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Dynamic kinetic resolution of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester via lipase-catalyzed hydrolysis in micro-aqueous isooctane

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Abstract

Candida rugosa lipases immobilized on polypropylene powders were employed as biocatalysts for the enantioselective hydrolysis of (*R, S*)-naproxen 2,2,2-trifluoroethyl ester in isooctane at 45 °C. An organic base of 1,5,7-triazabicyclo[4,4,0] dec-5-ene bound to polystyrene crosslinked with 2% DVB (p-TBD) was furthermore added as an in situ racemization catalyst for the remaining (*R*)-ester to carry out dynamic kinetic resolution for the racemate. The kinetic behavior of the lipase by considering enzyme stability and product inhibition and that of the base were first investigated. The results for the dynamic kinetic resolution process were then compared with those without adding p-TBD. Low enantiomeric excess (e.e.) for the product (i.e. $e.e._p = 58.1\%$) at the racemate conversion of 95.5% was obtained, which might be attributed to the decrease of racemization capacity of the base and increase of non-enzymatic hydrolysis. A comparison of the kinetic behavior was also made when employing (*R, S*)-naproxen 2,2,2-trifluorothioethyl ester and trioctylamine as the substrate and the base, respectively, for producing the desired (*S*)-naproxen.

Keywords: Dynamic kinetic resolution; Lipase; (*R, S*)-Naproxen ester; Hydrolysis

1. Introduction

The resolution of racemic compounds by using enzymes as practical catalysts continues to be a valuable method for obtaining optically pure pharmaceuticals, agricultural and other specialty chemicals [1–3]. However based on the racemic starting substrate, the standard kinetic resolution process has a disadvantage of maximum 50% yield in obtaining the desired enantiomer. To overcome this limitation, dynamic kinetic resolution [4–9] has been proved as a potentially efficient process in which the standard kinetic resolution is coupled with continuous in situ racemization of the starting substrate [10].

2-Arylpropionic acids (e.g. profens), as an important class of non-steroidal anti-inflammatory drugs, have their pharmacological activity mainly on the (*S*)-enantiomer [11]. Considerable efforts are made to obtain (*S*)-profens or their esters by using lipase-catalyzed kinetic resolution [12–17]. Recently, a facile dynamic kinetic resolution process was developed, in which a high yield and optical purity of (*S*)-naproxen, (*S*)-suprofen and (*S*)-fenoprofen from their respective (*R, S*)-thioesters was obtained by using a crude or immobilized lipase from *C. rugosa* and trioctylamine as the catalysts in isooctane [18–21]. However,

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Nomenclature

(B)	trioctylamine concentration (mM)
(E)	effective enzyme concentration (mg/ml)
$e.e._p$	enantiomeric excess for the naproxen product
$e.e._s$	enantiomeric excess for the ester substrate
E^*	enantiomeric ratio, defined as $k_{2A}K_{mB}/k_{2B}K_{mA}$
k_1, k_2	lipase deactivation constants (h^{-1})
k_{2A}, k_{2B}	kinetic constants for (*S*)- and (*R*)-substrate (mM ml/(h mg))
k_h	apparent constant for non-enzymatic hydrolysis (h^{-1})
k_{int}	apparent interconversion constant for racemization (h^{-1})
K_{mA}, K_{mB}	Michaelis–Menten constants for (*S*)- and (*R*)-substrate (mM)
K_p, K_Q	inhibition constants for naproxen and 2,2,2-trifluoroethanol (mM)
(P_A), (P_B)	(*S*)- and (*R*)-naproxen concentrations (mM)
(Q)	2,2,2-trifluoroethanol concentration (mM)
r	parameter in Eq. (3)
(S_A), (S_B)	(*S*)- and (*R*)-ester concentrations (mM)
t	time (h)
V_A, V_B	reaction rates of (*S*)- and (*R*)-ester, respectively (mM/h)
X_A	conversion of (*S*)-ester, defined as $(1-(S_A)/(S_A)_0)$
X_B	conversion of (*R*)-ester, defined as $(1-(S_B)/(S_B)_0)$
X_t	conversion of (*R*, *S*)-ester, defined as $(1-[(S_A)+(S_B)]/[(S_A)_0+(S_B)_0])$

Subscript

0	at the initial condition

drawbacks of relatively bad odor and expensive price of the thiol reagent as well as low lipase activity in comparison with the corresponding oxa-containing analogue were found. Therefore instead of using thioesters as the substrate in the present report, a *C. rugosa* lipase immobilized on polypropylene powders was first employed as the biocatalyst for the kinetic resolution of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester in micro-aqueous isooctane. An immobilized organic base of p-TBD was then added as a racemization catalyst for the remaining (*R*)-ester to perform the dynamic kinetic resolution of the racemate. Moreover, a comparison of the results with those when using (*R*, *S*)-naproxen 2,2,2-trifluoroethyl thioester as the substrate [21] was also made.

