BIOCATALYSIS

Edited by

Daniel A. Abramowicz

Van Nostrand Reinhold Catalysis Series

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Biocatalysis, edited by Daniel A. Abramowicz

Dedicated to my parents, Albert and Veda Abramowitz
and to my wife, Alice Abramowicz

Chapter Summaries

The Archeology of Enzymology **1**

This chapter represents a review of early work in the field of enzymology. It is written with the perspective that the more we know about the past, the better prepared we are to understand and learn from it. This work discusses efforts from the eighteenth century to present day.

Synthesis of Polyesters by Lipase-Catalyzed Polycondensation in Organic Media **25**

The application of hydrolases to the condensation of diesters with diols represents a new method for the preparation of $[AA\text{-}BB]_x$ polyesters. This transesterification reaction catalyzed by porcine pancreatic lipase appears to be general for the coupling of bis(2,2,2-trichloroethyl) alkanedioates with primary alkanediols to produce polyesters of moderate molecular weight (5,000–15,000 daltons). In addition, the method can be used to prepare optically active polyesters with a highly reactive epoxide group incorporated into the polymer backbone.

Regiospecific Hydroxylation of Biphenyl and Analogs by *Aspergillus parasiticus* **63**

Whole cell fermentations of the filamentous fungus *Aspergillus parasiticus* were used to catalyze the para hydroxylation of biphenyl and similar compounds. Broad substrate specificity was observed and rates of 1 g product/g fungus/day have been achieved. Product concentrations of >2 g/L and yields >95% may make this biosynthetic one-step process a potential economic alternative to current chemical methods.

Simple Carbohydrates as Starting Materials in Organic Synthesis: A Comparison of Strategies **93**

The synthesis of an organophosphate inhibitor of the enzyme DHQ synthase, 3-deoxy-D-arabino-heptulosonate-7-phosphonic acid, from simple carbohydrates is examined. The three strategies compared in-

cluded chemical synthesis, immobilized enzyme synthesis, and whole cell fermentations. The microbial whole cell synthesis using an *E. coli aroB* strain proved to be the most convenient synthesis on a larger scale.

The Production of Amino Acids by Transamination **115**
The application of transaminase enzymes to the preparation of chiral amino acids was investigated. Enzymes were immobilized onto silica-based supports that remained active for >1,000 h. These systems could catalyze the conversion of 2-ketoacids to the corresponding L-amino acids at high yields. In addition, the genes encoding this activity were cloned to facilitate the production of highly active enzyme preparations.

A Biocatalytic Approach to Vitamin C Production: Metabolic Pathway Engineering of *Erwinia herbicola* **135**
The microbial synthesis of 2-keto-L-gulonic acid (2-KLG), a key intermediate in the synthesis of ascorbic acid, is discussed. This was achieved by metabolic pathway engineering, combining portions of the catalytic pathway from two diverse organisms. By cloning the 2,5-diketo-D-gluconic acid (2,5-DKG) reductase gene from a *Corynebacter* species and expressing this gene in *Erwinia herbicola*, a strain was created that produces 2-KLG from glucose in a single fermentative step.

Chiral Synthons by Biocatalysis **157**
The application of certain pesticides as their single active stereoisomer rather than as racemates provides a real opportunity to utilize biocatalysis to the synthesis of key optically active intermediates. The production of phenoxyproponate herbicides as active stereoisomers requires S-2-chloropropanoic acid. This paper describes the application of a novel dehalogenase enzyme to affect kinetic resolution of racemic chloropropanoic acid to produce the desired isomer in high enantiomeric excess and in high yield.

Enzymatic Resolution of Ibuprofen in a Multiphase Membrane Reactor **167**
The use of enzymes to affect resolutions, via enzymatic hydrolysis of a racemic mixture of esters, has created engineering challenges in the

area of bioreactor design. A multiphase membrane reactor can be used to overcome the limitations of such immobilized enzyme systems with water-insoluble substrates. The application of this bioreactor for the enzymatic resolution of ibuprofen is described, along with process design improvements.

Microbial Reduction of Carbonyl Compounds: A Way to Pheromone Synthesis **178**

In an extension of previous efforts developed for the enzymatic reduction of carbonyl compounds, the chiral syntheses of natural insect pheromones has been demonstrated. These whole cell processes illustrate that fermentation conditions can dramatically alter the observed stereoisomer ratio. In all cases, the biosynthetic routes described are more efficient and convenient than previously reported chemical methods.

