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A mini-review of Chinese contributions to *Journal of Molecular Catalysis B: Enzymatic*

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Abstract

Since the birth of *Journal of Molecular Catalysis B: Enzymatic* in 1995, 13 papers contributed from Chinese authors have been published in this *journal* by the end of year 2001. These papers were summarized in this mini-review by dividing their contents into several subjects, including immobilization of biocatalysts, enzymatic polymerization, asymmetric biocatalysis, non-aqueous biotransformation, and overexpression and stabilization of enzymes. Though the number of annually published papers by Chinese authors in this *journal* is still very limited, it is increasing rapidly in the recent years.

Keywords: *Journal of Molecular Catalysis B: Enzymatic*; Special issue; Enzyme engineering in China; Chinese contributions; Mini-review

1. Introduction

At the very beginning of the 21st century, a new era of biology and biotechnology has been rapidly emerging along with the completion of sketch mapping of human being's genome, which will necessarily generate huge and revolutionary impacts on the development of enzymology and enzyme engineering in the future [1]. China has experienced great changes during the past two decades, in almost all areas including enzyme engineering. World has paid much attention and will pay more to any progress of enzyme engineering in China, because China has just been accepted as a new member of the World Trade Organization (WTO), indicating that China will interact with outside more strongly. As evidence of the developing trend of enzyme engineering research in China, the number of annually published papers by "Chinese authors" in this *journal* (*Journal of Molecular Catalysis B: Enzymatic*) is growing very rapidly, as illustrated in Fig. 1. It is worth noting that the so-called "Chinese authors" here is referred to only those authors with intellectual rights affiliating to China (including both the mainland and Taiwan regions of China), excluding those who study or work abroad and publish papers in names of foreign institutions.

One purpose of this mini-review is to reflect the whole profile of enzyme engineering research activities conducted in China by briefly summarizing the 13 already published papers by Chinese authors in the *Journal of Molecular Catalysis B: Enzymatic*, during the period from the birth (1995) of the *journal* to the end of the year 2001.

2. Immobilization of biocatalysts

A temperature-sensitive copolymer of *N*-isopropylacrylamide (NIPAAm) and *N*-acryloxy-succinimide

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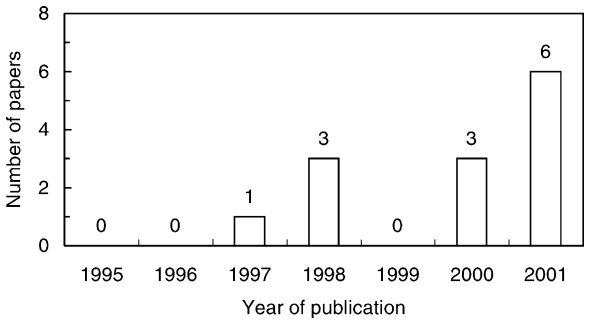

Fig. 1. Statistics of the number of papers annually published by Chinese authors in the *Journal of Molecular Catalysis B: Enzymatic*.

(NAS) was synthesized and utilized as a support for immobilization of α-chymotrypsin by Chen and Hsu [2] from the Chang Guang University (Taiwan, China). The chemically conjugated enzyme exhibited a lower critical solution temperature (LCST) of 35.5 °C, precipitated and flocculated in aqueous solution above the LCST and re-dissolved when cooled below that temperature. The immobilized enzyme showed enhanced thermal stability compared to the free enzyme.

In another paper contributed by Chen and Chen [3], phospholipase A_2 from cobra venom was immobilized by covalent binding to porous chitosan beads. The immobilized enzyme was packed into a circulating column reactor and was employed successfully for in vitro treatment of rabbit serum to lower the total serum cholesterol concentration via enzymatic hydrolysis of phospholipid on the surface of low density lipopotein particles.

