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Efficient resolution of (*R,S*)-1-(1-naphthyl)ethylamine by *Candida antarctica* lipase B in ionic liquids

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ABSTRACT

The resolution of (*R,S*)-1-(1-naphthyl)ethylamine ((*R,S*)-NEA) by *Candida antarctica* lipase B (CALB) in ionic liquids (ILs) containing 1-alkyl-3-methylimidazolium cations ($[C_n mim]^+$) and $[Tf_2N]^-$, $[BF_4]^-$, and $[PF_6]^-$ anions was investigated. When the alkyl chain on the cation contained less than six carbons, the lipase activity corresponded with the hydrophobicity of the ILs, but further increase in the chain length suppressed the enzyme activity. The enzyme activity decreased depending on the anion, where $[Tf_2N]^-$ > $[PF_6]^-$ > $[BF_4]^-$. The effects of acyl donors, pH, temperature, water activity, and substrate concentration on the resolution were determined. Under the optimal conditions, the conversion of (*R,S*)-NEA and enantiomer excess of (*R*)-*n*-octyl acyl-NEA was 49.3% and 99.2%, respectively. The resolution kinetics of (*R,S*)-NEA by CALB in $[C_6mim][Tf_2N]$ were studied and a ping-pong mechanism with a two substrate inhibition model was selected. The kinetic parameters of the fitting results were as follows: Michaelis constant of (*R,S*)-NEA K_{ma}, 461.8 mmol/L; Michaelis constant of vinyl *n*-octanoateK_{mb}, 262.1 mmol/L; inhibition constant of (*R,S*)-NEA K_{ia}, 8737.2 mmol/L; inhibition constant of vinyl *n*-octanoateK_{ib}, 62336.8 mmol/L; maximum reaction rate r_{max}, 0.352 mmol/(mg min). Moreover, circular dichroism revealed that incubation of CALB in $[C_6mim][Tf_2N]$ resulted in increased β-sheet content; its secondary structure was stable.

1. Introduction

(*R*)-1-(1-naphthyl)ethylamine ((*R*)-NEA) is an important pharmaceutical intermediate for the preparation of calcimimetic Cinacalcet Hydrochloride [1,2], and is used as a chiral intermediate for the resolution of Pregabalin intermediates [3]. It is also an important raw material for the preparation of heterogeneous chiral hydrogenation catalysts [4,5]. Therefore, considerable research has been carried out on preparation methods for chiral NEA, which can be divided into three categories. The first is chemical resolution methods; for example, (*D*)-tartaric acid was used as a chemical separation agent for the resolution of (*R,S*)-NEA [6]. The process was long and the optical purity of the product was low, and a large amount of chemical waste was generated. The second category is asymmetric synthesis methods; (*R,S*)-1-(1-naphthyl)acetophenone was used for the synthesis of (*R*)-NEA in the presence of a chiral catalyst [7]. This process was simple, but the chiral catalyst was structurally complex, expensive, and its large-scale production was difficult.The third category is biological methods; an enzyme catalyst transaminase was applied for the preparation of chiral NEA in the presence of 1-(1-naphthyl)ethanone [8] and *Candida antarctica* lipase B (CALB) was used for the resolution of (*R,S*)-NEA [9,10]. Compared to transaminase, CALB does not require a coenzyme, is inexpensive, and has other advantages [11].

Notably, organic solvents were used as reaction media for the resolution of (*R,S*)-NEA by CALB; environmental and safety concerns of the volatile organic solvents directly affected the enzymatic resolution of (*R,S*)-NEA. Ionic liquids (ILs), new reaction media with low saturated vapor pressures, exhibit incombustibility, hydrophobicity, and excellent thermal stability [12,13]. Recently, ILs have been reported to show favorable characteristics as reaction media in enzyme catalysis to improve enzyme enantioselectivity and thermal stability [14,15]. Therefore, in this study, CALB was selected as the catalyst and ILs were used as the reaction media for the resolution of (*R,S*)-NEA for the first time. The effects of the structure of ILs based on 1-alkyl-3-methylimidazolium ($[C_n mim]^+$) cations and ($[Tf_2N]^-$), $[BF_4]^-$, and $[PF_6]^-$ anions on the enzymatic catalytic activity were investigated. Moreover, the effects of the reaction conditions on the enzymatic activity were also investigated. Circular dichroism (CD) spectroscopy was used to measure changes in the secondary structure of CALB in different solvents, and the resolution kinetics of (*R,S*)-NEA by CALB in $[C_6mim]$

