

Lipase-Catalyzed Synthesis of S-Naproxenal oleins

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Abstract. S-Naproxenal oleins are compounds of S-Naproxen ((S)-2-(6-methoxy-2-naphthyl) propionic acid) and olein connected by an ester bond and can be used as potential prodrug in anti-inflammatory. In the present work, we describe for the first time the lipase-catalyzed synthesis of the S-Naproxenal oleins by transesterification between S-Naproxen and triolein in organic solvents medium. The ability of lipases to catalyze the transesterification between S-Naproxen and triolein was investigated. Only the lipase from *Candida rugosa* and the immobilized lipase B from *Candida antarctica* (Novozym 435) catalyzed the transesterification. The influences of reaction condition on the combined yield of S-Naproxenal diolein (NDO) and S-Naproxenal monoolein (NMO) were investigated. Water activity (a_w) had an obvious influence on transesterification efficacy. A maximal combined yield of 23.1 % of S-Naproxenal diolein (NDO) and S-Naproxenal monoolein (NMO) was obtained under optimum condition. These results indicated the industrial potential of the operation scheme developed in this study.

Introduction

(S)-2-(6-methoxy-2-naphthyl) propionic acid (S-Naproxen) is an important member of the family of 2-aryl propionic acid derivatives which are widely used as non-steroidal anti-inflammatory drug (NSAIDs)^[1]. Free carboxylic group of S-Naproxen has severe gastrointestinal side effects on oral administration that restricts its use^[2]. S-Naproxen can cause undesirable gastrointestinal toxic effects such as bleeding, dyspepsia and peptic ulcers. To overcome this, acidic group of S-Naproxen can be temporarily masked by synthesizing glyceride ester prodrugs, which can pass through the stomach without releasing active drug in significant quantity and also increase the absorption pertaining to the natural triglycerides^[3].

Earlier, the glyceride prodrugs of some NSAIDs like ibuprofen^[4], biphenyl acetic acid^[5], mefenamic acid^[6], aspirin^[7] could be synthesized by chemical methods using inorganic acid as catalysts at high temperature. The unstable NSAIDs resulted in the formation of by-products easily. As an alternative, to avoid any possible harmful effects from chemically synthesized product, the use of lipases to catalyze the synthesis of glyceride ester prodrugs of S-Naproxen may be a much more promising method.

S-Naproxenal oleins are compounds of S-Naproxen and olein connected by an ester bond and can be used as potential prodrugs in anti-inflammatory^[8]. In the present work, we describe for the first time the lipase-catalyzed synthesis of the S-Naproxenal oleins by transesterification between S-Naproxen and triolein in organic solvents medium (Figure1).

