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Enhancement of enantioselectivity on the synthesis of (*S*)-naproxen morpholinoalkyl ester prodrugs in organic solvents using isopropanol-dried immobilized lipase

Chun-Sheng Chang [a,*], Chien-Chih Su [a], Jia-Rong Zhuang [a], Shau-Wei Tsai [b]

[a] *Department of Biotechnology, Southern Taiwan University of Technology, Tainan Hsien 710, Taiwan, ROC*
[b] *Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan, ROC*

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Abstract

Enzymatic synthesis of a series of (*S*)-naproxen morpholinoalkyl ester prodrugs directly from racemic naproxen has been investigated. The decrease of enantioselectivity of the crude *Candida rugosa* lipase from 74.5 to 24 was observed with the increase of the straight alkyl chain length of hydroxyalkyl morpholines (acyl acceptor) from ethyl to butyl in the esterification reaction. The enantioselectivity for the isopropanol-dried immobilized lipase (IPA-dried IM-lipase) was all exceeded 100 for the different straight alkyl chain length of hydroxyalkyl morpholines in various organic solvents, but for the lyophilized IM-lipase was only reached about 11 in isooctane. The hydroxyalkyl morpholines still acts as an enzyme inhibitor at higher alcohol concentration and its optimal alcohol concentrations were ranged from 5 to 10 mM. In conclusion, IPA-dried IM-lipase shows good enzyme activity and excellent enantioselectivity in the synthesis of (*S*)-naproxen morpholinoalkyl ester prodrugs.

Keywords: Enantioselectivity; Esterification; Immobilized lipase; Naproxen; Prodrug

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a diverse group of drugs, have their pharmacological activity mainly on (*S*)-enantiomer that is widely used for the treatment of rheumatic diseases and related painful conditions [1]. 2-(6-Methoxy-2-naphthyl) propionic acid (naproxen) is the one of the best known. Gastrointestinal (GI) side effects produced by NSAIDs is generally believed to be resulted from the direct contact effect which can be attributed to a combination of local irritation produced by the acidic group of NSAIDs and local inhibition of prostaglandin synthesis in the GI tract [2,3]. Elimination of the GI side effects can be accomplished by drug derivation. The mean is to design bioreversible derivates such as prodrugs in order to decrease the toxicity induced by NSAIDs is necessary.

The prodrugs are bioreversible pharmacologically inactive derivates of a drug molecule that require a chemical or enzymatic transformation to release the active parent drug in situ [5]. Bundgaard and Nielsen have outlined some usefulness of the principles of esterification of acidic NSAIDs in which appropriate aqueous solubility and lipophilicity of prodrug is essential when administration via per oral route [4]. The bioreversible derivatives (prodrugs) of naproxen have been investigated to reduce their GI side effect and other possible systemic side effects due to high plasma peak levels after oral administration. Ester group of NSAIDs that is promising for reducing or abolishing the GI toxicity due to the localized effect replaces the carboxyl group of NSAIDs.

Rautio et al. have synthesized a series of morpholinyl- and methylpiperazinylacyloxyalkyl esters of naproxen and evaluated their biphasic solubility and the rate of enzymatic hydrolysis in vitro for topical drug delivery [6–8]. Morpholinoalkyl ester prodrugs of naproxen were less irritating to gastric mucosa than parent drug and increasing the aqueous solubility without altering the pharmacological profile of the parent compound for oral delivery [9]. Some cyclic

* Corresponding author. Tel.: +886-6-2533131x3920; fax: +886-6-2425741.
E-mail address: cs_chang@mail.stut.edu.tw (C.-S. Chang).

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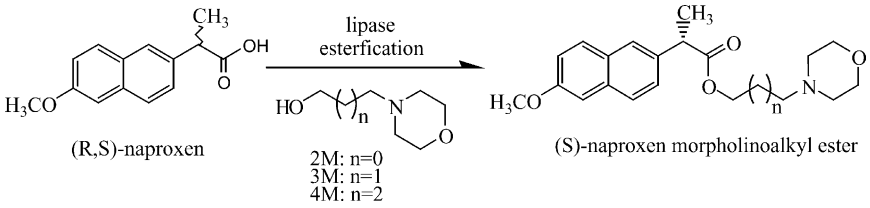

Scheme 1.

amide ester showed potentially useful derivates of naproxen to improve their therapeutic index for oral delivery [10].

