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Research review paper

Industrial applications of enzyme biocatalysis: Current status and future aspects

Jung-Min Choi [1], Sang-Soo Han [1], Hak-Sung Kim *

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Gusung-dong, Yusung-gu, Daejon 305-701, Republic of Korea

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ABSTRACT

Enzymes are the most proficient catalysts, offering much more competitive processes compared to chemical catalysts. The number of industrial applications for enzymes has exploded in recent years, mainly owing to advances in protein engineering technology and environmental and economic necessities. Herein, we review recent progress in enzyme biocatalysis, and discuss the trends and strategies that are leading to broader industrial enzyme applications. The challenges and opportunities in developing biocatalytic processes are also discussed.

Contents

1. Introduction

Enzymes are the most proficient catalysts, offering much more competitive processes compared to chemical catalysts. A number of enzyme-based processes have been commercialized for producing several valuable products since the biocatalysis was first introduced almost century ago (Bruggink et al., 1998; Estell et al., 1985; Jensen and Rugh, 1987; Sedlaczek, 1988). Despite great potential of enzymes, however, their industrial applications have been hampered mainly owing to undesirable property in terms of stability, catalytic efficiency, and specificity. To overcome such shortcomings, a variety of approaches have been attempted, including screening of enzymes from natural sources, random mutations, immobilization (Dincer and Telefoncu, 2007; Elleuche et al., 2014). During 1980s and 1990s, engineering of enzymes based on structural information allowed extension of their substrate ranges, enabling the synthesis of unusual intermediates. Accordingly, the use of enzymes has been expanded to the manufacture of pharmaceutical intermediates and fine chemicals (Griengl et al.,

* Corresponding author at: Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Gusung-dong, Yusung-gu, Daejon 305-701, Republic of Korea. Tel.: +82 042 350 2616.
E-mail address: hskim76@kaist.ac.kr (H.-S. Kim).
[1] Equally contributed.

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2000; Hills, 2003; Nagasawa et al., 1990). Although industrial applications of biocatalysis have expanded through structure-based rational design, a lack of structural and mechanistic knowledge about enzymes has limited widespread use of enzymes.

Since the mid 1990s, *in vitro* version of Darwinian evolution, so called directed evolution, has made a great contribution to the development of enzymes with great potential (Bornscheuer et al., 2012). Iterative cycles of random mutagenesis, followed by screening of a library enabled rapid and extensive improvement of various properties of enzyme, including stability, substrate specificity, and enantioselectivity. Directed evolution has thus made remarkable progresses in industrial applications of biocatalysis (Kumar and Singh, 2013). Recently, directed evolution tends to be merged with rational design and computational methods to enhance the efficiency of enzyme design by creating focused and smarter libraries (Bornscheuer et al., 2012). Rational design and computational methods based on the structure-function relationships are becoming popular in enzyme engineering. Structural complexity of valuable substances such as drugs and chiral intermediates requires biocatalysts with high stereo- and regio-specificities in industrial process. Accumulated knowledge on the structure-function and dynamics-function relationships are enabling *de novo* design of enzymes with new functions (Rothlisberger et al., 2008; Siegel et al., 2010), broadening the repertoire of enzymes.

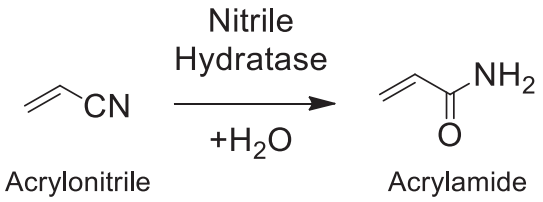
Soaring oil prices have led industries to seek alternative sources of raw materials, such as biomasses. Furthermore, public pressure on green technologies owing to environmental issues has strongly demanded the replacement of chemical processes with cleaner, safer, and more eco-friendly biocatalytic processes (Benkovic and Hammes-Schiffer, 2003). In these regards, enzyme biocatalysis has rapidly substituted traditional chemical processes in many areas, and such replacement is expected to be more accelerated through the development of new technologies in enzyme engineering. In this review, we describe recent advances in enzyme biocatalysis for industrial applications, and discuss the trends and strategies in engineering of industrial enzymes. The challenges and opportunities of enzyme biocatalysis in future will be also discussed.

