Lipase–catalyzed Kinetic Resolution of Naproxen

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Abstract—Naproxen is one of common non-steroidal anti-inflammatory drugs (NSAIDs). Its pharmacological properties vary with different configurations. In order to improve the drug efficacy, it is significant to prepare high enantiopure drugs. In this study, *carica papaya* lipase (CPL) and papain extracted from the crude papaya enzyme are used for the kinetic resolution of recemic Naproxen. The types of enzyme, the influences of reaction system, the amount of lipase and H_2O were studied in order to obtain the optimum conditions. The results showed that *carica papaya* lipase has resolution ability over Naproxen, but papain has no resolution ability over Naproxen. In the microaqueous-isooctane system (1.2ml H2O, 30ml isooctane, the concentration of Naproxen methyl ester is 10mg/ml), 1g CPL was used to catalyze the reaction. The reactions were carried out in a shaker (180r/min) at 30℃. In the condition described above, conversion of substrate nears 30% after 10d, and the enantiomeric ratio (E) is 30..

Keywords- Naproxen; Caria Papaya lipase; asymmetric hydrolysis; Chiral separation; Enantiomeric ratio

I. INTRODUCTION

Naproxen, 2-(6-methoxy-2-naphthyl)-propionic acid, is widely used in the clinical treatment of rheumatoid arthritis, rheumatoid spondylitis, postpartum and postoperative pain[1]. Naproxen has a part of enantiomer named R-Naproxen and S-Naproxen because there is an asymmetric α-carbon atom in chemical structure[2]. The efficacy of S-Naproxen is 28 times more active than that of R-Naproxen, and only the S-Naproxen is used as drugs for human[3]. Therefore, it is very important to make a single configuration Naproxen. Recently, the main method is chemical method for chiral resolution, and the disadvantages lie in complicated and costly production process and difficulty in separation and purification, so many people look for the other ways.

Lipase, also known as triacylglycerol hydrolase, is widely used in catalytic organic reactions and the kinetic resolution of a variety of racemic drugs due to its high catalytic efficiency and high stability[4-5]. Xin[6] used the lipase which was extracted from Bacillus, got S-Naproxen by asymmetric hydrolysis of Naproxen methyl ester. There is no significant difference between the aqueous phase systems and micro-water phase system by using CRL to separate recemic Naproxen according the study did by Zhao Yongjie[7]. Huang Zhuonan[8] produced S-Naproxen with the strereopreference of CRL and the lipase was immobilized on molecular sieve by adsorption. The maximum conversion rate was 45.8%, and the eep was 100% after 220h. Although lipase showed high activity and strereopreference in Chiral drugs, due to difficulty in separation and purification and high cost, its application was limited. Therefore, more and more scientists pay attention to carica papaya lipase (CPL), because of its adequate sources of raw materials and low prices. Many studies had shown that the activity of CPL which was extracted from papaya latex was not affected by the activity of papain[9], and could be used as a potential catalyst for separation of chiral drugs for its high activity, abundant.

In this paper, the aim of the present study is to achieve the kinetic resolution of chiral Naproxen by CPL, and explore the optimal reaction conditions.

II. MATERIALS AND METHODS

A. Materials

Carica papaya lipase was purchased from Nanning Dongheng Huadao Biological Technology Co., Ltd. Racemic Naproxen was purchased from Shanghai Meilan Industrial Co., Ltd. Esterification method of Naproxen was reported in literature[10]. All other chemicals were obtained commercially and were analytical grade. Isooctane, Benzene, Methanol and Petroleum ether were purchased from Tianjin Tianli Chemical Reagent Co., Ltd.

B. Synthesis of Naproxen methyl esterThe building of split reaction system

For the synthesis of Naproxen methyl, the authors did the following works: 5g racemic Naproxen was dissolved in 170ml anhydrous methanol, and then added 80ml benzene, 5ml concentrated sulfuric acid, reflux heating for 4h. Then added an appropriate amount of 5% Na2CO3 to remove the unreacted Naproxen and concentrated sulfuric acid, after dissolution with petroleum ether, rotary evaporated to dryness to give a pale yellow solid.

The carica papaya lipase was activated before use. The 100g crude papaya enzyme was added into 400ml 0.1mol/L pH7.5 PHB and stirred for thirty minutes. Then papain and CPL could be separated by 8000r/min centrifugation for 10min, the supernatant is papain, the precipitate is CPL. The above operation is repeated three times to get lipase milky precipitate, collected the precipitate and then lyophilized in vacuum to obtain the carica papaya lipase.

C. The building of split reaction system

1) Microaqueous-organic solvent system

The reactions were conducted in 100ml conical flask. Under standard conditions, 1g of the CPL was added into 30ml of isooctane containing 300mg Naproxen methyl ester and 0.8ml 0.2 mol/L pH7.0 of PHB. The reactions were carried out in a shaker (180r/min) at 30℃. These conditions were used unless otherwise stated. At 1-12d intervals, samples were withdrawn for the optical rotation (WZZ-1 automatic polarimeter) and visible spectrophotometric (UV5100B UV-visible spectrophotometer) to determine the yield and the enantiomeric excess of substrate and product.

