Biocatalysis in the Pharmaceutical and Biotechnology Industries

Edited by

Ramesh N. Patel

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Preface

There has been an increasing awareness of the enormous potential of microorganisms and enzymes for the transformation of synthetic chemicals in a highly chemo-, regio-, and enantioselective manner. Chiral intermediates are in high demand from pharmaceutical, agricultural, and other biotechnological industries for the preparation of bulk drug substances or fine chemicals. Bulk drug compounds or other fine chemicals can be produced by chemical or chemo-enzymatic synthesis. The advantages of biocatalysis over chemical synthesis are that enzyme-catalyzed reactions are often highly enantio- and regioselective. They can be carried out at ambient temperature and atmospheric pressure, thus avoiding the use of more extreme conditions which could cause problems with isomerization, racemization, epimerization, and rearrangement. Microbial cells and enzymes derived from microbial cells can be immobilized and reused for many cycles. Biocatalysis includes fermentation, biotransformation by whole cells or enzyme-catalyzed transformations, cloning and expression of enzymes, and directed evolution of enzymes to improve selectivity, substrate specificity, and stability. Various chapters in this book are contributed by internationally well-known scientists having many years of experience in different aspects of biocatalysis and biocatalytic applications in production of fine chemicals and chiral pharmaceutical intermediates.

This book contains 34 chapters with over 4000 references and more than 600 tables, equations, drawings, and micrographs. All the information cited in this book provides state-of-the-art knowledge and improves the ability of the reader to use different types of enzymatic reactions in synthesis of fine chemicals and chiral compounds and their application in biotechnological industries. Various chapters discuss the following important aspects in biocatalysis and its applications in various industries:

- Application of nitrilases and nitrile hydratases in synthesis of fine chemicals that describe cloning and expression of nitrilases and their use in production of chiral and achiral carboxylic acids, regioselective and chemoselective hydrolysis of nitriles, preparation of amides from nitriles, commercialized processes for preparation of nicotinamide, cyanovaleramide, acrylamide, and nitrile-containing polymers
- Biocatalytic deracemization processes that include dynamic kinetic resolution, stereoinversion processes, and enantioconvergent processes to prepare chiral compounds such as amino acids, amines, alcohols, diols, and epoxides in theoretical 100% yields
- Biocatalysis in pharmaceutical industries for synthesis of chiral intermediates and fine chemicals for chemoenzymatic synthesis of drugs such as anticancer, antiviral, antihypertensive, anticholesterol, anti-infective, anti-infammatory, antianxiety, and antipsychotic drugs
- Methods for directed evolution of lipases and esterases, assay development and screening of mutants for selection in esterification, transesterification, acylation and acyl hydrolytic reactions, and use of improved enzymes in organic synthesis
- Oxidative biocatalysis catalyzed by flavin-containing flavoprotein oxidases such as alcohol oxidases, amine oxidases, and sulfhydryl oxidases together with flavoprotein monooxygenases such as aromatic, heteroatom, and multicomponent monooxygenase in enzymatic oxygenation reactions