Immobilization always increased lipase thermo- and chemical stability. Immobilization of lipase secured the reusability and minimized the cost of production isolation [11]. Lipase immobilized onto a hydrophobic support by adsorption often shows good activity in organic media [12,13]. The 2-propanol treatment of *Candida rugosa* lipase may increase the activity and enantioselectivity by converting the closed form of lipase to the open form [14]. The very high activity *n*-propanol rinsed enzyme preparation can be obtained with both subtilisin Carlsberg and α-chymotrysin, since the different method of water removal can affect the amount of water left bound to the enzyme and the conformation of the dried enzyme [15].

Morpholinobutyl ester prodrug of naproxen among the series of morpholinoalkyl ester prodrugs of naproxen was the most significant reducing the GI side effect [9]. A facile enzymatic esterification process for the direct synthesis (*S*)-naproxen (and (*S*)-ibuprofen) 2-*N*-morpholinoethyl ester prodrug from racemic (*S*)-naproxen (and (*S*)-ibuprofen) has been developed using lipases as the biocatalyst in organic solvent [16,17]. The aim of the present work was to continue our earlier work on the enzymatic kinetic resolution process using lipases in the organic media to directly synthesize the morpholinobutyl and morpholinopropyl ester prodrugs of (*S*)-naproxen from racemic naproxen as shown in Scheme 1. We will further investigate the effect of the change of straight alkyl chain length of hydroxyalkyl morpholines on the enantioselectivity and activity for the different preparation types of immobilized enzyme.

2. Materials and methods

2.1. Materials

Optically pure (*S*)-naproxen was purchased from Sigma (St. Louis, MO). Bromopropanol and bromobutyl acetate were purchased from Lancaster (Lancashire, UK). Cyclohexane, *n*-hexane, *n*-heptane, isooctane (HPLC grade) were from TEDIA (Fairfield, OH). 4-(2-Hydroxyethyl) morpholine (2 M) was purchased from Aldrich (Milwaukee, WI, USA). All other chemicals were analytical grade. *C. rugosa* (Type VII, 901 U/mg) lipase was from Sigma. Polypropene powder (Accurel MP1000) was kindly donated by Akzo Nobel (Obernurg, Germany). Racemic naproxen was obtained by racemizing (*S*)-naproxen as previous described [18].

2.2. Analytical procedure

The esterification and transesterification were monitored by HPLC using a chiral column (Chiralcel OD-H, Daicel Chemical Industries Ltd., Japan) capable of separating the internal standard of nitrotoluene, (*R*)- and (*S*)-naproxen, (*R*)- and (*S*)-morpholinoethyl ester, (*R*)- and (*S*)-morpholinopropyl ester, (*R*)- and (*S*)-morpholinobutyl ester with the retention time as 4.95, 12.93, 14.44, 15.80, 18.69, 18.76, 21.47, 22.42, 24.55 min, respectively. The mobile phase was a (v/v) mixture of *n*-hexane:isopropanol:acetic acid (96.5:3.5:0.45) at a flow rate of 1.0 ml min^{-1}. UV detector at 270 nm was for quantification at the column temperature of 27 °C.

2.3. Synthesis of hydroxyalkyl morpholines [7]

2.3.1. 4-(3-Hydroxypropyl) morpholine (3 M)

Bromopropanol (2.87 g, 20.5 mmol) in 5 ml of benzene was added dropwise to a solution of morpholine (4.10 g, 41.0 mmol) in 5 ml of benzene and the solution was refluxed for 2 h. After cooling, the solution was filtered off and the filtrate was evaporated, and purified by liquid chromatography on silica gel, eluting with MeOH. The solution was dried with $MgSO_4$ overnight and evaporated to obtain an oil, 4-(3-hydroxypropyl)morpholine (1.42 g, 47.5%). ^{1}H NMR ($CDCL_3$) δ 1.66 (quint, $J = 6.0$ Hz, 2H, CH_2–CH_2OH), δ 2.25–2.51 (m, 4H, $(CH_2)_2N$–), δ 2.54 (t, $J = 6.0$ Hz, 2H, –N–CH_2), δ 3.51–3.68 (m, 4H, $(CH_2)_2O$), δ 3.70 (t, $J = 6.0$ Hz, 2H, CH_2–OH), δ 4.59 (s, br, OH).