2) Aqueous phase system

The reactions were conducted in 100ml conical flask. 1g Naproxen methyl ester was dissolved in 30ml distilled water, ultrasonic crushing 20min, then added 1g CPL and 1ml 5% Na2CO3 into the system. The reactions were carried out in a shaker (180r/min) at 30℃ after sealed. After 24h, the reaction solution was centrifuged at 14000r/min for 20min. The precipitate was added 30ml distilled water and 1ml 5% Na2CO3, after sealed and placed in the same shaker continued the reaction. The supernatant was added 1% hydrochloric acid until a milky white precipitate appeared, and then centrifuged at 8000r/min for 10min. The precipitate after drying was dissolved in 20ml anhydrous methanol, measured its optical rotation.

D. Determination of the conversion

Naproxen methyl ester was dissolved in isooctane and diluted to a concentration of 0.1, 0.2, 0.4, and 0.6mg/ml. Full wavelength scanning will be done with using isooctane as blank by UV-visible spectrophotometer when the concentration is 0.6 mg/ml, while the full wavelength scanning of isooctane also is done with using distilled water as a blank. From the result, the authors can obtain a wavelength that can be clearly distinguished Naproxen and Naproxen methyl ester. At this wavelength, the authors can get the standard curve after measured the visible spectrophotometric values of different concentrations of Naproxen methyl ester solution. By measuring the visible spectrophotometric of substrate can be obtained the concentration of Naproxen methyl ester, and then calculate the conversion.

E. Enantioselectivity Evaluation

The evaluation of lipase-catalyzed reaction can use the conversion, the enantiomeric excess of substrate and product (ees, eep), as well as the reaction of enantiomeric ratio (E). ee and E represents the ability to split enzymatic reactions. The calculation formula as follows:

$$E = \frac{\ln[(1-C)\times(1-ee_s)]}{\ln[(1-C)\times(1+ee_s)]} = \frac{\ln[1-C\times(1+ee_p)]}{\ln[1-C\times(1-ee_p)]} \quad (1)$$

$$ee_s = \frac{[\alpha]_D}{[\alpha]_{DS}} = \frac{\alpha}{C_0\times(1-C)\times L\times[\alpha]_{DS}} \quad (2)$$

$$\mathbf{ee_p} = \mathbf{ee_s}\times\frac{\mathbf{1-C}}{\mathbf{C}} \quad (3)$$

Where [α]D, [α]DS, C0, C, L represent the specific rotation, the specific rotation of a single isomer, the initial concentration, the final concentration, the length of polarimetry, respectively.

III. RESULTS AND DISCUSSION

A. Determination of the conversion rate

The results of full wavelength scan of Naproxen methyl ester and isooctane shown in Fig. 1, it can be seen from the figure that when the wavelength is 320nm, Naproxen and Naproxen methyl ester can be clearly distinguished. At this wavelength, the authors can get the standard curve shown in Fig. 2.

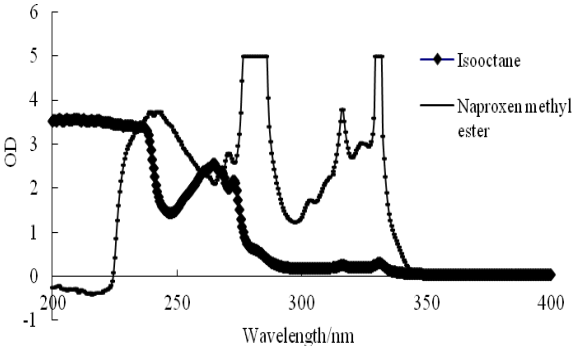

Figure 1. Full wavelength scan of Naproxen methyl ester

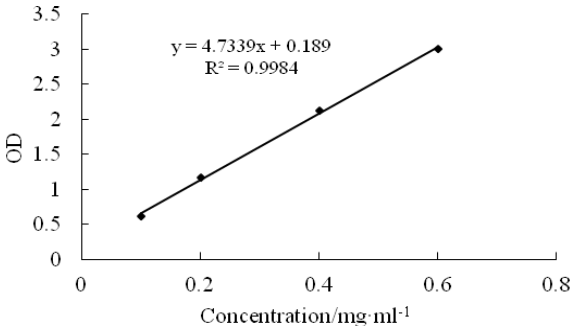

Figure 2. The standard curve of Naproxen methyl ester

B. *The initial test of CPL catalyzed capability*

The carica papaya lipase which is used in this study is from the unripe papaya fruit or the juice from papaya tree stems, and this juice containing both soluble papain and insoluble lipase. Thus, two different enzymes were tested to explore its resolution ability. The result shows in Fig. 3, Fig. 4. From this figure it can be seen that, the conversion rate and the enantiomeric excess value of products was increased by using CPL as the time went by. The conversion rate of the reaction catalyzed by papain is almost zero. Therefore, the authors can conclude that the CPL can catalyze chiral resolution of Naproxen, but papain cannot.