Resolution of Binaphthols and Spirobiindanols Using Pancreas Extracts **195**

The enantiospecific hydrolysis of binapthol and spirobiindanol diesters has been achieved with the enzyme cholesterol esterase. Synthetic scale (200 g) resolution of 1,1′-binaphthalene-2,2′-diol yielded each enantioner at greater than or equal to 99% enantiomeric excess (ee). Hydrolysis of the diesters to diols involves two enzyme-catalyzed steps, and calculations show that such multistep resolutions can yield higher purity stereoisomers.

Chiral Synthons by New Oxidoreductases and Methodologies **217**

The stereospecific microbial reduction of carboxyl compounds was investigated. Applications include the reduction of a range of 2-enolates with *Clostridium tyrobutyricum* and the reduction of a range of 2-oxo-carboxylates to (2R)-hydroxy-carboxylates with *Proteus vulgaris*. In addition, a newly detected tungsten containing enzyme has been shown to reduce carboxylic acids to aldehydes.

Enzymes from Extreme Environments **243**

Very stable enzymes are readily available from organisms growing in extreme environments, especially extreme thermophiles. Such thermostable enzymes isolated from extremely thermophilic archaebacteria display significant half-lives above 100°C. These enzymes should en-

able biosynthetic applications under harsh conditions. In addition, it has been shown that when cloned into mesophiles, heat treatment enables rapid, large-scale purification of the thermostable cloned enzymes.

Biocatalysis in Anaerobic Extremophiles **255**
Certain anaerobic microorganisms have developed unique biocatalytic mechanisms for adaptation to extreme environments (including temperature, pH, and salt concentration). This chapter describes these organisms and discusses the application of enzymes isolated from these organisms in biosynthesis. The Haloanaerobes and Acidoanaerobes appear to have evolved intracellular enzymes that function under high salt or acidic conditions. In addition, unique enzymes from Thermoanaerobes may have potential industrial utility due to their high physicochemical stability and broad substrate specificity.

Large-Scale Bioconversion of Nitriles into Useful Amides and Acids **277**
Nitrile-hydrolyzing enzymes, such as nitrile hydratase and nitrilase, have demonstrated great potential as catalysts for the conversion of nitriles into higher-value amides or acids. Recently, bacterial nitrile hydratase has been utilized for the production of the important chemical commodity acrylamide on an industrial scale. This work describes this enzymatic process as well as recent progress in the microbial transformation of nitriles.

Aldolases in Organic Synthesis **319**
The synthetic utility of adolases has been demonstrated for the synthesis of common and uncommon sugars with bacterial fructose-1,6-diphosphate aldolase and N-acetylneuraminic acid aldolase. Both thermodynamically and kinetically controlled C–C bond formations have been developed for the synthesis of C-alkyl and N-containing sugars. In addition, the use of enol esters in enzymatic transformations of sugar-related compounds has been shown.

Two-Liquid Phase Biocatalysis: Reactor Design **337**
The application of a two-liquid phase bioreactor to reactions involving compounds of low aqueous solubility is investigated. In this chapter,

the elucidation of the engineering parameters required for reactor design are discussed. The relevant design criteria are identified and methods suitable for obtaining such data are presented. The hydrolysis of benzyl acetate by pig liver esterase serves as a quantitative example of this methodology.

Enzymes That Do Not Work in Organic Solvents: Too Polar Substrates Give Too Tight Enzyme-Product Complexes **357**

The replacement of water by an apolar organic solvent largely effects the association between the enzyme and either substrate or product. This effect is important for enzyme complexes that demonstrate strong hydrogen bonding in aqueous solutions. It is believed that carbohydrate-converting enzymes are not catalytically active in organic solvents for this reason. This is supported by results that such enzymes are indeed active in organic solvents when the product formed is much less polar than the substrate.

Series Introduction

The action of enzymes fascinated mankind long before they were recognized for the complex chemicals that they are. The first application of these remarkable compounds to produce ethanol by fermentation is lost to antiquity. Payer and Persoz (*Ann. Chim. Phys., 53,* 73 (1833ii)) appear to have provided the first step toward understanding this complex area when they reported the isolation of diastase in 1833. These workers showed that diastase could catalyze the hydrolysis of starches to sugars. Somewhat earlier Kirchhoff (*Schwigger's Journal, 4,* 108 (1812)) had shown that a small amount of dilute acid could hydrolyze a seemingly endless amount of starch to sugars. The genius of Berzelius recognized the commonality of these two observations in connection with a few other isolated observations and in 1834 coined the term catalysis to describe such actions.

