Journal of Biotechnology 162 (2012) 183–190

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Use of hydrophilic ionic liquids in a two-phase system to improve Mung bean epoxide hydrolases-mediated asymmetric hydrolysis of styrene oxide

Wen-Jing Chen [a], Wen-Yong Lou [a,b,*], Chun-Yang Yu [c], Hong Wu [b], Min-Hua Zong [b,*], Thomas J. Smith [d]

[a] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510006, Guangdong, China
[b] Laboratory of Applied Biocatalysis, South China University of Technology, Guangzhou 510640, Guangdong, China
[c] School of Biosciences and Biotechnology, South China University of Technology, Guangzhou 510006, Guangdong, China
[d] Biomedical Research Centre, Sheffield Hallam University, Owen Building, Howard Street, Sheffield S1 1WB, UK

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form 5 September 2012
Accepted 8 September 2012
Available online 17 September 2012

Keywords:
Mung bean epoxide hydrolases
Styrene oxide
(*R*)-1-phenyl-1,2-ethanediol
Ionic liquids
Asymmetric hydrolysis

ABSTRACT

A comparative study was made of Mung bean epoxide hydrolases-catalyzed asymmetric hydrolysis of styrene oxide to (*R*)-1-phenyl-1,2-ethanediol in an *n*-hexane/buffer biphasic system containing various hydrophilic ionic liquids (ILs). Compared to the *n*-hexane/buffer biphasic system alone, addition of a small amount of hydrophilic ILs reduced the amount of non-enzymatic hydrolysis, and improved the reaction rate by up to 22%. The ILs with cation containing an alkanol group, namely [C_2OHMIM][BF_4] and [C_2OHMIM][TfO], and the choline amino acid ILs [Ch][Arg] and [Ch][Pro] were found to be the most suitable co-solvents for the reaction, owing to their good biocompatibility with the enzyme, which led to high initial rates (0.99–1.25 μmol/min) and high product *e.e.*s (95%). When substrate concentration was around 30 mM, where optimal performance was observed with the IL-containing systems, the product *e.e.* was improved from 90% without ILs to ≥95% in the presence of ILs.

1. Introduction

The asymmetric hydrolysis of epoxides is one of the most important and fundamental reactions for producing enantiomerically pure chiral vicinal diols, which are valuable and versatile chiral building blocks for production of many industrially important chemicals such as pharmaceuticals, pesticides, liquid crystals and flavors (Gong and Xu, 2005). The chiral vicinal diol (*R*)-1-phenyl-1,2-ethanediol ((*R*)-PED) is used as a key synthon for the preparation of the arylalkylamine calcimimetic (*R*)-(+)-NPS-R-568, NK-1 receptor antagonists (+)-CP-99,994, and the nucleoside analogs with antiviral activity (Jia et al., 2011). In addition, the opposite enantiomer (*S*)-PED can be employed for the synthesis of chiral biphosphines and chiral initiator for stereoselective polymerization (Nie et al., 2004). Recently, the preparation of enantiomerically pure chiral vicinal diols via green biocatalytic routes has attracted considerable interest due to the high enantioselectivity, mild reaction conditions and low environmental pollution that such technology offers. Among various biological methods, asymmetric hydrolysis of epoxides catalyzed by epoxide hydrolases (EHs) can be an efficient and economic option for synthesis of enantiopure vicinal diols. EHs, which are widespread in nature, are cofactor-independent enzymes that catalyze rapid asymmetric hydrolysis of a variety of epoxides to the corresponding vicinal diols (Kumar et al., 2011). Pedragosamoreau et al. first reported the asymmetric hydrolysis of styrene oxide (SO) to (*R*)-PED with the EHs from *Aspergillus niger* LCP 521 cells, but the product *e.e.* was only 51% (Pedragosamoreau et al., 1993). Subsequently, EHs isolated from *Bacillus alcalophilus* and other microorganisms, plants (cress, potato etc.) and animal tissues (including mouse and duck liver) have been widely used for the biocatalytic kinetic resolution of epoxides and higher *e.e.*s have been achieved (Chiappe et al., 2007; Chimni et al., 2010; Lu et al., 2011). One of the major problems with using EHs for such kinetic resolutions is that in aqueous solutions non-enzymatic hydrolysis of the epoxides and the relatively poor water-solubility of many such substrates can lead to a substantial fall in the product *e.e.* and yield. Recently, two novel EHs capable of effectively catalyzing enantioconvergent hydrolysis of racemic *p*-nitrostyrene oxide to (*R*)-*p*-nitrophenyl glycol have been discovered from Mung bean (*Phaseolus radiatus* L.), which is inexpensive and available in large quantities (Xu et al., 2006). We previously attempted to address problems of poor aqueous solubility of SO and its extensive non-enzymatic hydrolysis in aqueous phase by performing Mung bean EHs-catalyzed asymmetric hydrolysis of

