

Lipase Catalyzed Naproxen Methyl Ester Enantioselective Hydrolysis in Ionic Liquids

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Abstract. A series of dialkylimidazolium ionic liquids with various anions including hexafluorophosphate alkane sulfonate and tetrafluoroborates were studied in the lipase-catalyzed enantioselective hydrolysis. Lipase activity, stability and enantioselectivity were determined by both of alkane and anions. In the best aqueous- C4mimPF6 biphasic system lipase resolution of Naproxen proceed with much effective. It was observed that the activity of lipase in aqueous- C4mimPF6 system was comparable with that in aqueous-organic system, but the enantiomeric ratio of Lipase-catalysis hydrolysis in aqueous - C4mimPF6 biphasic system ($E > 300$) was much higher than that in aqueous -organic biphasic system ($E \approx 90$).

Introduction

(S)-(+)-2-(6-Methoxy-2-naphtyl) propionic acid (Naproxen) is a non-steroidal anti-inflammatroy drug and the physiological activity of the S-Naproxen is 28-fold higher than that of R-form[1]. The methods for product S-Naproxen has been intensively explored and enzymatic resolution of Naproxen is efficient and can be performed by hydrolysis[2,3] and esterification[4,5]. As the two methods from the standpoint of high productivity, easy product separation, and few reaction steps, lipase-catalyzed asymmetric hydrolysis has been judged to be superior for the practical resolution of racemic acids [6]. Anterior studies of asymmetric hydrolysis often carry through in aqueous system or in aqueous-organic biphasic system. In the first system the relatively low solubility of substrate cause a low reaction rate [7]. In the second system the substrate can be dissolved in an organic phase, while the emulsion made difficult to separate the product and substrate, decreased the stability of the enzyme, the volatile organic is dangerous and it does not accord with the "Green chemistry" tenet [8].

To overcome the aforementioned problems, room-temperature ionic liquids are an appreciate choice. Ionic liquids have been reported as non-volatile, non-flammable, low toxicity, and good solubility for many organic and inorganic materials. They also be reported can enhance activity, selectivity and stability of catalysts in chemical reactions [9, 10], and can dramatically reduce the use of hazardous and volatile polluting organic solvents.

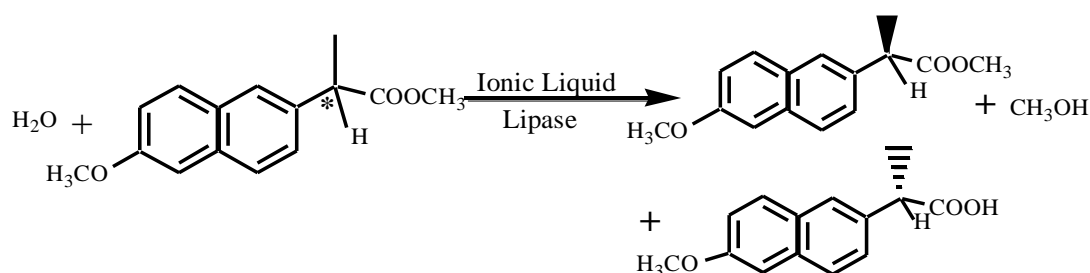


Figure 1. Lipase catalyzed enantioselective hydrolysis of Naproxen methyl ester in aqueous-ionic liquids biphasic system

Materials and Methods

Reagents: Lipase type VII L-1754 from *Candida rugosa* (1140 units/mg Sigma), naproxen methyl ester purified by recrystallization from petroleum ether and dried in vacuum, ionic liquid synthesized and purified according to Parks[11], other reagents were analytical degree and used without any purification. Synthesis of sucrose esters by microwave-assisted lipase-catalyzed reaction.

Analytical Methods In both cases, samples were withdrawn at specified time intervals for measurement of the conversion (C) and enantiomeric excess of the reaction. Firstly, water in aqueous phase was removed by freeze-dryer, and then methanol was used to extract the Naproxen. Hexane was used to extract the remained substrate from ionic liquids phase. The enantiomeric excess of product (eep) and remain substrate (ees) were determined by HPLC using chiral column respectively. Analytical conditions: naproxen methyl ester used Chiralcel OD-H, 250×4.6mm hexane/2-propanol 90/10(v/v) flow rate 0.5 mL/min UV 254nm at room temperature; naproxen used Chiralcel R-NGLY&DNB, 250×4.00mm, acetic ammonia dissolve in methanol with 30mMol/L, flow rate 0.5 mL/min UV 254nm at room temperature.. The enantiomeric ratio (E) values were calculated using equation: $E = \ln[1 - c(1 + eep)] / \ln[1 - c(1 - eep)]$, [12] where $c = ees / (ees + eep)$.