A research group of Duan and co-workers at the Beijing University of Chemical Technology (Beijing, China) reported two types of new inorganic supports, mesoporous MCM-41 [4] and calcined layered double hydroxides (CLDH) [5], for immobilization of penicillin G acylase. MCM-41 is a member of the M41S family of silicate/aluminosilicate mesoporous materials, which have well ordered long-range structure, large pore diameter, narrow pore-size distribution and high pore volume and specific surface area. The penicillin G acylase can be immobilized on MCM-41 through either direct adsorption or covalent coupling with glutaraldehyde, with higher activity observed using the former method. CLDH, prepared by calcination of layered double hydroxides ($Mg(OH)_2/Al(OH)_3$ or $Zn(OH)_2/Al(OH)_3$), also have porous structures, large specific surface area and abundant basic sites for binding with an enzyme. Mg/Al-CLDH had a higher affinity for penicillin G acylase than Zn/Al-CLDH. The activity of CLDH-adsorbed enzyme decreased with increasing Mg/Al molar ratio of the CLDH. The basic nature of the CLDH support markedly increased the pH-stability of the penicillin G acylase against acid-induced inactivation which is easy to happen in enzymatic hydrolysis of penicillin G acylase into 6-APA and phenyl acetic acid.

3. Enzymatic polymerization

Zhu et al. at the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (Changchun, China) contributed two papers concerning the mechanism of cytochrome *c* (acting as peroxidase) catalyzed oxidative polymerization of *o*-phenylenediamine [6] and hydroxylated species of *m*-aminophenol [7] through isolation and identification of their polymerization products, as illustrated in Fig. 2.

4. Asymmetric biocatalysis

A simple and practical additive method has been developed by Liu et al. [8] at the East China University of Science and Technology (Shanghai, China) to significantly improve the enantioselectivity of a commercially available crude lipase preparation (Lipase OF, from *Candida rugosa*) for preparation of optically

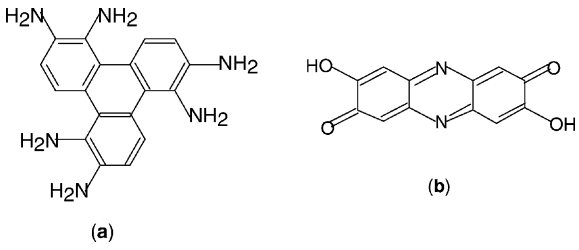

Fig. 2. Proposed structure of the polymerization products of *o*-phenylenediamine (a) and *m*-aminophenol (b) catalyzed by cytochrome *c*.

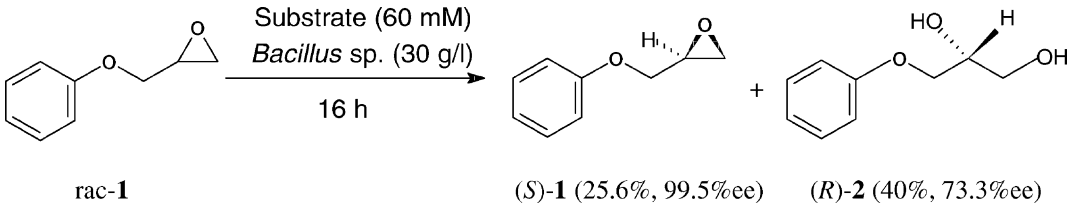

Fig. 3. Biocatalytic preparation of (*S*)-phenyl glycidyl ether using resting cells of a newly isolated *Bacillus megaterium* ECU1001 producing novel *R*-enantioselective epoxide hydrolase.

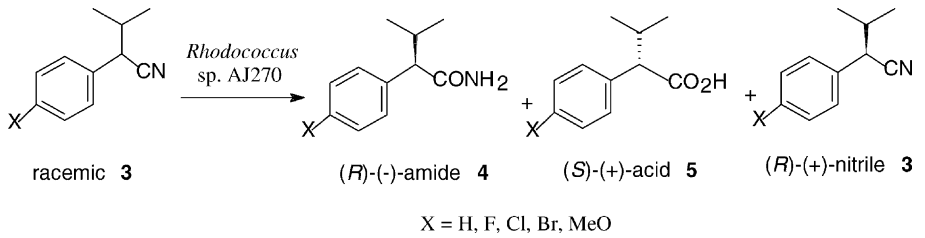

Fig. 4. Synthesis of enantiopure (*S*)-(+)-2-aryl-3-methylbutyric acids and (*R*)-(−)-2-aryl-3-methylbutyramides using *Rhodococcus* sp. AJ270.