* Corresponding authors at: Laboratory of Applied Biocatalysis, South China University of Technology, Guangzhou 510640, Guangdong, China.
Tel.: +86 20 8711 1452; fax: +86 20 2223 6669.
E-mail addresses: wylou@scut.edu.cn (W.-Y. Lou), btmhzong@scut.edu.cn (M.-H. Zong).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.09.006

SO in a two-phase system (Chen et al., 2011), where an aqueous phase contained the Mung bean EHs and a water-immiscible organic phase acted as a reservoir for the substrate. The optimal *n*-hexane/buffer biphasic system partially inhibited non-enzymatic hydrolysis of SO and thus enhanced the product *e.e.* to some extent, but the enzymatic reaction was slowed down substantially owing to mass transfer limitations between the phases. Because of the lower concentration of substrate in the aqueous phase, the enzymatic reaction needed a longer time. The longer reaction time may also have reduced the product *e.e.* substantially by increasing the time during which non-enzymatic hydrolysis could occur. Therefore, we reasoned that additional development of the solvent systems for the reaction was needed to minimize these problems and further optimize the reaction.

Ionic liquids (ILs) have been studied extensively as solvents for a great variety of biocatalytic transformations and many biocatalysts have exhibited high activity, enantioselectivity and stability in IL-containing systems (Chiappe et al., 2007; van Rantwijk and Sheldon, 2007; Zhang et al., 2012a; Chen et al., 2012). ILs are nonvolatile, non-flammable, highly stable, and able to dissolve a variety of polar and apolar compounds. One of the supreme advantages of ILs is the ability to alter the structures of the cations and anions in order to tune the solvent properties of the IL to match the specific requirements of each particular process (Welton, 1999). In recent years, the use of hydrophilic ILs as additives for enzyme and microbial cell catalysis has been investigated, with many notable successes. For instance, the presence of certain hydrophilic ILs as additives in reaction systems can facilitate the biocatalytic reduction reactions with alcohol dehydrogenases from *Lactobacillus brevis* and *Rhodococcus rubber* and with whole cells of *Rhodotorula* sp. AS2.2241, *Trigonopsis variabilis* AS2.1611 and *Candida parapsilosis* CCTCC M203011, and provide significant increases in reaction rate, yield, and product *e.e.* (Kohlmann et al., 2011; de Gonzalo et al., 2007; Zhang et al., 2012b; Lou et al., 2009a,b). In our previous report (Lou et al., 2004), the addition of small amount of hydrophilic [C_4MIM][BF_4] could improve the activity, enantioselectivity and stability of papain during asymmetric hydrolysis of D,L-*p*-hydroxyphenylglycine methyl ester. Similarly, the presence of [C_nMIM][BF_4] (n = 3–5) at 2–6% (v/v in the aqueous phase) in an aqueous/diisopropyl ether biphasic system greatly promoted transcyanation reactions catalyzed by the hydroxynitrile lyase from *Prunus amygdalus* and inhibited undesired non-enzymatic reactions, thus markedly increasing the product *e.e.* and the yield (Lou et al., 2005). To our knowledge, the use of ILs during asymmetric hydrolysis of epoxides using EHs has been so far largely unexplored, with only few published accounts (Chiappe et al., 2004, 2007). Those studies did not involve SO or Mung bean EHs, and where substituted derivatives of SO were used as substrates, the product *e.e.*s reported were relatively low (≤90%) and yields were generally rather low (in most cases ≤40%, compared with the theoretical maximum of 50%).