2. Material and methods

2.1. Materials

Optically pure (*S*)-naproxen ((*S*)-2-(6-methoxy-2-naphthyl) propionic acid) and phenyl dichlorophosphate were purchased from Sigma (St. Louis, MO). Lipase MY (triacylglycerol ester hydrolases, EC 3.1.1.3) from *C. rugosa* (30 units/mg of solid) was provided by Meito Sangyo (Tokyo, Japan). Polypropylene powders (Accurel MP 1000) were kindly donated from Akzo Nobel (Obernburg, Germany). The particles of size between 0.4 and 0.6 mm were screened and employed as the support for lipase immobilization. Other chemicals of analytical grade were commercially available as follows: 2,2,2-trifluoroethanol from Aldrich (Milwaukee, WI); isooctane, sodium chloride, thionyl chloride from Merck (Darmstadt, Germany); chloroform, 1,2-dimethoxyethane from Tedia (Fairfield, OH); anhydrous pyridine from Riedel-deHaen (Seeize, Germany); 2-nitrotoluene and 1,5,7-triazabicyclo[4,4,0] dec-5-ene bound to polystyrene crosslinked with 2% DVB (p-TBD, 2.6 mmol/g resin and average diameter of 0.167 mm) from Fluka (Buchs, Switzerland). In order to increase the specific surface area, p-TBD was ground and an average diameter of 0.067 mm was measured by microscopy. Racemic naproxen was obtained by racemizing (*S*)-naproxen at 140 °C in ethylene glycol containing NaOH [15].

2.2. Analysis

Hydrolysis of (*R*, *S*)-naproxen ester in micro-aqueous isooctane was monitored by HPLC using a

chiral column from Regis ((*S*, *S*)-WHELK-01; Morton Grove, IL) capable of separating the internal standard of 2-nitrotoluene, (*R*)- and (*S*)-naproxen, (*R*)- and (*S*)-ester with the retention time as 4.5, 12.2, 21.5, 7.2 and 8.9 min, respectively. The mobile phase was a mixture of *n*-hexane/isopropanol/acetic acid (80/20/0.5, v/v/v) at a flow rate of 1.0 ml/min. UV detection at 270 nm was for quantification at the column temperature of 25 °C.

2.3. Synthesis of (S)- and (R, S)-naproxen 2,2,2-trifluoroethyl ester

By following a standard procedure [22], the acid chloride of (*S*)- or (*R*, *S*)-naproxen was prepared by refluxing 20 ml of benzene containing 3.45 g of the acid and 3.20 g of thionyl chloride for 1.5 h. The resultant solution was evaporated to dryness under vacuum, added 30 ml of benzene containing 2.7 g of 2,2,2-trifluoroethanol and 1.19 g of pyridine, and refluxed for 4 h. By cooling down the reaction solution, an aqueous solution (50 ml) containing 6 mM sodium carbonate and deionized water (100 ml) were successively employed four times and two times, respectively, to extract the excessive alcohol and remaining naproxen. The organic layer was separated, dried over magnesium sulfate, filtered and concentrated under vacuum. After purification in silica gel chromatography with the mobile phase of hexane/ethyl acetate (2/1, v/v) and concentrated by vacuum, the desired (*S*)- or (*R*, *S*)-naproxen ester in white powder was obtained.

2.4. Lipase immobilization on Accurel MP 1000

The immobilized lipase was prepared by adsorbing Lipase MY on Accurel MP 1000 with a particle size distribution between 0.4 and 0.6 mm. Briefly, enzyme solution (50 ml) by dissolving 2.2 g of the crude lipase in 55 ml of deionized water was centrifuged at 3000 rpm for 10 min to remove the precipitate. The solution was brought in contact with 2.0 g of the support pre-wetted with 99.5% ethanol, washed with 50% ethanol–water solution and pure deionized water in succession for 1.5 h, respectively. The lipase-support system was mildly shaken at 4 °C for 6 h. The supports were then separated from the solution by filtration, lyophilized for 12 h and stored at 4 °C for use.