- Biotransformation (hydroxylation, dealkylation, N-oxide formation, and *O*-demethylation reactions) of natural and synthetic compounds for the generation of molecular diversity and drug metabolites
- Enzymatic acylation of alcohols and amines in preparation of pharmaceuticals such as anticonvulsant agents, anticancer agents, immunosuppressive compounds, analgesic drugs, antidepressants, anticholesterol drugs, antibiotics, β-adrenergic blockers, calcium channel blockers, serotonin uptake inhibitors, antifungal agents, anti-Alzheimer's agents, antiulcer agents, α-adrenoceptor agonists, and other drug substances
- Enzyme–metal complex-catalyzed asymmetric biotransformations and dynamic resolution processes to prepare chiral alcohols, amines, and acetates
- Applications of aromatic hydrocarbon dioxygenases such as toluene dioxygenase, naphthalene dioxygenase, chlorobenzene dioxygenase in synthesis of fine chemicals, and pharmaceuticals with continuous cofactor regeneration during biotransformations
- Investigation on baker's yeast reduction processes by genomic approach and preparation of chiral alcohols and synthesis of anticancer, anticholesterol, and antihypertensive drugs
- Techniques and applications of immobilization of enzymes as cross-linked enzyme aggregates (CLEA) in synthesis of fine chemicals
- Application of C–C bond forming enzymes such as aldolases and transketolases in synthesis of fine chemicals and pharmaceuticals
- Biocatalytic synthesis of nucleoside analogs by modification of base, sugar and oxidation reactions, and applications of nucleoside analogs as antiviral agents
- Biocatalytic reduction of carboxylic acids by carbonyl reductases, mechanism of carbonyl reductases, and cloning, and expression of enzyme with application in synthesis of fine chemicals
- Application of dehalogenases in biocatalysis and biodegradation with emphasis on haloalkane dehalogenases, haloacid dehalogenases, and halohydrin dehalogenases and their application in preparation of chiral compounds
- Enzymatic synthesis of sugar esters and oligosaccharides from renewable resources that includes regioselective synthesis of fatty acid sugar ester using lipases and proteases, and synthesis of oligosaccharides by transglycosidases and transglucosidases
- Efficient methodology and instrumentation for engineering custom enzymes using directed evolution and solid-phase screening to maximize high throughput and selection of evolved enzymes and application of highly active enzymes in synthesis of intermediates for pharmaceuticals
- Biocatalytic enantioselective and diastereoselective deaminations for chemoenzymatic synthesis of antiviral agents using adenosine deaminase or adenylate deaminase
- Enzymatic resolution of lactones by lactonases and synthesis of chiral alcohols by carbonyl reductases and effective cofactor regenerating systems for synthesis of pharmaceutical intermediates
- Enzymatic acyloin condensations by decarboxylases and rational design of arylmalonate decarboxylase for synthesis of fine chemicals and chiral intermediates
- Enantioselective biocatalysis for synthesis of pheromones and juvenile hormones
- Stereoselective and regioselective modifications of polyhydroxylated steroids by dehydrogenases, lipases, and proteases to prepare novel steroidal compounds
- Industrial enzymatic processes for C–C, C–N, C–O bond formations by lyases such as phenylalanine ammonia lyase, fumarase, malease, hydratases, and dehydratases
- State of the art and application in enantioselective synthesis of chiral cyanohydrins by hydroxynitrile lyases

- Chiral switches strategies, opportunities, and experiences, and biocatalysis in preparation of ibuprofen, ketoprofen, esomeprazole, methylphenidate, doxyzosin, levofloxacin, and other chiral molecules
- Cutting-edge methodology for gene shuffling, family of genes shuffling, directed evolution, and high-throughput screening of mutants to increase the selectivity, activity, and stability of enzymes
- Biocatalytic preparations of chiral amines by kinetic resolution, dynamic kinetic resolution; deracemization and stereoinversion processes using transaminases, amine oxidases, and lipases

Biocatalysis in the pharmaceutical and biotechnological applications is an indispensable resource for organic chemists, biochemists, microbiologists, biochemical engineers, biotechnologists, medicinal chemists, pharmacologists, and upper-level undergraduate and graduate students in these disciplines.

It is my pleasure to acknowledge sincere appreciation to all the authors for their contribution to this book. I would like to acknowledge continual support from David Fausel (production coordinator) and Anita Lekhwani (acquisition editor) and Richard Tressider (project editor) of the Taylor & Francis Group and Vinithan Sethumadhavan (project manager) of SPi. My interest in biocatalysis was developed and stimulated by David Gibson, Derek Hoare, Nicholas Ornston, Allen Laskin, Ching Hou, Laszlo Szarka, Christopher Cimarusti, John Scott, Richard Mueller, and many of my colleagues at the University of Texas, Yale University, Exxon Research and Engineering Company, and Bristol-Myers Squibb. I acknowledge their support and encouragement over the last 35 years. Finally, I would like to express my sincere thanks to my wife, Lekha, and my daughter, Sapana, for their support and encouragement while I worked on this book.