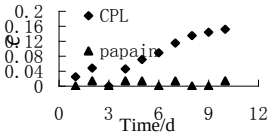

Figure 3. Effect of CPL and papain on the conversion

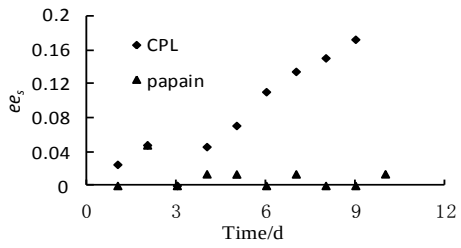

Figure 4. Effect of CPL and papain enzyme on the ee_s

C. *Effect of two different reaction systems*

Fig. 5, Fig. 6 shows the time course of conversion and ees in the aqueous phase system and the micro-water phase system. From the Fig. 5, the authors can get that the aqueous phase system is better, because the aqueous phase of the conversion rate reached the highest value of 18.5% at 6d, and micro-water phase system conversion rate is 9.8% at 6d. But lipase was inactivated after 6 days in aqueous phase system, and the lipase cannot be recycled. The lipase still has good activity and recyclability in organic phase after 15d. Therefore, the authors choose the micro-water phase system for its stability and recyclability.

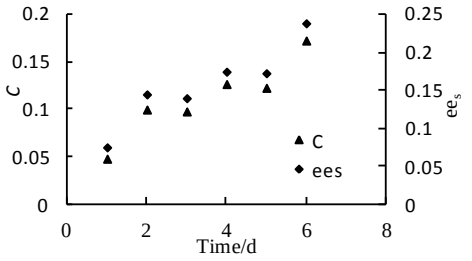

Figure 5. The time course of conversion and ee_s in the aqueous phase system

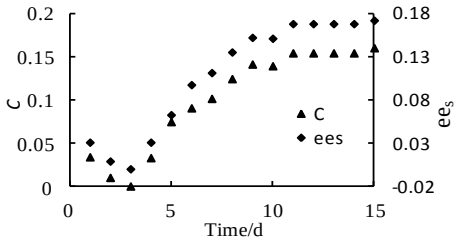

Figure 6. The time course of conversion and ee_s the micro-water phase system

D. *Effect of the amount of CPL*

Fig. 7 shows the variety of conversion and ees with the amount of CPL. It can be seen from the figure, the conversion and ees was increased with the amount of CPL, and when the amount of lipase continue to increase, the conversion rate and ees presented a downward trend. The reason is that the amount of lipase molecules being assigned to each of the buffer is reduced with the increase of the amount of CPL at a condition of a certain amount of H2O, and the H2O will affect lipase activity, then the conversion and ees reduced. Finally, the authors choose 1g for the optimum amount of lipase.

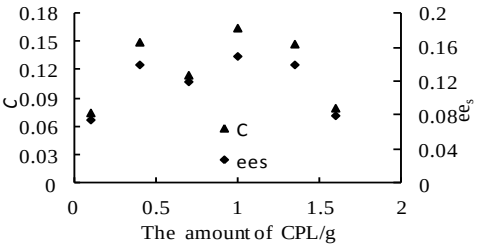

Figure 7. Effect of the amount of CPL on the conversion and ee_s

E. *Effect of the amount of H_2O*

In chiral resolution, water content of themedium influences not only the rate of reaction but also the activity of lipase. Suitable water content can keep the enzyme active configuration, but more water will inactivate the activity of lipase. Fig. 8 shows the variety of conversion and ees with the amount of H2O. It can be seen from the

figure that the conversion increased with the amount of the H2O. When the H2O amount increased to 1.2ml, the conversion rate is almost 17%, the value of enantiomeric excess of substrate is 18.68%. Then begin to decline and stabilize, so the best dosage is 1.2 ml.

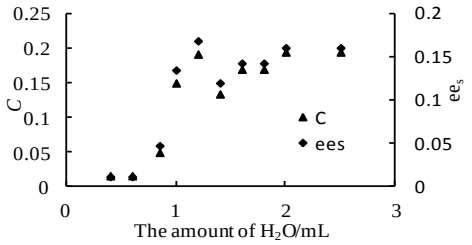

Figure 8. Effect of the amount of H_2O on the conversion and ee_s

IV. CONCLUSIONS

The results showed that carica papaya lipase has resolution ability over Naproxen, but papain has no resolution ability over Naproxen. It is better to choose the micro-water phase system than the aqueous phase system for the high strereopreference and stability. Overall, the racemic Naproxen methyl ester was hydrolyzed selectively to (S)-Naproxen by CPL in microaqueous- isooctane system. To 1g CPL was added in the concentration of 10mg/ml of Naproxen methyl ester, 30ml isooctane, 1.2ml H2O. The reactions were carried out in a shaker (180r/min) at 30℃. The maximum conversion rate is about 30%, E-value is 30 after 240h, and the enantiomeric excess of product is greater than 99%.

ACKNOWLEDGEMENTS

The authors thank the Scientific Research Fund of Heilongjiang Province (GC 13C111) for support.

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