functional groups of amino acids to form specific binding sites and catalytic centers, and it provides flexibility to promote formation and stabilization of transition states and to induce conformational changes for modulation of the catalytic efficiency. The 20 proteinogenic amino acids with their hydrophilic, hydrophobic, acidic, and basic side chains permit most enzymes to realize both functions such as specific binding of substrates and regulator molecules and catalytic conversion. More difficult catalytic mechanisms cannot be brought forth only by the amino acid side chains; rather, nonproteinogenic compounds are included, which can either be dissociable as *coenzymes*,[1] or nondissociable as *prosthetic groups*. Dissociable coenzymes are NAD(P), thiamine diphosphate, or coenzyme A, while FAD, cytochromes, porphyrins, pyridoxamine, lipoic acid, biotin, and tetrahydrofolic acid function as nondissociable, partly covalently bound prosthetic groups. Often also metal ions are required, both for catalysis and for stability of the enzyme, Mg^{2+} serves to neutralize the phosphate groups in compounds such as ADP, ATP, and thiamine diphosphate and mediates their binding to the enzyme. Iron (in cytochromes), cobalt (in the corrin ring system), copper (e.g., in cytochrome oxidase and tyrosinase), zinc (in carboanhydase and alcohol dehydrogenase), molybdenum (in nitrogenase), manganese (in arginase and xylose/glucose isomerase), and selenium ion (in glutathion peroxidase) support the enzyme reactions.

The protein nature enables enzymes to adapt their specificity to any desired ligand by mutations. This feature is applied in biotechnology using *site-directed mutagenesis* to modify the specificity and function of enzymes. By the method of *molecular modeling* (protein design) distinct modifications are simulated and thereafter the respective mutations are executed. An example is hydroxyisocaproate dehydrogenase, an enzyme catalyzing the reductive conversion of α-oxo acids to chiral hydroxycarbonic acids as hydroxyanalogs of amino acids. Its preferred substrate is α-oxocaproic acid. α-Isocaproic acid, an analogous compound, is accepted only with reduced efficiency. By site-directed mutagenesis the catalytic efficiency (k_{cat}/K_m) for this compound has been increased by four orders of magnitude, as compared to the physiological substrate (Feil, Lerch, and Schomburg, 1994).

Owing to their protein nature, enzymes are very sensitive to environmental influences such as pH, ionic strength, and temperature and, consequently, to attain optimum activity, stringent conditions must be established. In the physiological milieu of the living cell, these conditions are maintained as far as possible, although with respect to temperature, this cannot be permanently guaranteed (with the exception of warm-blooded vertebrates). However, enzymes are remarkably

1) The terms *coenzyme* and *cosubstrate* are not always clearly differentiated. Coenzymes, in contradistinction to cosubstrates, are supposed to support the catalytic mechanism and should not be converted. For example, pyridoxal phosphate in transamination reactions accepts an amino residue becoming pyridoxamine phosphate, but in the second step of the reaction the amino group is transferred to an α-oxoacid and the coenzyme regains its original form at the end of the reaction. NAD(P), on the other hand, is reduced in a dehydrogenase reaction and must be reoxidized by a separate enzyme reaction, therefore it is more a cosubstrate than a coenzyme.

able to adapt to extreme conditions. Although proteins are regarded as being very temperature sensitive, distinct microorganisms such as *Thermus, Thermotoga*, and *Thermoplasma*, including their complete enzymatic inventory, persist in temperatures up to 100 °C. It must be assumed that during evolution the ancient organisms have had to bear much higher temperatures. The ancient precursors of the present enzymes must have all been thermophilic, but obviously they lost this feature with the decrease in environmental temperature. This can also explain the fact that proteins, instead of the more stable nucleic acids, are preferred by nature as biocatalysts, although some catalytic activities are retained in RNA.

As an introduction to the practical work with enzymes, at least some fundamental theoretical rules must be discussed. They will be addressed in the first part, followed by a description of the general features of enzymes, which must be considered when dealing with them. This is followed by a presentation of the most important techniques. This general part should enable the reader to work with enzymes; for instance, to develop an assay for a newly isolated enzyme without further need to consult the literature. The following special part presents detailed descriptions of enzyme assays and related methods such as protein determination. A multitude of assays corresponding to the immense number of different enzymes exists, which cannot all be considered within the scope of a laboratory manual; rather, only a selection can be presented. Criteria for the selection are not only the frequency of application, but also the broad variety of enzyme types and methods. Certainly, such a selection cannot satisfy all expectations and the choice will sometimes appear rather arbitrary. For further information the reader is referred to standard books and databases of enzymology (see References section below). Procedures for immobilization of enzymes and special aspects of analysis of immobilized enzymes, principles of enzyme reactors, and enzyme electrodes are presented in separate sections of the book.

References

Alberty, W.J. and Knowles, J.R. (1976) *Biochemistry*, **15**, 5631–5640.
Feil, I.K., Lerch, H.P., and Schomburg, D. (1994) *Eur. J. Biochem.*, **223**, 857–863.
Menger, F.M. (1993) *Acc. Chem. Res.*, **26**, 206–212.
Radzicka, A. and Wolfenden, R. (1995) *Science*, **267**, 90–93.

Standard Books, Series, and Databases

Advances in Enzymology and Related Areas of Molecular Biology, John Wiley & Sons, Inc., New York.
Bergmeyer, H.U. (ed.) (1983) *Methods of Enzymatic Analysis*, 3rd edn, Verlag Chemie, Weinheim.
Methods in Enzymology, Academic Press, San Diego.
Schomburg, D. (ed.) (2001f) *Springer Handbook of Enzymes*, Springer, Berlin.
www.expasy.org/enzymes.
www.brenda-enzymes.org.