pure (*S*)-ketoprofen as a chiral anti-inflammatory drug. Among various surfactants tested, two non-ionic emulsifiers, Tween-80 and OP-10 (nonyl phenol polyethyleneoxy ether), could enhance the activity of the crude enzyme by 13 and 15 times, respectively, at their optimal concentrations of 2 and 3% (w/v). In the presence of 2–5% (w/v) Tween-80, the enantiomeric ratio (*E*-value) increased from 1.2 to 6.7 for the crude lipase and from 8 to >100 for the partially purified lipase component (L2).

A bacterial strain (*Bacillus megaterium* ECU1001) capable of producing a novel epoxide hydrolase [EC 3.2.2.3] was isolated by Tang et al. [9] from soil samples through two steps of screening utilizing phenyl glycidyl ether (PGE) as sole carbon and energy sources. The epoxide hydrolase was biosynthesized in parallel with the cell growth, reaching a maximum of 31 U/l or 3.6 U/g DCW at 30 h of culture. When the lyophilized cells of *B. megaterium* ECU1001 were used as a chiral biocatalyst for enzymatic resolution of the racemic epoxide **1** (PGE), the (*R*)-enantiomer of PGE was preferentially hydrolyzed into (*R*)-diol **2** with high enantioselectivity ($E = 47.8$) and the optically pure (*S*)-epoxide was remained with >99.5% e.e. in a theoretical yield of 44% and an isolation yield of 25.6% (Fig. 3).

Wang et al. [10] at the Institute of Chemistry, Chinese Academy of Sciences reported that optically pure (e.e. >99%) (*S*)-(+)-2-aryl-3-methyl butyric acids **5** were successfully synthesized through enantioselective biotransformations of racemic 2-aryl-3-methylbutyronitriles **3** using nitrile hydratase and amidase-containing whole-cell of *Rhodococcus* sp. AJ270 as a robust biocatalyst. It was shown that the nitrile hydratase displays a low (*S*)-enantioselectivity against nitriles, whereas, the amidase exhibits a strict (*S*)-enantioselectivity against 2-aryl-3-methylbutyramides **4**, affording highly enantiopure (*S*)-(+)-2-aryl-3-methyl butyric acids **5** which are very important intermediates of pharmaceutical and agrochemical significance (Fig. 4).

5. Non-aqueous biotransformation

The biodiesel fuel made from vegetable oil is expected to replace conventional diesel because of some environmental advantages of the former. By collaborating with his Japanese colleagues (M. Iso), Chen and co-workers [11] at the University of Shanghai for Science and Technology (Shanghai, China) reported the production of biodiesel fuel from triglycerides by

transesterification reaction with short-chain alcohols in microaqueous media, using the immobilized *Pseudomonas fluorescens* lipase on porous kaolinite particle (Toyonite^{TM} 200-M) as biocatalyst. The product of this reaction, long-chain fatty acid ester, can be used as a diesel fuel that does not produce sulfur oxide and minimize the soot particulate. When methanol and ethanol were used as alcohol, organic solvent such as 1,4-dioxane was required. Whereas, the reaction could be performed in the absence of solvent when 1-propanol and 1-butanol were used as short-chain alcohols. The immobilized enzyme could be repeatedly used without significant loss of activity and troublesome separation of the product.