2.3.2. 4-(4-Hydroxybutyl) morpholine (4 M)

4-(4-Hydroxybutyl) morpholine was prepared as described for 4-(3-hydroxypropyl) morpholine from bromobutyl acetate (4.00 g, 20.5 mmol) and morpholine (4.10 g, 41.0 mmol). Liquid chromatography (MeOH) proceeded morpholino butyl acetate as a viscous oil, which was refluxed with 70 ml of 2 M NaOH for 24 h. To a cooling solution, 5 g NaCl was added, and a solution was extracted (1:2 ethanol:chloroform, 3 × 60 ml), dried, and evaporated to yield 4-(4-hydroxybutyl) morpholine (2.22 g, 68.1%). ^{1}H NMR ($CDCL_3$) δ 1.60–1.64 (m, 4H, CH_2CH_2–OH), δ 2.33 (t, $J = 6.9$ Hz, 2H, –N–CH_2), δ 2.41–2.62 (m,

4H, $(CH_2)_2$N–), δ 3.54 (t, $J = 6.0$ Hz, 2H, CH_2–OH), δ 3.62–3.81 (m, 4H, $(CH_2)_2$O), δ 4.85(s, br, OH).

2.4. Lipase immobilization with different dry method

The immobilized lipase (IM-lipase) was prepared by adsorbing *C. rugosa* lipase on the adsorbent of Accurel MP 1000 of particle size distribution between 355 and 425 mm. Briefly, dissolving 1200 mg of the crude lipase in 30 ml phosphate buffer pH 7, centrifuging at 4000 rpm for 15 min and removing the precipitate obtained 25 ml of clear enzyme solution. The solution was brought in contact with 1000 mg of the support that had been pre-wet with 99.5% ethanol and pre-washed with 50% ethanol–water solution and pure deionized water, in succession. The lipase–support system was shaken at 4 °C for 6 h. Then, the support particles were separated from the solution by filtration. There are two different methods to dry the immobilized lipase. The first method is to lyophilize IM-lipase for 12 h and stored at 4 °C. The second method used isopropanol, 20 ml/g, to rinse and decant six times, and then to rinse IPA-dried IM-lipase with reaction solvent and decant six times. The equivalent enzyme laden on the support was determined after assaying the residual absorption intensity of the filtrate by employing the Bio-Rad Protein Assay method with bovine serum albumin as the standard.

2.5. Reaction media and hydroxyalkyl morpholines screening in esterification

Unless specified, 75 mg of the crude *C. rugosa* lipase was added to 15 ml of reaction media containing 0.2 mM racemic naproxen and 5 mM hydroxyalkyl morpholines at 37 °C. The resultant mixture was stirred with a magnetic stirrer, and samples were removed for HPLC analysis at different time intervals.

2.6. Operation stability test of IPA-dried IM-lipase

To 15 ml of cyclohexane was added 7.7 mg/ml (equivalent to 5 mg/ml of *C. rugosa* lipase) of IPA-dried IM-lipase, 0.5 mM racemic naproxen and 5 mM 4-(4-hydroxybutyl) morpholine at 37 °C. Stability tests were carried out by a series of successive esterification reactions, using the same amount of IPA-dried IM-lipase. Each esterification run for 48 h, its IPA-dried IM-lipase is washed with cyclohexane three times. Stability run tests were repeated eight times.

3. Results and discussion

3.1. Screening of the reaction media and hydroxyalkyl morpholines

From the previous research, we know *C. rugosa* lipase and lipase MY that have good enzyme activity and enantioselectivity for the synthesis of (*S*)-naproxen 2-*N*-morpholinoethyl ester prodrug from racemic naproxen [16]. Several studies indicated that substrate selectivity and enantioselectivity of the enzyme have been altered in different polarity of organic solvents [19–21]. In general, there is no single parameter for solvent polarity that can consistently correlate the enzyme activity with the hydrophobicity of solvents. Among the parameters of solvent polarity, logarithm of partition coefficient of the solvent ($\log P$) between octanol and water was recommended the best parameter relating enzyme activity to solvent nature [22]. Table 1 shows the enantioselectivity (V_S/V_R) of crude *C. rugosa* lipase for the esterification of naproxen with hydroxyalkyl morpholines in various organic solvents. The polarity of solvents had marked effect on the enzyme enantioselectivity and activity. The highest enzyme enantioselectivity and activity was provided by the most hydrophobic isooctane. Clearly, the increase of the solvent hydrophobicity for straight chain hydrocarbons results in the improvement of the enantioselectivity (V_S/V_R), and the enhancement of enzyme activity. These results indicated the enantioselectivity and activity of enzyme largely decreased as the polarity of the solvent increased. The crude *C. rugosa* lipase had a higher enantioselectivity in less hydrophobic cyclohexane than in hexane. Nakamura et al. discovered the enantioselectivity of a lipase depends on the structure of solvent molecules [21,23]. Cycloalkanes may be incorporated more easily into the hydrophobic pocket of lipase than the corresponding normal alkanes of the same carbon number because of the compact structure of solvent molecules.