2.5. Racemization of (S)-naproxen ester

To 10 ml isooctane was added 1 mM of (*S*)-naproxen ester and p-TBD of various concentrations. The resultant solution was stirred with a magnetic stirrer at 45 °C. Samples were removed and injected onto the above HPLC system at different time intervals for analysis. From the time-course data of the enantiomeric excess $e.e._s$, the apparent interchange constant k_{int} can be estimated.

2.6. Kinetic resolution of (R, S)-naproxen ester

To 10 ml of isooctane was added 50 mg of the immobilized lipase (containing 2.0%, w/w of water based on the enzyme mass) and (*R*, *S*)-naproxen ester of concentration varied from 1 to 10 mM. The resultant solution was stirred with a magnetic stirrer at 45 °C. Samples were removed and injected onto the above HPLC system at different time intervals for analysis. Similar experiments by employing 1 mM of (*R*, *S*)-naproxen ester were carried out except that various concentrations of (*S*)-naproxen or 2,2,2-trifluoroethanol were added. From the time-course conversions of (*R*)- and (*S*)-naproxen ester, the initial rates were estimated and employed for kinetic analysis.

2.7. Non-enzymatic hydrolysis of (R, S)-naproxen ester

To 10 ml of isooctane containing trace of water was added 1 mM of (*R*, *S*)-naproxen ester and 3.846 mg/ml of p-TBD. The resultant solution was stirred with a magnetic stirrer at 45 °C. Samples were removed and injected onto the above HPLC system at different time intervals for analysis. From the time-course variation of the conversions for the racemate, the apparent rate constant for hydrolysis k_h can be estimated and employed for the kinetic analysis.

2.8. Dynamic kinetic resolution of (R, S)-naproxen ester

To 10 ml of isooctane was added 1 mM of (*R*, *S*)-naproxen ester, 3.846 mg/ml of p-TBD and various concentrations of the immobilized lipase. The resultant solution was stirred with a magnetic stirrer

at 45 °C. Samples from the organic phase were removed and injected onto the above HPLC systems at different time intervals for analysis. Then, the time-course conversions for both enantiomers X_A and X_B, the racemate conversion X_t and the enantiomeric excess for the substrate e.e.$_s$ were calculated. After the reaction, p-TBD was separated by filtration and added to isooctane containing acetic acid to strip the adsorbed naproxen. Samples were removed for HPLC analysis to determine the concentrations of (*R*)- and (*S*)-naproxen and hence the enantiomeric excess for the product e.e.$_p$.

3. Model development

3.1. Kinetic resolution of (R, S)-naproxen ester

As the hydrolysis product of 2,2,2-trifluoroethanol is a good leaving moiety, an irreversible Michaelis–Menten mechanism coupled with product inhibition effect can be employed to describe the kinetic behavior of lipase-catalyzed hydrolysis of (*R, S*)-naproxen ester in isooctane [18]. By furthermore assuming that both (*R*)- and (*S*)-naproxen have the same inhibition effect to the enzyme, one may derive the rate equation for each substrate as follows:

$$V_A = -\frac{d(S_A)}{dt} = \frac{(S_A)(E)k_{2A}/K_{mA}}{1 + (S_A)/K_{mA} + (S_B)/K_{mB} + [(P_A) + (P_B)]/K_p + (Q)/K_Q} \quad (1)$$

$$V_B = -\frac{d(S_B)}{dt} = \frac{(S_B)(E)k_{2B}/K_{mB}}{1 + (S_A)/K_{mA} + (S_B)/K_{mB} + [(P_A) + (P_B)]/K_p + (Q)/K_Q} \quad (2)$$

Notations (E), (Q), (P_A), (P_B), (S_A) and (S_B) denote the concentrations of effective enzyme, 2,2,2-trifluoroethanol, (*S*)- and (*R*)-naproxen, (*S*)- and (*R*)-ester, respectively. Moreover, k_{2A}, K_{mA} and k_{2B}, K_{mB} are the rate constants for (*S*)- and (*R*)-ester, respectively, in the Michaelis–Menten mechanism; K_p and K_Q are the product inhibition constants.