Abbreviations: (R,S)-NEA, (R,S)-1-(1-naphthyl)ethylamine; CALB, Candida antarctica lipase B; ILs, ionic liquids; $[Tf_2N]^-$, bis[(trifluoromethyl)sulfonyl]imide; $[BF_4]^-$, tetrafluoroborate anion; $[PF_6]^-$, hexafluorophosphoric acid anion; $[Cnmim]^+$, 1-alkyl-3-methylimidazolium cations; CD, circular dichroism

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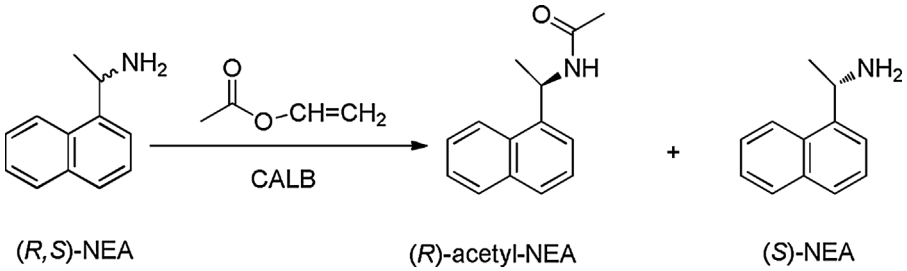
Scheme 1. Route of resolution of (*R,S*)-NEA by CALB.



[Tf_2N] was investigated.

2. Experimental

2.1. Materials

(*R,S*)-NEA (98%), ionic liquids[C_4mim][BF_4], [C_4mim][PF_6], [$C_n$mim][Tf_2N] (n = 2,4,6,8,10) were purchased from Alfa Aesar chemical Co. Ltd (Shanghai, China). The purity of IL was 99%. Recombinant lipase B from *Candida antarctica* from *Aspergillus oryzae* (9 U/mg) was obtained from Sigma-Aldrich(Shanghai, China). All other reagents were of analytical grade, and were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

2.2. (R,S)-NEA kinetic resolution study

Unless otherwise indicated, in a typical resolution of (*R,S*)-NEA by CALB (Scheme 1), reactions were carried out in a 10 mL flask with a stopper; 200 mmol/L(*R,S*)-NEA and 120 mmol/Lvinyl acetate were added to 5 mL IL (or 5 mL toluene). The reaction was catalyzed in the presence of 100 mg CALB at 40 °C with shaking at 180 rpm. The water activities of substrates, solvents and enzyme were not specially controlled. The pH of CALB was also not specially controlled.

2.3. Analysis conditions

Aliquots were withdrawn from the reaction mixture at various intervals and the samples were analyzed using HPLC equipped with a Chiralcel® OJ-H chiral column (250 mm × 4.6 mm, 5 μm) and detected using UV at 222 nm at 25 °C. The mobile phase was composed of a mixture of hexane, isopropanol, and ethanol (300:50:0.8, V/V) with a flow rate of 1 mL/min. The retention times of (*R*)-NEA and (*S*)-NEA were 6.5 and 7.7 min, respectively; the retention time of (*R*)-*n*-octyl acyl-NEA and its enantiomer were 11.4 (*R*) and 14.6 (*S*) min, respectively.

The enantiomeric excess (*ee*) formulas for (*R*)-*n*-octyl acyl-NEA and (*S*)-NEA were $ee_P = \frac{c_{PR} - c_{PS}}{c_{PR} + c_{PS}} \times 100\%$ and $ee_S = \frac{c_S - c_R}{c_S + c_R} \times 100\%$, respectively. The conversion of *c* was calculated according to the changes in the number of substrates. C_{PR} and c_{PS} were the contents of *R* and *Sn*-octyl acyl-NEA, and c_R and c_S were the contents of (*R*)- and (*S*)-NEA, respectively. The enantioselectivity was determined using the following equation: $E = \frac{\ln[1-c(1+ee_p)]}{\ln[1-c(1-ee_p)]}$.