Professor Leibig was one of the giants of the chemical world in 1840. In addition to his own work, Liebig was training the world's next generation of chemists in his laboratory in Giessen. This cadre of chemists were very impressed by the master teacher so that is it only natural that Liebig's views should dominate with this next generation of chemists. Leibig was, in the 1830s and 1840s, developing his mastery of agricultural chemistry. The mechanism of putrefication was of great concern to Leibig, and he turned to the newly defined area of catalysis for an explanation. However, Liebig did not adopt Berzelius' explanation of catalysis involving a new force—a catalytic force. Instead, Liebig viewed catalysis to be an induction of activity in an inactive body by the actions of a nearby active body. Thus, an unreacting mass could be made to react by placing it so that the motions of a highly active reacting mass could be transmitted to the unreacting body, and thereby activate it. Simply stated: place a rotting apple into a barrel of good apples and you soon have a barrel of rotten apples—to Liebig the rotting apple was in a highly active vibrating state and thus induced

vibrations in the good apples so that they also became activated and rotten. This induced-vibration theory spread just as the graduates of Liebig's school spread throughout the scientific world.

Some 50 years later Ostwald, a future Nobel Prize winner, would refute this vibration theory as one that is worthless since it could not be subjected to experimental verification. Ostwald offered instead a kinetic definition of catalysis (*Z. Physik Chem., 15,* 705 (1894)). In Ostwald's view, a catalyst acted only to increase the rate of a reaction that was already occurring at a slow rate. Ostwald's view worked very well where the catalytic species could be described on a quantitative basis as, for example, the hydrogen ion. Thus, in the early 1900s the future Nobel Prize winner Langmuir was establishing the kinetic laws for heterogeneous catalysis (*Trans. Faraday Soc., 17,* 621 (1921)). Even earlier, Michaelis and Menton (*Biochem. Z., 49,* 333 (1913)) were making a definition of the kinetic law for enzyme action that was identical to the form deduced independently by Langmuir, but their law did not immediately attract the attention of the catalytic world.

Thus, in the early 1900s, a description of the kinetics in catalytic and enzymatic reactions was at about the same stage. However, the understanding to enzyme action was destined to progress at a slower rate, and this was primarily due to the complexity of the enzyme. Difficu[illegible] in purification and structure identification ensured that the progress would be slow, and that advances could only come with great effort. But in recent years the situation has changed rapidly. Sophisticated instrumentation now allow a determination of composition, both chemical sequence and three-dimensional configuration, that early workers could not even imagine in their wildest dreams. With this rapid advance in defining the structure of the enzyme, and even the catalytic site, understanding of enzyme action has speedily advanced. This volume defines much of these recent advances as it applies to the synthesis of organic products, both at the fundamental level and at the level important for commercial applications.

BURTRON H. DAVIS

Preface

This volume contains the applications of enzymes (biocatalysts) to the synthesis of specialty chemicals. These efforts involve whole cell as well as isolated and immobilized enzyme systems. By definition this work excludes the synthesis of low value bulk chemicals (methane, ethanol, biomass, etc.) and of hormone and protein products (human growth hormone, insulin, etc.). Instead, this volume focuses on the relatively new application of enzymes to the synthesis of organic chemicals with intermediate value (~$1–20/lb), an area of great commercial importance. The major themes presented in this work include:

- History of biocatalysis
- Applications in plastics (monomer and polymer synthesis)
- Biosynthesis of biotic metabolites (carbohydrates, peptides, vitamins)
- Chiral resolutions (herbicides, pharmaceuticals, chiral auxiliaries)
- Enzymes from extremophiles (thermophiles, acidophiles, halophiles)
- Applications of aldolases, enzymes in organic solvents, bioengineering concepts, and large-scale applications.

The volume is an outgrowth of the first international conference on the biocatalysis of organics and selected papers are included. The conference "Biocatalytic Synthesis of Organic Compounds" was held at Skidmore College in Saratoga Springs, New York, August 8–12, 1988. The conference was organized by an Executive Committee consisting of:

Conference Co-chairman	Daniel A. Abramowicz (GE)
	Alexander M. Klibanov (MIT)

Organizing Committee	David L. Anton (duPont)
	Arnold Demain (MIT)
	Charles R. Keese (GE)
	Saul Neidleman (Cetus)

I would also like to take this opportunity to acknowledge the assistance of Herman L. Finkbeiner (GE) who conceived of the Biocatalysis Conference and was therefore responsible for its success and, indirectly, for this volume.

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