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Ionic liquids as cosolvents for the lipase-catalyzed kinetic resolution of ketoprofen

Saerom Park[a], Thanh Thi Ngoc Doan[b], Yoon-Mo Koo[b], Kyeong Keun Oh[c], Sang Hyun Lee[a,*]

[a] Department of Biological Engineering, Konkuk University, Seoul 05029, South Korea
[b] Department of Biological Engineering, Inha University, Incheon 22212, South Korea
[c] Department of Chemical Engineering, Dankook University, Yongin, 16890, Gyeonggi, South Korea

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ABSTRACT

This study examined the use of ionic liquids (ILs) as cosolvents for the *Candida rugosa* lipase-catalyzed enantioselective hydrolysis of racemic ketoprofen ethyl ester. To determine the effect of the IL anion structures on the lipase activity and enantioselectivity, six ILs containing the 1-butyl-3-methylimidazolium ([Bmim]) cation were used as cosolvents and their solvent properties were correlated with various reaction characteristics. The highest lipase activities were obtained using [Bmim][BF_4] and [Bmim][$MeSO_4$] as cosolvents. The enantioselectivity (E) of lipase was enhanced by a factor of 50 with 5% [Bmim][$MeSO_4$]. Although the conversion, enantiomeric excess of product (ee_p), and E value in 5% [Bmim][$MeSO_4$] were 47.3%, > 99%, and ~300, respectively, the corresponding lipase stability is inadequate for commercial application. Excluding [Bmim][$MeSO_4$], the lipase enantioselectivity increased with decreasing hydrogen bond basicity (β) of the IL when the log E values of lipase in 5% ILs were correlated with IL solvent parameters. With [Bmim][PF_6] as the cosolvent, which has the lowest β value among all ILs examined, the E value was enhanced by a factor of 32. The conversion and ee_p in 20% [Bmim][PF_6] were 48.0% and 96.9%, respectively. Additionally, the lipase stability in aqueous [Bmim][PF_6] solution was much higher than that in aqueous [Bmim][$MeSO_4$] solution.

1. Introduction

Organic solvents have long been investigated as reaction media for non-aqueous enzymatic reactions since the pioneering results of Klibanov [1]. Organic solvents as reaction media can greatly enhance the stability and selectivity of enzymes, and even shift the chemical equilibrium of a reaction. At present, many studies are concerned with discovering new reaction media for non-aqueous enzyme reactions because of the environmental toxicity, flammability, and volatility of traditional organic solvents [2]. In this regard, much research has been dedicated over the past 15 years to the identification of ionic liquids (ILs) as alternative reaction media for non-aqueous enzyme reactions [3]. ILs are organic salts that melt below 100 °C. Interest in ILs stems from their potential as "green solvents". Specifically, their non-volatile character and thermal stability make them attractive alternatives for volatile organic solvents [4]. Moreover, their synthetic flexibility has also led to ILs being referred to as "designer solvents", because of the vast number of combinations of anions and cations encompassed by ILs [5].

It has been observed that ILs could successfully enhance the activity, selectivity, and stability of enzymes when ILs were used as anhydrous reaction media. For example, various esterification reactions using proteases and lipases have been reported. Many ILs containing BF_4^-, PF_6^-, and Tf_2N^- anions have been demonstrated as good anhydrous reaction media for enzymatic reactions [3,5–7]. Meanwhile, non-aqueous systems using ILs as cosolvents have also been developed to enhance the activity and selectivity of enzymes. For example, He et al. reported that higher reaction yield and higher enantiomeric excess were obtained in the microbial reduction of ethyl acetoacetate to ethyl (*R*)-3-hydroxybutyrate in a 2.5% [Bmim][BF_4] solution [8]. Lai et al. showed the activation and stabilization of *Penicillium expansum* lipase using 0.63 M [Choline][Acetate] and 0.27 M [$NHMe_3$][$MeSO_3$] as cosolvents [9]. Wang et al. reported the hesperidinase-catalyzed hydrolysis of rutin into isoquercitrin in a 10% [Bmim][BF_4]-containing buffer system to enhance the substrate solubility and product yield [10]. The Mao group investigated the esterase-catalyzed kinetic resolution of ibuprofen ethyl ester using ILs as cosolvents. In this case, 1-octyl-3-methylpyridinium tetrafluoroborate was identified as an optimal cosolvent as it increased the enantioselectivity of esterase from 3 to 135 [11]. The same group also reported the fructosyltransferase-catalyzed

* Corresponding author.
E-mail address: sanghlee@konkuk.ac (S.H. Lee).