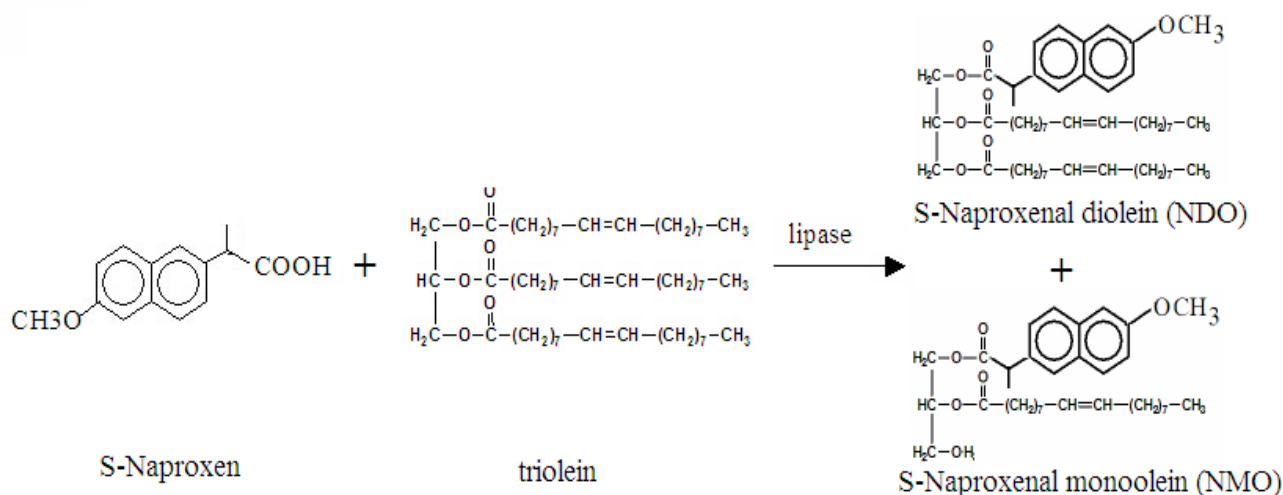


Figure. 1 Lipase-catalyzed transesterification of Naproxen and triolein

Naproxenal group substitution at the glycerol sn-1 position is shown for simplicity as the regioselectivity of the reaction is unknown.

Materials and methods

Lipase. Lipase from *Aspergillus niger* (Lipase AS “Amano” powder, $\geq 12,000$ U/g), Lipase from *Pseudomonas fluorescens* (Lipase AK “Amano” powder, $\geq 20,000$ U/g), Lipase from *Burkholderia cepacia* (Lipase PS “Amano” IM, immobilized on diatomite, 500 U/g solid enzyme) were purchased from Amano International Enzyme Co. (Nagoya, Japan). Novozym 435 (*Candida antarctica* lipase immobilized on polyacrylic resin, with an activity of $\geq 10,000$ U/g solid enzyme), was purchased from Novozymes A/S (Bagsvaerd, Denmark). Porcine pancreas lipase Type II (powder, ≥ 30 U/mg) and *Candida rugosa* lipase Type VII (powder, ≥ 700 U/mg) were purchased from Sigma.

Water activity pre-equilibration of reaction medium. Pre-equilibration was done at 25°C. The solid adsorbent was 3 Å molecular sieves ($a_w < 0.01$). The saturated salt solutions used were prepared with LiBr (a_w : 0.07), LiCl (a_w : 0.11), CH₃COOK (a_w : 0.23), (MgNO₃)•6H₂O (a_w : 0.54), NaCl (a_w : 0.75), KCl (a_w : 0.85), K₂Cr₂O₇ (a_w : 0.98)^[9]. By equilibrating lipase and substrates (i.e. S-Naproxen and triolein) with saturated salt solutions or 3A molecular sieves, the initial a_w for the reaction can be fixed over a broad range from < 0.01 to 0.98.

Procedure for lipase catalyzed transesterification. Transesterification reactions were conducted in 100-mL closed, screw-capped glass vials. S-Naproxen and triolein were dissolved together in organic solvent medium. Before the start of the reaction, the substrates (i.e. S-Naproxen and triolein), organic solvent and the lipase were pre-equilibrated for at least 120h in separate sealed containers enclosed with saturated salt solutions or solid adsorbent to establish fixed a_w for transesterification.

Reactions were performed through the addition of lipase to the organic solvent medium containing S-Naproxen and triolein in a 100-mL flask. When the lipase was added, glass vials were placed up right on a magnetic stirrer (200 rpm) and incubated at 30-32°C.

Monitoring and analysis of reaction mixtures. Analysis of the mixture composition was performed regularly. Samples were diluted 1:10 (vol/vol) in acetone and analyzed by thin-layer chromatography (TLC). The TLC migration was carried out with a solvent mixture of ethyl ether/hexane/ice acetic acid (15:85:1, v/v/v). The TLC plates were visualized under UV. Results were estimated from intensity of spots on TLC. The yields were calculated based on the total peak area of Naproxenal diolein (NDO), Naproxenal monoolein (NMO), and Naproxen. The sum of all Naproxenal species peak areas remained constant over the time course of the reaction, allowing accurate NDO and

NMO yields to be calculated as the percentage ratio of the NDO and NMO peak area to the total peak area of all Naproxen species.