The esterification reaction rate, as well as enantioselectivity, varied largely with different acyl acceptors. Such an effect of straight alkyl chain length of alcohol for enantioselectivity has been showed opposite tendency that may be attributable to the different in the type of reactions (transesterification versus esterification) or the nature of enzyme changed [24,25]. Long-chain aliphatic alcohols may coordinate to the acyl-binding tunnel of the *C. rugosa* lipase, thereby selectivity inhibiting the turnover of the fast-reacting *S*-enantiomer, thus resulting in a lowered enantioselectivity in the resolution [26]. As listed in Table 1, an increase in straight alkyl chain length of morpholinoalkyl alcohols led to increase the reaction rate in the esterification of (*R*)-naproxen but only slightly affected the reaction rate in the esterification of (*S*)-naproxen. The decrease of enantioselectivity of the crude *C. rugosa* lipase from 74.5 to 24 was observed with the increase of the straight alkyl chain length of hydroxyalkyl morpholines (acyl acceptor) from ethyl to butyl in the esterification reaction. It indicated that hydroxypropyl and hydroxybutyl morpholine act as enantioselective inhibitors in the *C. rugosa* lipase catalyzed resolution of racemic naproxen. Several studies indicated that a long-chain aliphatic alcohol competes with acyl donor for the same binding site in the active site of lipase and acts as enantioselective inhibitors for giving a higher enantioselectivity for the *R*-enantiomer. The alcohol coordinated into the active tunnel might have resulted in the changed

Table 1
Enantioselectivity of crude *C. rugosa* lipase for the esterification of naproxen with hydroxyalkyl morpholines in various organic solvents at 37 °C

Alcohol		Reaction medium			
		Isooctane	Heptane	Hexane	Cyclohexane
2 M	X_t (%)[a]	45.1	25.6	22.9	37.0
	V_S (mM mg^{-1} h^{-1})	1.07×10^{-4}	2.81×10^{-5}	2.26×10^{-5}	5.46×10^{-5}
	V_S/V_R	74.5	16.8	22.5	76.9
3 M	X_t (%)	56.5	35.9	33.9	46.7
	V_S (mM mg^{-1} h^{-1})	8.85×10^{-5}	2.29×10^{-5}	2.20×10^{-5}	5.09×10^{-5}
	V_S/V_R	22.4	4.7	4.3	18.4
4 M	X_t (%)	61.2	37.5	34.9	45.2
	V_S (mM mg^{-1} h^{-1})	1.02×10^{-4}	2.58×10^{-5}	2.91×10^{-5}	4.85×10^{-5}
	V_S/V_R	24.2	5.6	4.8	21.2

(*R*, *S*)-naproxen = 0.2 mM, hydroxyalkyl morpholines = 5.0 mM, crude *C. rugosa* lipase = 5 mg/ml.
[a] X_t: the conversion of (*R*, *S*)-naproxen at reaction time 24 h, $X_t = X_R + X_S$, where X_R and X_S are the yields of (*R*)-naproxen morpholinoalkyl esters and (*S*)-naproxen morpholinoalkyl esters, respectively.

enantioselectivity of lipase [27,28]. It is said that an acyl donor (acid) attacks lipase and forms an acyl-enzyme intermediate, then an acyl acceptor (alcohol) reacts with the acyl enzyme intermediate to yield an ester. If the results obtained in this work are explained on the basis of the above mechanism, the reaction rate and enantioselectivity should be determined at the step of deacylation rate of acyl-enzyme intermediate by the alcohol to be the second substrate.

3.2. Effect of preparation type of IM-lipase on the enantioselectivity and activity

From the view of process, the question that we must consider the enantioselectivity of crude *C. rugosa* lipase for the synthesis of (*S*)-naproxen morpholinopropyl and morpholinobutyl ester prodrugs on the low side. Table 2 shows a comparison of the enantioselectivity and initial rate obtained for the esterification of racemic naproxen in various organic solvents using immobilized lipase with different methods of water removal. The enantioselectivity for the IPA-dried IM-lipase were all exceeded 100 in various hydrophobic organic solvents, but for the lyophilized IM-lipase are only reached about 11 in isooctane. Immobilized lipase was dried by lyophilization which is the most common dry method, that results in the large decrease of the enantioselectivity of the crude lipase and the initial rate (V_S) of (*S*)-naproxen. Comparison of lyophilized IM-lipase and IPA-dried IM-lipase, the large difference existed at the