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synthesis of sucrose-6-acetate in phosphate buffer containing 20% 1-decyl-3-methylimidazolium hexafluorophosphate with enhanced synthetic yield [12].

Many ILs have been used as anhydrous media or cosolvents for various non-aqueous enzyme reactions. However, there is no clear strategy for choosing optimal ILs for biocatalysis. Various factors such as the type of IL anions, the alkyl chain length of the cations, IL polarity, viscosity, ion kosmotropicity, hydrophobicity, amphiphilicity, and the IL network can influence the activity, selectivity, and stability of enzymes. Moreover, the interactions among these factors play an important role in determining the characteristics of biocatalytic systems [7]. In this work, the *Candida rugosa* lipase (CRL)-catalyzed enantioselective hydrolysis of racemic ketoprofen ethyl ester was chosen as a model reaction system. To understand the effect of ILs as cosolvents on the CRL, the statistical analysis of the correlation between lipase action and solvent properties of ILs was investigated.

Ketoprofen (2-(3-benzoylphenyl)-propionic acid) is one of the most useful nonsteroidal anti-inflammatory drugs (NSAIDs). (*S*)-Ketoprofen reduces inflammation and relieves pain, whereas (*R*)-ketoprofen can be used as a toothpaste additive to prevent periodontal disease. Therefore, efforts are underway to obtain optically pure ketoprofen [13]. Enzyme-catalyzed kinetic resolution of ketoprofen has been widely investigated as a green process to obtain enantiopure ketoprofen over the last 20 years. The enantioselective esterification of racemic ketoprofen with an alcohol as acyl acceptor can produce an ester form of enantiopure ketoprofen, while enantioselective hydrolysis of racemic ketoprofen ester can produce an acid form of enantiopure ketoprofen [14]. Commercially available CRL is very useful for the kinetic resolution of profens such as ibuprofen, ketoprofen, and naproxen due to its high reactivity. However, because the enantioselectivity of CRL is not sufficient for commercial production of enantiopure ketoprofen, several approaches have been investigated for the enantioselective hydrolysis of ketoprofen esters, including solvent pretreatment of CRL, use of surfactant, immobilization of CRL, and the development of an optimal ketoprofen ester [15–17].

In this study, six ILs containing the same [Bmim] cation were used as cosolvents for the hydrolysis of ketoprofen ethyl ester in order to enhance the solubility of ketoprofen ester and increase the lipase enantioselectivity. In addition, the effect of the ILs on the activity and enantioselectivity of lipase was also investigated in terms of the solvatochromic parameters, hydrophobicity, and polarity of the ILs.

2. Experimental

2.1. Materials

Lipase from *Candida rugosa* (Type VII), (R,S)-ketoprofen, (*S*)-ketoprofen, sodium phosphate monobasic, sodium phosphate dibasic, citric acid, sodium citrate, ethanol, silica gel (particle size: 40–63 μm), acetonitrile, *n*-hexane, ethyl acetate, *p*-anisaldehyde, isopropanol, acetic acid, and phosphoric acid were purchased from Sigma-Aldrich (St. Louis, USA). The ethyl ester of racemic ketoprofen was prepared with the spontaneous chemical esterification described by Jin et al. [13]. In brief, 20 mmol of ethanol was added to 20 mmol of racemic ketoprofen in 50 ml vial. The mixture was heated on a hotplate with stirring until all substrates liquefied, and then further incubated at 60 °C in a water bath. Product was purified by flash chromatography using a column packed with silica gel (particle size: 40–63 mm), with *n*-hexane and ethyl acetate (16:1) as eluent. After purification of product, the collected pool was evaporated in a rotary evaporator at 60 °C. The authenticity and purity of the ketoprofen ethyl ester were determined by ^{1}H NMR and HPLC, respectively. All ILs were synthesized and purified by C-TRI (Suwon, Korea) and had a residual chloride content of less than 30 ppm. All other chemicals used were of analytical grade and were used without further purification.