Editor

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Robert DiCosimo

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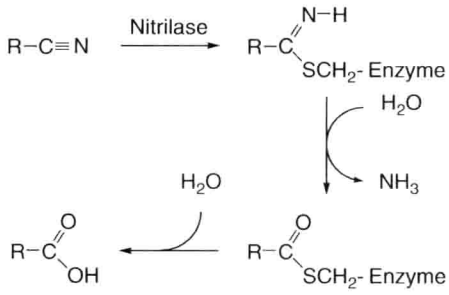
1.1 INTRODUCTION

Nitrilase and nitrile hydratase (NHase) are two classes of enzymes that are finding increasing use as catalysts for the conversion of nitriles to carboxylic acids and amides, respectively; in addition, NHases are often used in combination with amidases to produce carboxylic acids. These reactions can be enantioselective, chemoselective, and/or regioselective, and there are often no equivalent chemical catalysts that can produce the desired product with the same selectivity afforded by the enzyme-catalyzed reactions. A large number of publications, patent applications, and patents describe the preparation and use of these enzyme catalysts, and numerous reviews of nitrilase- and NHase-catalyzed reactions have been published. The most recent work in this area has been summarized here, with a focus on the application of these enzymes as catalysts in synthesis, as well as in process development or in commercial processes.

1.2 NITRILASES

Nitrilases have been the subject of a number of recent reviews [1–7]. All known nitrilases share a highly conserved region of amino acid sequence which includes a cysteine that is responsible for the catalytic activity of the enzyme [1,8]. The mechanism that has been proposed for conversion



SCHEME 1.1 Proposed mechanism for hydrolysis of nitrile to carboxylic acid by nitrilase. (Redrawn from Kobayashi, M., Goda, M. and Shimizu, S., *Biochem. Biophys. Res. Commun.*, 253, 662, 1998.)

of a nitrile to a carboxylic acid by nitrilase is depicted in Scheme 1.1, where, after binding in the enzyme active site, the nitrile reacts with a cysteine sulfhydryl residue to produce an intermediate thioimidate; subsequent hydrolysis of this thioimidate produces the ammonium salt of the carboxylic acid [9]. A crystal structure has not yet been reported for nitrilase.

1.2.1 Heterologous Nitrilase Expression

A number of recent patent applications describe the preparation of nitrilase catalysts, where either the nitrilase gene was isolated from a wild-type cell and expressed in a transformant such as *Escherichia coli*, or variants of the wild-type nitrilase gene were created through directed evolution techniques and heterologously expressed. The nitA gene encoding an enantioselective nitrilase from *Rhodococcus rhodochrous* NCIMB 11216 was cloned and expressed in *E. coli*, and the resulting transformants screened for activity against a variety of aliphatic and arylaliphatic nitriles [10]. A "PnitA-NitR" system for regulatory gene expression in *Streptomycetes* has been developed, based on the expression mechanism of *R. rhodochrous* J1 nitrilase, which is highly induced by ε-caprolactam [11]; heterologous protein expression yielded nitrilase levels of as high as 40% of soluble protein. The nitrilase from *Acidovorax facilis* 72W has been cloned and expressed in *E. coli*, where the amount of nitrilase protein produced (active and inactive) was 58% of total soluble protein, and active nitrilase comprised 12% of total soluble protein [12].

The nitrilase gene from the photosynthetic cyanobacterium *Synechocystis* sp. strain PCC6803 has been expressed in *E. coli*, and the purified nitrilase isolated from this recombinant strain was characterized [13]. The observed substrate specificity of the purified nitrilase most closely resembled that of previously described aliphatic nitrilases, and the temperature optima (40 to 45°C) and pH optima (pH 7 to 7.5) were similar to the nitrilases of the mesophilic bacteria *R. rhodochrous* J1 or *Alcaligenes faecalis* JM3. The purified enzyme was active in the presence of a wide range of organic solvents; for example, after incubation of the nitrilase for 10 min in a mixture of 50 mM phosphate buffer and solvent, the rate of hydrolysis of benzonitrile to benzoic acid was not significantly affected by 40% dimethyl sulfoxide or *n*-heptane, 20% methanol, or 10% ethanol or dimethyl formamide. The turnover rates of substrates with poor water solubility, e.g., dodecanoic acid nitrile and naphthalenecarbonitrile, were increased in the presence of both water-soluble and water-immiscible solvents.

Two nitrilase genes, ZmNIT1 and ZmNIT2, have been isolated from maize (*Zea mays*), and heterologously expressed in *E. coli*. [14]. ZmNIT2 and *Arabidopsis* NIT4 have a relatively high homology (69.3%) but ZmNIT2 had no activity toward β-cyanoalanine, the substrate of *Arabidopsis* NIT4, and instead hydrolyzed indole-3-acetonitrile (IAN) to indole-3-acetic acid (IAA), where AtNIT4 had no activity for hydrolysis of IAN.