Enzymatic Resolution of Naproxen. In typical experiments, 125mg of *Candida rugosa* lipase and 75mg of substrate Naproxen methyl ester were added into 3ml of sodium phosphate buffer and 3ml of ionic liquids the resolution reaction was started by shaking the flask at 32 °C (170 rpm). As comparing, the same reaction was also performed in aqueous-organic biphasic system containing 3ml of sodium phosphate buffer and 3ml of isooctane.

Results

Selection of Ionic Liquids. We synthesized a series of ionic liquids according to Parks[11] in order to study the feasibility of lipase resolution of naproxen in ionic liquids system. The results were given in Table 1

Table 1 the effect of ionic liquid composition on the lipase-catalyzed naproxen methyl ester

ionic liquids	ee _s (%)	ee _p (%)	E (10 ²)	C (%)
C ₄ mimPF ₆	35.60	99.40	3.0	26.40
C ₄ mimBF ₄	8.00	98.70	1.6	7.50
C ₆ mimPF ₆	17.50	98.80	1.9	15.00
C ₆ mimC ₇ SO ₃	7.40	98.00	1.0	7.00
C ₆ mimBF ₄	6.20	99.00	2.1	5.90
C ₈ mimBF ₄	4.80	98.50	1.4	4.60

hydrolysis^a

L-1754=125mg; naproxen methyl ester=75mg; PH=7.0; temperature 32.0 °C; 170r/min; sample in the fifth day. Ionic liquid/sodium phosphate buffer (v/v) = 1/1. (3ml/3ml)

With the same alkylmethylimidazolium the enzyme activity and diastereomeric selectivity have relations with the anion, in our experimentation was PF₆ > BF₄ > C₇SO₃; with the same anion condition the relationship of enzyme activity and diastereomeric selectivity with the alkyl length was C₄ > C₆ > C₈. For this reason we choose c₄mimPF₆ for the follow experience.

According to our previous report [13] water content is a sensitive factor of lipase activity in biphasic system. So the effect of water content in the reaction medium on the conversion (C), the

enantiomeric excess of product (eep)、remain substrate (ees) and enantiomeric ratio (E) was studied. As shown in Table 2

Table2 The effects of the water content in the biphasic system on the Conversion, enantiomeric excess and enantiomeric ratio (E)

ionic liquids:Aqueous (v/v)	ee _s (%)	ee _p (%)	E (10 ²)	C (%)
3:1	18.40	99.00	2.4	15.70
2:1	27.40	99.10	2.9	21.70
2:2	28.60	99.30	3.0	22.40
2:3	22.00	98.70	1.9	18.20
2:4	21.80	99.00	2.5	18.00

keep the concentration of lipase L-1754 and naproxen methyl ester invariablenes; PH7.0 ; Temperature32℃; 170r/min, determined at the fifth day.

When the ratio of ionic liquid to aqueous was 1:1 (v/v), both of the conversion and enantioselective was high, however the decline of conversion and enantioselective was found when the water content in the reaction medium was too big or too small. We suspect that it maybe the influence of the reaction interfaces.

In the aqueous -ionic liquids biphasic system, lipase could keep the same activity as in aqueous -organic biphasic system. The advantages of use aqueous -ionic liquids biphasic system are that they can be readily reused but have no infection to the enzyme; and the enantioselective was much higher than that in aqueous -organic biphasic system. Also, ionic liquids are not environment pollutions as organic solvents. The aqueous -organic biphasic system was so facilitate and strong emulsification with water that remain substrate, product and enzyme could hardly be separated after reaction, but the aqueous -ionic liquids biphasic system was not. And using no detectable vapor pressure ionic liquids instead of the volatile organic can make the reaction much safer.

Summary

In summary, from our research we can observe that aqueous -ionic liquids biphasic system can work as aqueous -organic biphasic system for lipase-catalyzed enantioselective hydrolysis with advantages enhancing the enantioselectivity, avoiding the emulsification and separating the product and substrate easily.

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