2.2. Enzymatic hydrolysis of ketoprofen ethyl ester in aqueous solutions containing ILs

Ketoprofen ethyl ester (10 mM) was dissolved in 1 mL of 0.1 M phosphate buffer (pH 7.0) containing various contents of ILs. The reaction was initiated by adding 25 mg of lyophilized lipase, and the reaction mixture was sustained at 30 °C in a water bath with shaking at 160 rpm. After a predetermined incubation time, the reaction mixture was diluted with 1 mL of acetonitrile in order to terminate the enzyme reaction and fully dissolve any unreacted ketoprofen ethyl ester and the product ketoprofen. The reaction mixture was then centrifuged to obtain the supernatant, which was subjected to HPLC analysis. The activity was expressed as an initial rate by measuring the concentration of ketoprofen produced after reaction time of 30, 60, 90, and 120 min. The initial rates were calculated by non-linear regression using SigmaPlot (10.0) and measurements were carried out in triplicate.

2.3. Analytical methods

The conversion and enantioselectivity of the lipase-catalyzed hydrolysis reaction was determined by HPLC. Unreacted ketoprofen ethyl ester and the product ketoprofen were quantified by a Shimadzu HPLC system (Model LC-10 A, Japan) equipped with a reverse-phase C18 column (SYMMETRY®, Waters, USA) with UV detection at 254 nm. The mobile phase was acetonitrile/water (80/20, v/v) containing 50 μL phosphoric acid. The flow rate was maintained constant at 1.0 mL/min. The conversion (c) was calculated using Eq. (1):

$$c(\%) = \frac{[\text{ketoprofen}]}{[\text{ketoprofen ethyl ester}] + [\text{ketoprofen}]} \times 100 \quad (1)$$

Chiral resolution of ketoprofen enantiomers was accomplished using a HPLC system equipped with a Chiralcel OJ-H column (4.6x250 mm) and a UV detector at 254 nm. The mobile phase was *n*-hexane/2-propanol/acetic acid (90/10/0.5, v/v/v), and the flow rate was maintained at 1 mL/min. The enantiomeric excess of the product (ee_p) and enantioselectivity (E) were calculated using Eqs. (2) and (3) [13]:

$$ee_p(\%) = \frac{[(S)\text{-ketoprofen}] - [(R)\text{-ketoprofen}]}{[(S)\text{-ketoprofen}] + [(R)\text{-ketoprofen}]} \times 100 \quad (2)$$

$$E = \frac{\ln[1\text{-}c(1 + ee_p)]}{\ln[1\text{-}c(1\text{-}ee_p)]} \quad (3)$$

2.4. Stability of lipase in aqueous solutions containing ILs

The stability of lipase in aqueous solutions containing ILs was measured based on its residual activity after various incubation times. Lipase (25 mg) was dissolved in 1 mL of 0.1 M phosphate buffer (pH 7.0) containing 5% [Bmim][$MeSO_4$] or [Bmim][PF_6]. The solution was incubated at 30 °C for a predetermined period of time (0, 10, 20, 34, 40, or 50 h), and then the reaction was initiated by adding 10 mM ketoprofen ethyl ester. The residual activity of lipase was determined with the same procedure described above.

3. Results and discussion

3.1. Effect of IL properties on lipase activity and enantioselectivity

The effect of the anion structures of ILs containing the [Bmim] cation on the activity and enantioselectivity of lipase was investigated. Four hydrophilic ILs including [Bmim][BF_4], [Bmim][$MeSO_4$], [Bmim][CF_3SO_3], and [Bmim][Cl] and two hydrophobic ILs including [Bmim][Tf_2N] and [Bmim][PF_6] were used as cosolvents for the lipase-catalyzed hydrolysis of ketoprofen ethyl ester in aqueous media (Table 1).

The lipase activities, which are expressed as initial rates, decreased