2. Biocatalysts in industrial biotechnology

2.1. Fine and bulk chemical industries

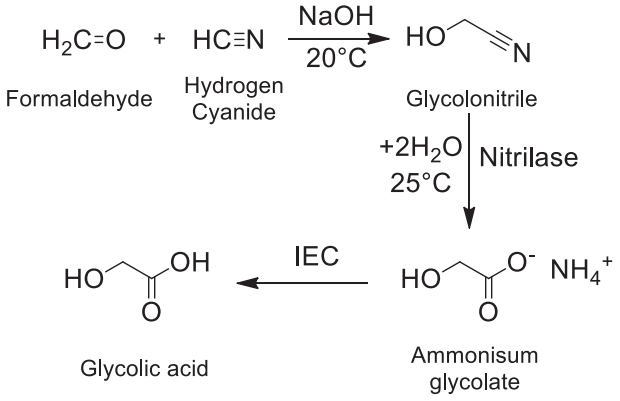
Applications of enzymes and whole cell biocatalysis for producing diverse types of chemical and biological substances have become a proven technology in chemical and pharmaceutical industries because enzyme-based processes usually lead to a reduction in the process time, number of reaction steps, and amount of waste (Wohlgemuth, 2010). In particular, enzymes provide a more powerful way of producing enantiomerical pure compounds mainly through high chemoselectivity, regioselectivity, and streoselectivity (Nestl et al., 2011). Some examples showing the contribution of biocatalysis to fine and bulk chemical fields are described below.

Acrylamide is an important commodity chemical for synthesizing polyacrylamide used for petroleum recovery, wastewater treatment, papermaking, pesticide formulation, soil erosion prevention, and gel electrophoresis (Asano, 2002; Leonova et al., 2000). Traditionally, acrylamide can be produced chemically by oxidizing acrylonitrile using copper and sulfuric acid as a catalyst at high temperature (Padmakumar and Oriel, 1999). However, both methods are known to cause several types of environmental pollution. The discovery of nitrile hydratase (EC 4.2.1.84) and its application in nitrile hydration has offered a novel process for the production of acrylamide (Asano et al., 1982; Nagasawa et al., 2000). *Rhodococcus rhodochrous* J1 overexpressing nitrile hydratase efficiently converts acrylonitrile into acrylamide at up to 45% (W/W) under mild conditions (Scheme 1). This biotranformation process produces over 650,000 t annually in Japan (Ogawa and Shimizu, 2002; Groger et al., 2012; Reetz, 2013). To further increase the productivity, Cui et al. recently engineered nitrile hydratase from *Pseudomonas putida* NRRL-18668, and showed improvements in thermal stability and catalytic activity of 3.5- and 1.5-fold, respectively (Cui et al., 2014). Kang *et al.* also reported the overexpression of nitrile hydratase from *Rhodococcus rhodochrous* in *Corynebacterium glutamicum*, and the recombinant cells resulted in a conversion yield of 93% and final acrylamide concentration of 42.5% in 6 h (Kang et al., 2014).



Scheme 1. Conversion of acrylonitrile into acrylamide using nitrile hydratase.

Glycolic acid is a C2 chemical building block that has found a wide range of applications in cosmetics, food industry and as a precursor for biopolymers (Koivistoinen et al., 2013; Panova et al., 2007). Glycolic acid can be polymerized into polyglycolic acid (PGA), which has high strength and thermo-tolerance as well as low gas permeability suitable as an ideal packaging material for food and other goods. The glycolic acid market in 2011 was $93.3 million, and the total production amount was 40 million kg. The market is expected to reach $203 million in 2018 (Koivistoinen et al., 2013). The conventional method of glycolic acid production relied on the reaction of formaldehyde and carbon monoxide through an acid catalysis at high pressure and temperature (Panova et al., 2007). An alternative method is the use of heterologous host expressing nitrilase (EC 3.5.5.1), lactoaldehyde reductase (EC 1.1.1.77), and lactoaldehyde dehydrogenase (EC 1.2.1.22) for the hydrolysis of glycolonitrile and the oxidation of ethylene glycol, followed by the conversion of glycolic acid (Chauhan et al., 2003). However, conventional chemical and biotransformation methods for glycolic acid have certain drawbacks such as high impurity. For the production of high-purity glycolic acid, Panova et al. attempted a chemo-enzymatic process using *E. coli* cells overexpressing nitrilase from *Acidovorax facilis* 72 W (Panova et al., 2007). This chemo-enzymatic process comprises the synthesis of glycolonitrile from formaldehyde and hydrogen cyanide using NaOH, followed by the conversion of glycolonitrile into ammonium glycolate by nitrilase at room temperature, which is further converted to glycolic acid by ion exchange chromatography (IEC) (Scheme 2). This process enables the productivity of more than 1 kg of glycolic acid/g dry cell weight. In addition, to further increase the catalytic activity of nitrilase, a directed evolution was attempted, resulting in a 125-fold increase (Wu et al., 2008).



Scheme 2. Synthesis of glycolic acid from formaldehyde and hydrogen cyanide using nitrilase.