From the variations of V_A with (S_A) and V_B with (S_B) at the initial stage without adding the product, the kinetic constants of k_{2A}, k_{2B}, K_{mA} and K_{mB} may be estimated. Similarly, product inhibition constants can be determined from Eq. (1) when (*S*)-naproxen or 2,2,2-trifluoroethanol is added initially. Then by using a fourth-order Runge–Kutta method, the theoretical time-course conversion for each substrate can be solved from Eqs. (1) and (2), in which (P_A), (P_B) and (Q) are eliminated from the material balances $(P_A) = [(S_A)_0 - (S_A)]$, $(P_B) = [(S_B)_0 - (S_B)]$ and $(Q) = [(S_A)_0 + (S_B)_0 - (S_A) - (P_B)]$ with $(S_A)_0$ and $(S_B)_0$ as the initial concentrations of (S_A) and (S_B), respectively.

When lipase deactivation effect for (*S*)-ester (but not for (*R*)-ester) is considered, an irreversible series mechanism is employed to derive the effective lipase concentration (E) in Eq. (1) as follows [23]:

$$(E) = (E)_0 \left\{ \left[1 - \frac{rk_1}{k_1 - k_2} \right] \exp(-k_1 t) + \left[\frac{rk_1}{k_1 - k_2} \right] \exp(-k_2 t) \right\} \quad (3)$$

Notations $(E)_0$, r, k_1 and k_2 represent the initial lipase concentration, fraction of enzyme activity of the intermediate lipase, deactivation constants for the initial and intermediate lipases, respectively. These constants can be estimated from comparing the time-course variations of substrate concentration with the best-fit predictions solved numerically from Eqs. (1)–(3).

3.2. Non-enzymatic hydrolysis of (R, S)-naproxen ester

An irreversible first-order kinetics is employed to describe the non-enzymatic hydrolysis of (*R, S*)-naproxen ester by using p-TBD as the catalyst. Therefore, the apparent rate constant for hydrolysis k_h can be estimated from the time-course data of $[(S_A) + (S_B)]$ coupled with the equation $\ln\{[(S_A) + (S_B)]/[(S_A)_0 + (S_B)_0]\} = -k_h t$.

3.3. Dynamic kinetic resolution of (R, S)-naproxen ester

A reversible first-order kinetics is employed to describe the interconversion of (*S*)- and (*R*)-naproxen esters [18]. The apparent interconversion constant k_{int} is then estimated from the time-course racemization of (*S*)-naproxen ester coupled with the equation $\ln[\text{e.e.}_s/\text{e.e.}_{s0}] = -2k_{int}t$. Therefore, an irreversible Michaelis–Menten kinetics for the lipase, an irreversible first-order non-enzymatic hydrolysis and a reversible first-order racemization kinetics for the base are employed to simulate the dynamic kinetic resolution of (*R*, *S*)-naproxen ester as follows [21]:

$$-\frac{d(S_A)}{dt} = \frac{(S_A)(E)k_{2A}/K_{mA}}{1 + (S_A)/K_{mA} + (S_B)/K_{mB} + [(P_A) + (P_B)]/K_p + (Q)/K_Q} + k_{int}[(S_A) - (S_B)] + k_h(S_A) \quad (4)$$

$$-\frac{d(S_B)}{dt} = \frac{(S_B)(E)k_{2B}/K_{mB}}{1 + (S_A)/K_{mA} + (S_B)/K_{mB} + [(P_A) + (P_B)]/K_p + (Q)/K_Q} + k_{int}[(S_B) - (S_A)] + k_h(S_B) \quad (5)$$

$$-\frac{d(P_A)}{dt} = \frac{(S_A)(E)k_{2A}/K_{mA}}{1 + (S_A)/K_{mA} + (S_B)/K_{mB} + [(P_A) + (P_B)]/K_p + (Q)/K_Q} + k_h(S_A) \quad (6)$$

By furthermore combining the material balances $(Q) = [(P_A)+(P_B)] = [(S_A)_0+(S_B)_0-(S_A)-(S_B)]$ to eliminate (P_B) and (Q) in Eqs. (4)–(6) and considering the lipase deactivation (i.e. Eq. (3)) for Eqs. (4) and (6), the time-course variations of (S_A), (S_B) and (P_A) are solved numerically when employing (*R*, *S*)-naproxen ester as the substrate.