2.4. CD spectroscopy

CALB powder was incubated in [C_6mim][Tf_2N] (or toluene) at 4 °C for 2 h, washed with cooled ethyl acetate to remove the IL, and subsequently washed with cooled ether to remove ethyl acetate; the ether was volatilized at room temperature. The treated CALB powders were dissolved in phosphate buffered solution (50 mM) to a concentration of 0.1 mmol/L, and the enzyme solution was used for CD measurements. Spectra were recorded at 4 °C from 260 to 190 nm (0.5 nm increments) with a 0.1 cm path-length cell, 20 nm/min scan rate, 4 s response time, and 2 nm bandwidth. The mean residue ellipticity, $[\Theta]_{MR}$, was expressed in deg·cm^2·dmol^{-1}. A buffer sample containing no protein was subtracted from all spectra to account for any background signal. Spectra in all the region were averaged after three accumulations.

2.5. Water activity

The substrates and ILs were separately pre-equilibrated at different water activities (a_w) prior to the resolution of (*R,S*)-NEA. All samples were placed in a closed vessel above saturated salts, and equilibrium was reached overnight at room temperature. The salts used in this test included $Na_2HPO_4{\cdot}2H_2O/Na_2HPO_4$, $a_w = 0.17$; $NaAc{\cdot}3H_2O/NaAc$, $a_w = 0.28$; $CuSO_4{\cdot}5H_2O/CuSO_4{\cdot}3H_2O$, $a_w = 0.42$; $Na_2HPO_4{\cdot}7H_2O/Na_2HPO_4{\cdot}2H_2O$, $a_w = 0.63$; and $Na_2HPO_4{\cdot}12H_2O/Na_2HPO_4{\cdot}7H_2O$, $a_w = 0.78$.

2.6. Dynamic parameter fitting

The initial enzymatic reaction rates were determined at different substrate concentrations with certain amounts of (*R,S*)-NEA (50 ~ 400 mmol/L) and vinyl *n*-octanoate (50 ~ 240 mmol/L) respectively, according to the method described in Section 2.2. The dynamic parameters were fitted according to the dynamic model, substrate concentration, and initial reaction rate using Origin 11.0 software.

3. Results and discussion

3.1. Effect of solvent on resolution of (R,S)-NEA catalyzed by CALB

As (*R,S*)-NEA has poor solubility in hydrophilic ILs, several hydrophobic ILs with various anions and cations were used. The initial reaction rates of the resolution of (*R,S*)-NEA catalyzed by CALB in ILs and toluene are shown in Table 1. The initial reaction rates of the resolution by CALB in ILs were lower than that in toluene. Organic solvents, like toluene, have lower viscosities than ILs [12], thus the enzyme and substrates have higher mass transfer coefficients in toluene than in ILs. However, the conversion of (*R,S*)-NEA was not dominant in toluene with prolonged reaction times, and the conversion of (*R,S*)-NEA in [C_6mim][Tf_2N] gradually caught up and exceeded that in toluene (Fig. 1). The *ee* value of *R*-*n*-octyl acyl-NEA remained relatively stable (95.1%–99.5%) in [C_6mim][Tf_2N]; however, the *ee* value of *R*-*n*-octyl acyl-NEA rapidly decreased as the resolution time increased in toluene.

Table 1
Effect of solvents on resolution of (*R,S*)-NEA catalyzed by CALB.

Entry	Solvent	Conversion/%	ee_p/%	*E*	v_0/mmol mg^{-1} min^{-1}
1	[C_4mim][BF_4]	0.347	78.1	12	0.025
2	[C_4mim][PF_6]	0.377	79.8	14	0.027
3	[C_2mim][Tf_2N]	0.426	92.0	49	0.034
4	[C_4mim][Tf_2N]	0.455	88.6	37	0.045
5	[C_6mim][Tf_2N]	0.494	95.1	137	0.052
6	[C_8mim][Tf_2N]	0.439	84.1	23	0.038
7	[C_{10}mim][Tf_2N]	0.401	86.5	24	0.031
8	Toluene	0.465	71.2	11	0.056

E = Enantiomeric ratio, v_0 = Initial reaction rate, reaction time 6 h.