Table 2
Effect of preparation type on the enantioselectivity and activity of *C. rugosa* lipase for the esterification of naproxen with hydroxyalkyl morpholines in various organic solvents at 37 °C

Alcohol			Reaction medium			
			Isooctane	Heptane	Hexane	Cyclohexane
2 M	Lyophilized IM-lipase	V_S (mM mg^{-1} h^{-1})	4.10×10^{-5}	8.84×10^{-6}	6.30×10^{-6}	1.83×10^{-5}
		V_R (mM mg^{-1} h^{-1})	6.20×10^{-7}	4.89×10^{-7}	3.24×10^{-7}	2.4×10^{-7}
		V_S/V_R	66.1	18.1	19.5	75.9
	IPA-dried IM-lipase	V_S (mM mg^{-1} h^{-1})	1.29×10^{-4}	2.52×10^{-5}	2.21×10^{-5}	6.98×10^{-5}
		V_R (mM mg^{-1} h^{-1})	8.59×10^{-8}	4.30×10^{-8}	3.75×10^{-8}	5.62×10^{-8}
		V_S/V_R	>100	>100	>100	>100
3M	Lyophilized IM-lipase	V_S (mM mg^{-1} h^{-1})	5.39×10^{-5}	1.43×10^{-5}	2.12×10^{-5}	3.72×10^{-5}
		V_R (mM mg^{-1} h^{-1})	5.15×10^{-6}	5.80×10^{-6}	5.01×10^{-6}	3.70×10^{-6}
		V_S/V_R	10.5	2.5	4.2	10.0
	IPA-dried IM-lipase	V_S (mM mg^{-1} h^{-1})	1.21×10^{-4}	2.26×10^{-5}	2.06×10^{-5}	6.07×10^{-5}
		V_R (mM mg^{-1} h^{-1})	6.75×10^{-8}	2.58×10^{-8}	3.01×10^{-8}	3.59×10^{-8}
		V_S/V_R	>100	>100	>100	>100
4 M	Lyophilized IM-lipase	V_S (mM mg^{-1} h^{-1})	6.46×10^{-5}	1.49×10^{-5}	1.11×10^{-5}	3.41×10^{-5}
		V_R (mM mg^{-1} h^{-1})	4.72×10^{-6}	5.25×10^{-6}	5.29×10^{-6}	2.89×10^{-6}
		V_S/V_R	13.7	2.8	2.1	11.8
	IPA-dried IM-lipase	V_S (mM mg^{-1} h^{-1})	1.11×10^{-4}	1.71×10^{-5}	1.60×10^{-5}	5.83×10^{-5}
		V_R (mM mg^{-1} h^{-1})	5.84×10^{-8}	2.42×10^{-8}	2.53×10^{-8}	8.18×10^{-8}
		V_S/V_R	>100	>100	>100	>100

(*R*, *S*)-naproxen = 0.2 mM, hydroxyalkyl morpholines = 5.0 mM, IM-lipase concentration is equivalent to 5 mg/ml of crude *C. rugosa* lipase.

methods of water removal that the former removed water by lyophilization, the latter used isopropanol to strip water and then replaced isopropanol by hydrophobic solvent. The procedure of lyophilization for immobilized lipase led to undesirable change in the conformation of active site. The IPA-dried IM-lipase does not easily happen to the change of conformation of active site because its last step replaced isopropanol by hydrophobic solvent that provides the more strict surroundings of immobilized lipase. We may, therefore, reasonably conclude that IPA-dried method can increase the enzyme activity and improve its enantioselectivity because the isopropanol dehydration process leaves the enzyme conformation close to active form; the following replacement procedure of hydrophobic solvent sustains the active conformation of immobilized lipase.