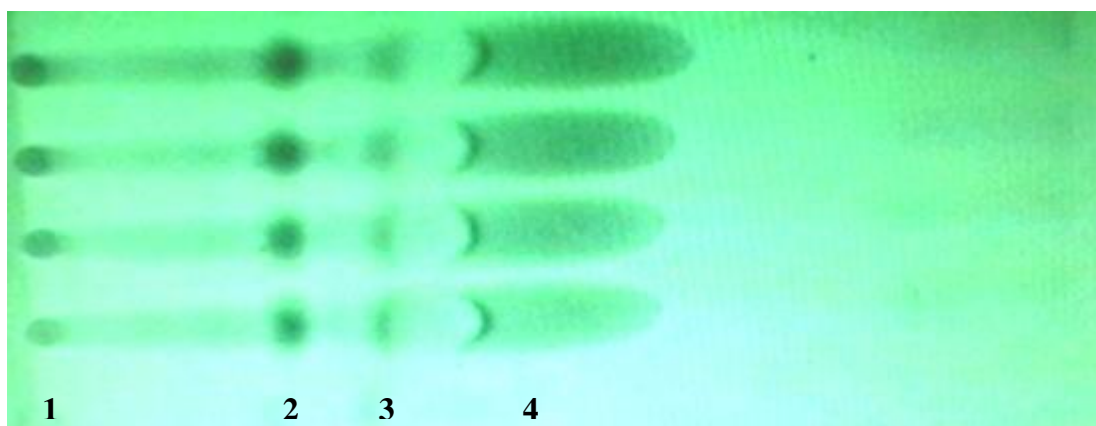


Figure. 2 Thin-layer chromatography (TLC) of lipase-catalyzed transesterification between S-Naproxen and triolein

1. origin; 2. Naproxen; 3 Naproxenal monoolein (NMO); 4. Naproxenal diolein (NDO)

Results and discussion

Six lipases from different sources (*Candida rugosa* lipase, Lipase AS, Lipase AK, Lipase PS, Novozym 435 lipase and Porcine pancreas lipase) were screened and evaluated for their ability to catalyze the transesterification reaction. Only *Candida rugosa* lipase and Novozym 435 lipase catalyzed the transesterification. However, Novozym 435 lipase gave rise to slower transesterification rate than *Candida rugosa* lipase. Therefore, *Candida rugosa* lipase was selected as the biocatalyst in the following experiments.

By using *Candida rugosa* lipase as catalyst, an enzymatic synthesis of S-Naproxen oleins from S-Naproxen with triolein was successfully carried out. It has been observed that water activity (a_w) had obvious influence on transesterification efficacy. The combined yield of Naproxenal diolein (NDO) and Naproxenal monoolein (NMO) decreased with increasing a_w from < 0.01 to 0.98 .

The influences of reaction medium on the yield of Naproxenal oleins was also studied. Isooctane has been found to be the optimal organic solvent of all the solvents that had been studied. The yield 16.5% achieved in isooctane ($\text{Log } P = 4.5^{[10]}$), which was higher than the corresponding reactions yield that used n-hexane ($\text{Log } P = 3.5^{[10]}$) or toluene ($\text{Log } P = 2.5^{[10]}$).

The molar ratio of one substrate to another is an important parameter affecting the reaction equilibrium. The effect of molar ratio of S-Naproxen to triolein on the lipase-catalyzed transesterification was investigated. The Naproxen concentration was fixed at 1.0 mmol while the triolein concentration varied from 0.5 to 4.0 mmol . When the triolein/Naproxen molar ratio increased, the combined yield of Naproxenal diolein (NDO) and Naproxenal monoolein (NMO) increased and reached a maximum at a ratio of $3/1$. The influence of the molar ratio of the substrates on the yield may be explained by a thermodynamical shift of the equilibrium in favour of the synthesis of the ester due to triolein excess. However, for a further increase in triolein content, the synthesis yield was declined slightly.

Finally, a maximal combined yield of 23.1% of S-Naproxen diolein (NDO) and S-Naproxen monoolein (NMO) was obtained using a $3/1$ mole ratio of trioleins to S-Naproxen in isooctane under $30\text{-}32^\circ\text{C}$ at 96h .

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