Herein, we for the first time report the use of a wide range of hydrophilic ILs, including choline/amino acid ILs recently developed in our group (Liu et al., 2012), as additives to improve the performance of Mung bean EHs for asymmetric hydrolysis of SO in a phosphate buffer/*n*-hexane biphasic system. The results show significant increases in reaction rate upon adding the ILs, reduction in the rate of non-enzymatic hydrolysis, and the ability to increase substrate concentration substantially without significant loss of *e.e.*

2. Experimental

2.1. Biological and chemical materials

Mung beans were purchased from a local supermarket in Guangzhou. Racemic SO (98% purity) and (±)-PED (98% purity) were obtained from Guangzhou Qiyun Bioscience Co. Ltd., China. 4′-Chloroacetophenone (99% purity) was from Sigma–Aldrich (USA). The fourteen ILs, 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-propyl-3-methylimidazolium tetrafluoroborate ([PMIM][BF_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-(2-cyanopropyl)-3-methylimidazolium tetrafluoroborate ([CPMIM][BF_4]), 1-allyl-3-methylimidazollium tetrafluoroborate ([AMIM][BF_4]), 1-(2′-hydroxyl)ethyl-3-methylimidazolium tetrafluoroborate ([C_2OHMIM][BF_4]), 1-(2′-hydroxyl)ethyl-3-methylimidazolium trifluoromethanesulfonate ([C_2OHMIM][TfO]), 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), 1-butyl-3-methylimidazolium bromide ([BMIM][Br]), 1-butyl-3-methylimidazolium dihydrogenphosphate ([BMIM][H_2PO_4]), 1-butyl-3-methylimidazolium perchlorate ([BMIM][ClO_4]), 1-ethyl-3-methylimidazolium saccharinate, ([EMIM][Sac]), 1-butyl-3-methylimidazolium saccharinate ([BMIM][Sac]) and 1-butyl-3-methylimidazolium acesulfamate ([BMIM][Ace]) were purchased from Lanzhou Institute of Chemical Physics (China) and were all of over 98% purity. The six cholinium amino acid ILs used in this work, cholinium threonine ([Ch][Thr]), cholinium arginine ([Ch][Arg]), cholinium lysine ([Ch][Lys]), cholinium proline ([Ch][Pro]), cholinium aspartate ([Ch][Asp]) and cholinium glutamate ([Ch][Glu]) were synthesized in our laboratory (Liu et al., 2012). All other chemicals were from commercial sources and were of analytical grade.

2.2. Preparation of the crude EHs from Mung beans

The protocol used to prepare crude EH from Mung beans was based on previously reported methods (Ju et al., 2008; Devi et al., 2008). Mung beans (100 g) were soaked in distilled water (1 L) for 12 h, dehusked, and ground to a fine paste, which was then suspended in 300 mL of Tris–HCl buffer (50 mM, pH 7.4) containing 1 mM EDTA and stirred at 0 °C for 1 h. The mixture was centrifuged (10,000 rpm) for 15 min at 4 °C and the sediment discarded. Remaining particulate matter was removed from the supernatant by filtering through a 0.45 μm filter on a Millipore labscale TFF system and then though a 0.22 μm filter using the same system. The clarified supernatant (270 mL) was then fractionated via ultrafiltration with membranes of different molecular weight cut-offs (100 kDa) to effect partial purification of the EH. The concentrated slurry was freeze-dried to obtain a pale yellow powder (757.2 mg) containing the crude EH enzymes.

2.3. Analytical methods

Substrate concentration and product *e.e.* were assayed with a Shimadzu 2010 GC (Japan) equipped with a chiral column (30 m × 0.25 mm, 10% permethylated β-cyclodextrin, Hewlett-Packard, USA) and a flame ionization detector. The column temperature was held at 140 °C. Nitrogen was used as the carrier gas at a flow rate of 1.2 mL/min. The split ratio was 1:50 (v/v). Injector and detector temperatures were set at 280 °C. The retention times for SO, 4′-chloroacetophenone (an internal standard), (*S*)-PED and (*R*)-PED were 3.74, 7.17, 19.89 and 20.46 min, respectively. Product concentration was analyzed with an Agilent 1100 HPLC using a Zorbax Extend-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) and UV detection at 256 nm. The mobile phase (flow rate 0.5 mL/min) was a mixture of water and methanol (70/30, v/v). The retention time for PED was 14.4 min. All data were averages of experiments performed at least in duplicate, with no more than 1% standard deviation.

2.4. EH activity assay

The crude enzyme powder prepared as described above was added to 2 mL phosphate buffer (100 mM, pH 6.5) containing SO (10 mM). The enzymatic reaction was conducted for 10 min at 35 °C