Reverse micelle is also an important type of micro-aqueous system which has been widely used as the medium for non-aqueous biotransformation with various enzymes such as lipase [12]. Chen et al. [13] at the East China University of Science and Technology (Shanghai, China) described the biocatalytic synthesis of galacto-oligosaccharides (GOS) from lactose by β-galactosidase solubilized in AOT/*iso*-octane reverse micellar solution. The increase in initial lactose concentration resulted in the enhancement of GOS yield in both aqueous and reverse micellar systems. The enzymatic transgalactosylation in reverse micelles was dependent on the molar ratio of water to surfactant (w_0). Under an optimized condition ($w_0 = 15$, pH 7.0, 45 °C), a maximum GOS yield of 51.2% (w/w) from 45% (w/v) lactose solution could be reached in reverse micelles, in contrast to a 31% yield of GOS in the case of aqueous solution, though the volumetric productivity of GOS in the later case was substantially larger than the former.

6. Overexpression and stabilization of enzymes

Cytidine 5′-monophosphate *N*-acetylneuraminic acid (CMP-NeuAc), as an important component of glycoproteins or glycolipids in mammalian tissue, plays a prominent role in a variety of biological processes including cell–cell communication, cell–matrix interaction, and protein targeting. In vitro, CMP-NeuAc can be synthesized enzymatically by a bacterial CMP-NeuAc synthetase. In order to increase the yield of enzyme production, the gene coding for CMP-NeuAc synthatase from *Escherichia coli* 44277 was cloned and overexpressed in *E. coli* BL21(DE3)pLysS through a primer-directed polymerase chain reaction, by Xia et al. [14] at the Institute of Microbiology, The Chinese Academy of Sciences (Beijing, China). Upon the induction of IPTG, the recombinant enzyme was shown to be 26% of the total bacterial proteins, which was 850-fold higher than that of the wild strain. Under the optimal inducing condition, the enzyme yield could reach 100 U/l cell culture and CMP-NeuAc could be synthesized in 90% yield by using the partially purified enzyme.

The stability and stabilization of an enzyme are also very important for its application. By combining the traditional approach of thermal inactivation kinetic analysis with the modern molecular modeling computation technique, Zhang et al. [15] at the Tianjin University (Tianjin, China) investigated the molecular mechanism responsible for the stabilization effect of trypsin caused by methoxypolyethylene glycol (MPEG) modification. It was shown by kinetic analysis that the enhanced thermal stability of MPEG modified trypsin is resulted from the decrease in both autolysis and thermal denaturation. The results of molecular modeling computation indicated that the steric hindrance caused by MPEG chain would result in the decreased rate of autolysis and the decreased rate of thermal denaturation should be ascribed to the increased number of hydrogen bond, instead of the increased molecular rigidity.

7. Concluding remarks

Though the annual and total numbers of papers published by Chinese enzyme engineers in this *journal* are still very limited at present stage, a larger number of papers are expected to appear in the near future, as can be seen from the rapidly increasing trend as shown in Fig. 1. In the past, *Journal of Molecular Catalysis B: Enzymatic* was not so known among Chinese biotechnologists because this *journal* is put together with other chemical journals (e.g. *Journal of Molecular Catalysis A: Chemical*) and, thus remote from other biotechnological journals (e.g. *Enzyme and Microbial Technology*) in many (if not all) China libraries. It is believed that the edition and publication of the special issue for enzyme engineering in China will significantly improve this unfavorable situation

and will, therefore, attract more Chinese scientists to notice at first, and then to contribute papers to this young journal which provides a good international forum for researchers who are interested in biocatalytic transformation.

In addition, it is worth noting that an increasing number of papers contributed from the Chinese enzyme engineering researchers have also been published by other biotechnology-related journals, such as *Biotechnology and Bioengineering*, *Enzyme and Microbial Technology*, *Applied Biochemistry and Biotechnology*, *Process Biochemistry*, and so on. A China–Japan joint Symposium on Enzyme Engineering has been performing every 2 years, since 1990 and the 14th International Enzyme Engineering Conference (Enzyme Engineering XIV) was also successfully held in Beijing (1997). More importantly, Chinese government is spending more money in supporting both basic research and industrial development of biotechnology including, of course, enzyme technology. Therefore, more and better papers from Chinese enzyme engineering scientists could be expected in the future, not only in *Journal of Molecular Catalysis B: Enzymatic*, but also in other related international journals.

Acknowledgements

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