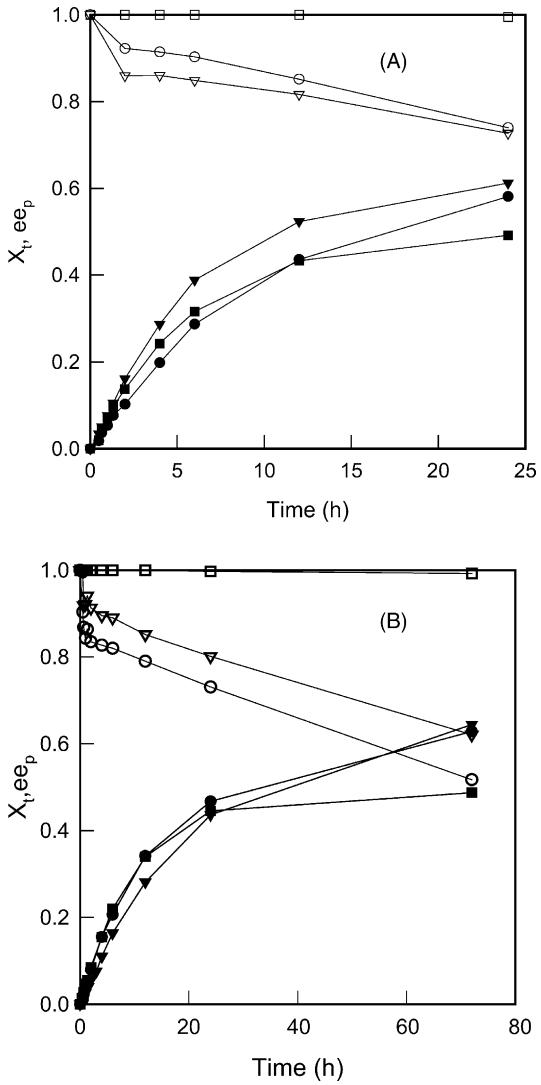

Fig. 1. Effect of different preparation types of lipase on the conversion of (*R*, *S*)-naproxen and ee_p of the morpholinobutyl and morpholinopropyl ester prodrugs of (*S*)-naproxen in different organic media. Conversion of (*R*, *S*)-naproxen X_t (filled); ee_p (empty); free lipase (circle); lyophilized IM-lipase (triangle); IPA-dried IM-lipase (square). Conditions: (*R*, *S*)-naproxen = 0.2 mM, 37 °C, IM-lipase concentration is equivalent to 5 mg/ml of crude *C. rugosa* lipase. (A) hrdroxypropyl morpholine = 5 mM in isooctane, (B) hydroxybutyl morpholine = 5 mM in cyclohexane. ee_p: the enantiomeric excess for the product, $ee_p = (X_S - X_R)/(X_S + X_R)$.

Fig. 1(A) and (B) illustrates the typical time course results of the conversions of racemic naproxen (X_t) and the enantiomeric excess of ester product (ee_p) for comparison of the different preparation types of lipase catalyzed the esterification between (*R*, *S*)-naproxen and hydroxyalkyl morpholines in isooctane and cyclohexane, respectively. The enantiomeric excess of morpholinobutyl and morpholinopropyl ester prodrugs of (*S*)-naproxen during the kinetic resolution using the IPA-dried IM-lipase can constantly maintain over 99% even though the (*S*)-naproxen was almost exhausted. Apparently, the IPA-dried IM-lipase suppressed the initial rate of (*R*)-naproxen that result in the sharply increase of enantioselectivity for racemic naproxen.

The lyophilized IM-lipase with the low initial rate revealed initially inactive. The lyophilized IM-lipase was further treated with different ratios of water/isopropanol that expected to supply lipase with the adequate water and then to regenerate its activity which procedure is the similar as the isopropanol dehydration process that IPA rinse IM-lipase preparation. From Fig. 2, it showed that the enzyme activity and enantioselectivity of lyophilized IM-lipase could not be recovered by the above regenerated procedure. The results indicated the change of the conformation of active site of enzyme is very sensitive to the method of water removal.

3.3. Effects of alcohol concentration

The variation of the specific initial rate of (*S*)-naproxen with the hydroxyalkyl morpholine concentration in cyclo-

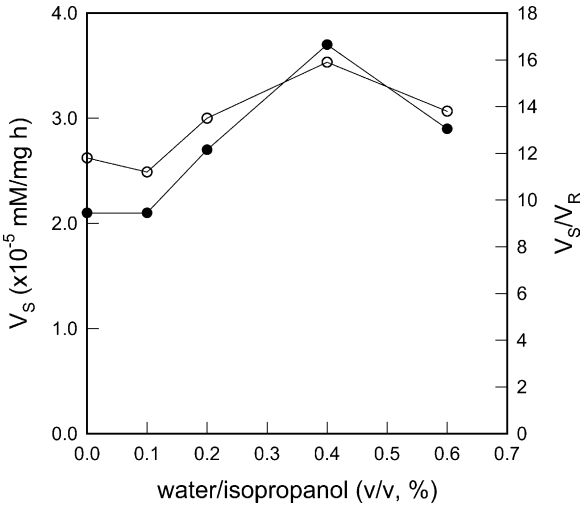

Fig. 2. Using different volume ratios of water/isopropanol rinsed the lyophilized IM-lipase to regenerate its enzymatic activity and enantioselectivity. Conditions: (*R*, *S*)-naproxen = 0.5 mM, hrdroxypropyl morpholine = 5 mM, 37 °C, IM-lipase concentration is equivalent to 5 mg/ml of free *C. rugosa* lipase in cyclohexane. The rinsed procedure is the same as the second method of Section 2.4. Initial rate V_S (●); V_S/V_R (○).

