Lipase Catalyzed Synthesis of High Optical Purity (S) - Naproxen Starch Ester

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Abstract. The high optical purity naproxen starch ester has been synthesized by *Candida rugosa* lipase (CRL) in iso-octane. The starch was treated with Urea /NaOH / H₂O solution at low temperature to improve its chemical reactivity. Under the reaction condition that the ratio of racemic naproxen methyl ester to activation corn starch was 1:1, the amount of CRL was 10%(w/w), the reaction temperature was 65 °C, the stirred speed was 100r/min, and the reaction time was 144 hours. The conversion of racemic naproxen methyl ester was 8.01% and the enantiomeric excess of the product (ee) was high to 96.01%. The production could be used as prodrug.

Introduction

Naproxen ((S)-(+)-2-(6-methoxy-2-naphthyl) propionic acid;) is a non-steroidal anti-inflammatory drug (NSAIDs) derived from propionic acid[1,2]. Naproxen is widely used in therapeutics as analgesic and antipyretic and it is also used for relief of symptoms of rheumatoid arthritis and osteoarthritis in addition to treatment of dysmenorrhea, among other indications[3]. The (S)-enantiomer is 28-fold more active than the corresponding (R)-enantiomer, (R)-isomer shows adverse side effects including renal impairment, gastrointestinal bleeding and platelet inhibition with altered haemostasis, warranting elimination of the unwanted enantiomer from the formulation [4]. Thus it was a good solution to generate (S) - naproxen starch ester. This can improve the efficacy, and can reduce drug damage to the stomach [Fig. 1].

Fig. 1 Preparation of (S)- naproxen starch ester using naproxen methyl ester, activated corn starch and CRL

Materials and methods

Materials. *Candida rugosa* lipase (CRL) was purchased from Novozymes, Denmark. Corn starch was purchased from a Chinese reagent company. The processing method is obtained in the literature[5]. Racemic Naproxen was purchased from Shanghai Mei Lan Industrial Co. Ltd. Esterification method of naproxen was reported in literature [6].

Analysis method. The Agilent 1200 HPLC Chiralcel OD-H, chiral column (250 mm x 4.6 mm, 5 microns) (Dacel Chemical Ind. Ltd) was used. The chromatographic conditions for separation of (R)-

and (S)- Naproxen methyl ester were a mobile phase composed of n-heptane/2-propanol (99/1 v/v) at a flow rate of 1 mL/min. Sample quantity was 5 μ L. A UV-VIS detection, model UV-2550 from shimadzu was set at 298 nm. The enantiomeric excesses of the substrate (ees), the product (eep) and the conversion of substrates(C) were calculated with the equations described in the literature[7-9].

Naproxen Starch Ester Synthesis

To choose the reaction substrates, naproxen or naproxen methyl ester, native starch or activated corn starch was selected. Set up four reactions, respectively naproxen and native starch; naproxen methyl ester and native starch; naproxen and activated corn starch; naproxen methyl ester and activated corn starch. Under the reaction condition that the ratio of racemic naproxen methyl ester to activation corn starch was 1:1, the amount of CRL was 10%(w/w), the reaction temperature was 65 °C, the stirred speed was 100r/min, and the reaction time was 144 hours.

Calculation of conversion rate

By UV-VIS detection measured absorbance, the comparison standard curve, calculated the quality of naproxen methyl ester.

$$C\% = (1 - \frac{m_1}{m_0}) \times 100\%$$

Where C is the conversion of naproxen starch ester; m_0 is the quality of naproxen starch ester at the beginning, mol; m_1 is the quality of naproxen starch ester in the end, mol.

Calculation of ee_s and ee_p

$$ee_s \% = \frac{S_R - S_S}{S_R + S_S} \times 100\%$$

Where ee_s is the enantiomeric excesses of the substrate, S_R is the peak area of (R)- naproxen starch ester, S_S is the peak area of (S)- naproxen starch ester.

$$ee_p = ee_s \times \frac{1 - C}{C}$$

Where ee_p is the enantiomeric excesses of the product.

Results and Discussion

The effect of the substrate. Results show that the native starch cannot attend reaction naproxen starch ester. Activated starch can do it very well. As the Table 1 showed that the CRL only could catalyze the esterification of naproxen methyl ester with activation starch. The naproxen and the native starch could not been catalyzed by CRL in isooctane. In the FT-IR spectrum, the differences between native starch and Naproxen starch ester were that there was new peak present at 1739.9cm⁻¹. That is the characteristic absorption band of ester bond.

Table 1 Generate naproxen starch ester reaction parameters

Substrate	Lipases	Time/h	Temperature	ee _s	C	ee _p
			[°C]	[%]	[%]	[%]
Naproxen+ native starch	CRL	144	65	-	0.0	-
Naproxen+activated starch	CRL	144	65	-	0.0	-
naproxen methyl ester+ native starch	CRL	144	65	-	0.0	-
naproxen methyl ester+ activated starch	CRL	144	65	8.36	8.01	96.01

The effect of naproxen methyl ester was better than naproxen. The CRL has a specific selectivity in this reaction. At the beginning of the reaction the peak area of (R)-naproxen methyl ester was 43.987%, (s)-naproxen methyl ester was 47.326% [Fig. 2]. After 144 hours the peak area of (R)-naproxen methyl ester was 47.562%, (s)-naproxen methyl ester was 42.633% [Fig. 3]. According to the formula to calculate the ee_s was 8.36%. Conversion rate was 8.01% and the ee_p was 96.01%.

Fig. 2 HPLC chromatograms of (R,S)- naproxen methyl ester at the beginning

Fig. 3 HPLC chromatograms of (R,S)- naproxen methyl ester at the end

The Reaction Proceeding

With the increase of reaction days, conversion and ee_p were increased, and the reaction rate increased from the third day, but when the reaction reached after 6 days, ee_p began to decline. Specific selectivity of CRL began to reduce after 6 days. The enzyme structure may be changed by reaction days and the temperature.

Fig. 4 The influence of reaction days on C and EEP
(10% CRL and 15mL isooctane added, in oil bath pot at 65 °C, 100r/min for 144 hours.)

Summary

The study showed that the high optical purity naproxen starch ester could be synthesized by CRL in isooctane when the naproxen methyl ester and activation starch as the substrate. There was new ester bond peak at 1739.9cm⁻¹ in the FT-IR spectrum, and the ee_p of naproxen starch ester was high to 96.01% that could be used as prodrug.

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