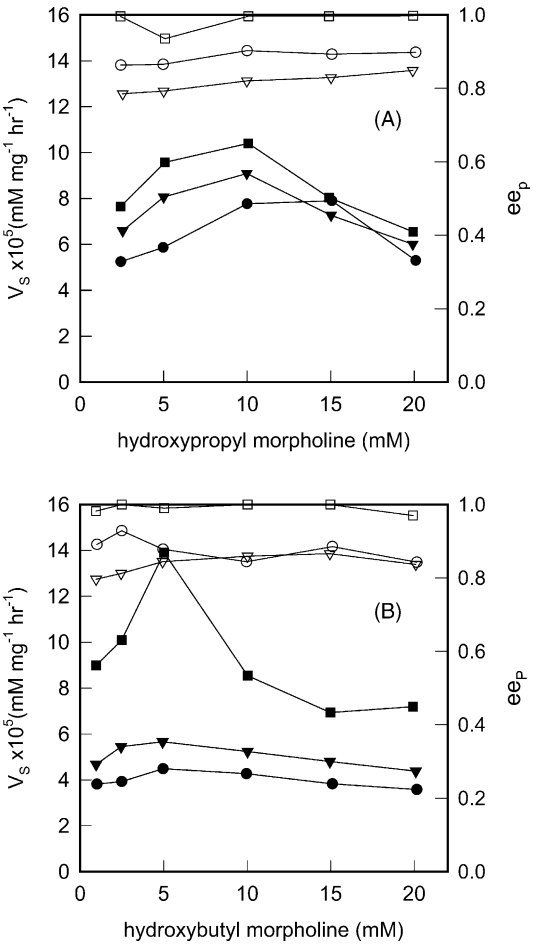

Fig. 3. Alcohol concentration effect on the initial rate V_S and ee$_p$ of the morpholinobutyl and morpholinopropyl ester prodrugs of (*S*)-naproxen in cyclohexane for different preparation types of lipase. Initial rate V_S (filled); ee$_p$ at reaction time 24 h (empty); free lipase (circle); lyophilized IM-lipase (triangle); IPA-dried IM-lipase (square). Conditions: (*R*, *S*)-naproxen = 0.5 mM, 37 °C, IM-lipase concentration is equivalent to 5 mg/ml of crude *C. rugosa* lipase. (A) hydroxypropyl morpholine = 5 mM, (B) hydroxybutyl morpholine = 5 mM.

hexane when using crude lipase, lyophilized IM-lipase and IPA-dried IM-lipase is shown in Fig. 3. Their maximums are observed at around 10 and 5 mM for hydrxypropyl morpholine and hydroxybutyl morpholine, respectively. As previous report, the optimal 4-(2-hrdroxyethyl) morpholine concentration around 8 mM was found [16]. This implies that hydroxyalkyl morpholines might acts as an enzyme inhibitor at higher alcohol concentration and its optimal alcohol concentrations were ranged from 5 to 10 mM. It clearly showed that their individual initial rates would be influenced differently by a non-chiral acyl acceptor and its concentration. The shift of optimal concentration may result from the alkyl chain length of hydroxyalkyl morpholine difference. Meanwhile, the initial rate and enantioselectivity of IPA-dried IM-lipase was obviously higher than other preparation type of lipases at the equivalent enzyme concentration. It indicated that using isopropanol removes water associated with the enzyme

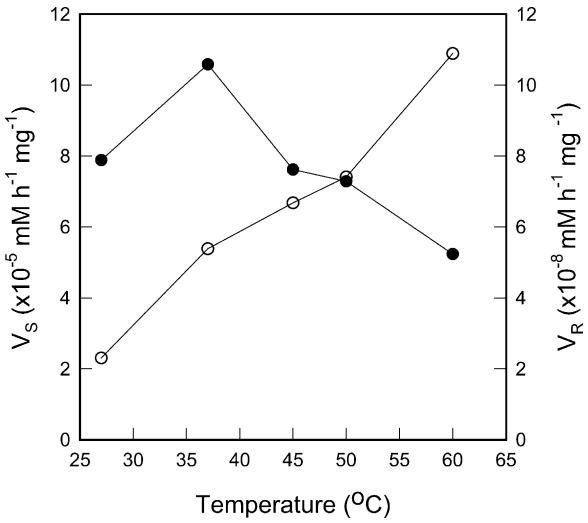

Fig. 4. Effect of temperature with IPA-dry IM-lipase on the initial rate in cyclohexane V_S (filled) and V_R (empty), respectively. Conditions: (*R*, *S*)-naproxen = 0.5 mM, hydroxybutyl morpholine = 5 mM, 37 °C, IM-lipase concentration is equivalent to 5 mg/ml of crude *C. rugosa* lipase.

by a mechanism that changed the conformation of active site of enzyme molecules to slightly increase its original crude enzyme activity and sharply enhance its enantioselectivity.

3.4. Effect of temperature on the enantioselectivity and activity IPA-dried IM-lipase

As shown in Fig. 4, the temperature to give a maximum initial rate V_S for the enzymatic esterification of racemic naproxen with hydroxybutyl morpholine in cyclohexane was around 37 °C. The initial rate V_R increases with temperature elevated, which results in the decrease of the value of enantioselectivity. The lipase-catalyzed esterification is a temperature-dependent reversal of stereochemistry. However, the enantioselectivity was still high enough to obtain high enantiomeric excess for the production of (*S*)-naproxen morpholinobutyl ester prodrugs by IPA-dried IM-lipase.

3.5. Operational stability of IPA-dried IM-lipase

Operational stability tests were carried out by a series of successive esterification runs, rinsed the IPA-dried IM-lipase three times with cyclohexane for removing the residual substrate and product from the immobilized lipase. Fig. 5 shows the operational stability of IPA-dried IM-lipase. The activity decreased rapidly to about 30% of their initial value after four runs. However, when using the washing solvent replaced cyclohexane by isopropanol, IM-lipase activity decreased more rapid to only 10% of their initial value after two runs (data was not showed). Al-Duri et al. indicated that lipase immobilized on polypropylene-based support maintain their activities for >8 successive runs almost unchanged using hexane to rinse the immobilized lipase [12]. The difference of washing solvent may affect the operational stability.

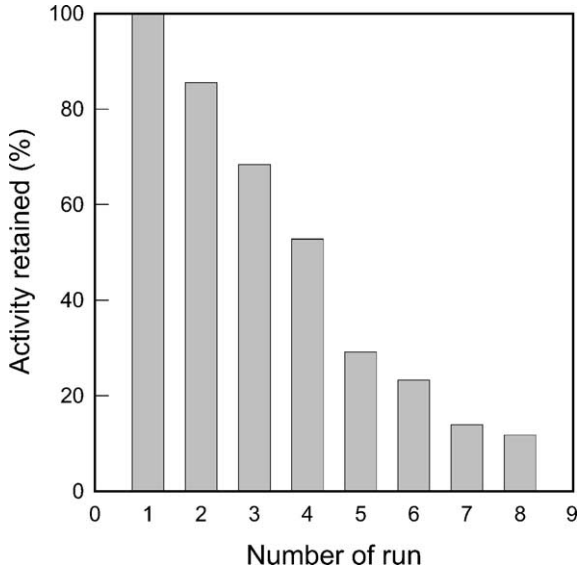

Fig. 5. Operational stability test of IPA-dried IM-lipase. For experimental detail see text.

More studies on the procedures of regeneration of IPA-dried IM-lipase for operation stability and activity are needed.

4. Conclusions

Lipase-catalyzed enantioselective syntheses of a series of (*S*)-naproxen morpholinoalkyl ester prodrugs directly from racemic naproxen were studied. The enantioselectivity of the crude *C. rugosa* lipase was affected by the change of the straight alkyl chain length of hydroxyalkyl morpholines. The enantioselectivity of crude *C. rugosa* lipase for the synthesis of (*S*)-morpholinobutyl ester prodrug of naproxen with the most significant reducing the gastrointestinal side effect was only reached 24 in isooctane. A screening of different preparation types of lipase and organic solvents considered enzyme enantioselectivity and activity. IPA-dried IM-lipase and isooctane were selected as the best preparation type of lipase and reaction medium, respectively. The enantioselectivity of IPA-dried IM-lipase was all exceeded 100 for the different straight alkyl chain length of hydroxyalkyl morpholines in the enzymatic esterification reaction with various hydrophobic organic solvents. This suggested the isopropanol dehydration process was a simple and effective way for preparing immobilized lipase in organic media. Here, the hydroxyalkyl morpholines at higher alcohol concentration still acts as an enzyme inhibitor for the different preparation types of lipase.

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