4. Results and discussion

4.1. Kinetic resolution of (R, S)-naproxen ester

Fig. 1 illustrated some time-course conversions of (*R*)- and (*S*)-naproxen ester (i.e. X_B and X_A, respectively) at various substrate concentrations for the kinetic resolution of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester in isooctane at 45 °C. The initial rates varied with the substrate concentration were then determined and demonstrated in Fig. 2. The kinetic constants were estimated from Eqs. (1) and (2) and represented in Table 1, giving a high enantiomeric ratio of $E^* = k_{2A}K_{mB}/k_{2B}K_{mA} = 309$ for the lipase. A high enantiomeric ratio of more than 100 for the crude *C. rugosa* lipase has been reported when (*R*, *S*)-naproxen 2,2,2-trichloroethyl ester was the substrate in potassium phosphate buffer at 22 °C [12].

Similar results as Fig. 1 (not shown here) were obtained when different concentrations of (*S*)-naproxen or 2,2,2-trifluoroethanol were added initially when using 1 mM of (*R*, *S*)-naproxen ester as the substrate. The initial rates of (*S*)-naproxen ester varied with the added product concentration were then determined. From the variations of V_A^{-1} with $(S_A)^{-1}$ at the initial stage (Fig. 3) coupled with Eq. (1), one might furthermore determine the inhibition constants K_p and K_Q for the acid and alcohol products, respectively (Table 1).

By substituting the kinetic constants into Eqs. (1) and (2), the theoretical time-course variations of X_A and X_B at various substrate concentrations were calculated (data not shown), where large deviations with the experimental were found. However when considering the enzyme deactivation effect for (*S*)-ester, good agreements between the experimental data and best-fit results were obtained (Fig. 1). This really implies that a minute change of enzyme conformation in the active site due to lipase deactivation has a profound influence on the affinity of (*S*)- but not (*R*)-naproxen ester to the enzyme.

The kinetic constants previously obtained for the kinetic resolution of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl thioester in isooctane by employing the same immobilized lipase were listed in Table 1 [21]. A comparison of the second-order rate constant revealed that about 2 order of magnitude higher k_{2A}/K_{mA} for (*S*)-naproxen ester than that for (*S*)-naproxen thioester was obtained. A similar result of a higher k_{2B}/K_{mB} for (*R*)-ester compared with (*R*)-thioester was also found in the table. The low value of k_{2A}/K_{mA} for (*S*)-thioester (or k_{2B}/K_{mB} for (*R*)-thioester) corresponds

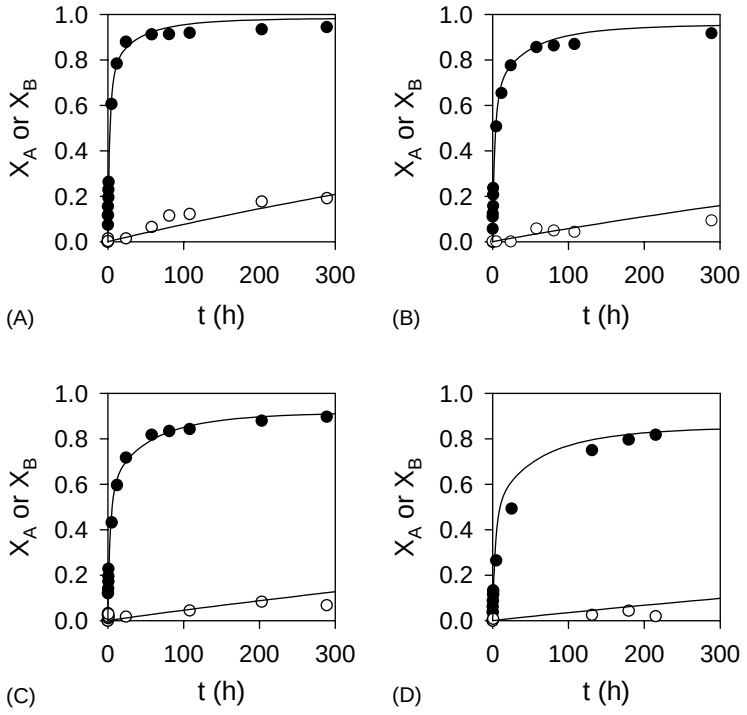

Fig. 1. Effects of initial concentration of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester on time-course variations of X_A (●) and X_B (○) with $(E)_0 = 5$ mg/ml at 45 °C: (A) $(S_A)_0 = (S_B)_0 = 0.5$ mM, (B) $(S_A)_0 = (S_B)_0 = 1$ mM, (C) $(S_A)_0 = (S_B)_0 = 1.5$ mM, (D) $(S_A)_0 = (S_B)_0 = 2.5$ mM. Best-fit results (—).

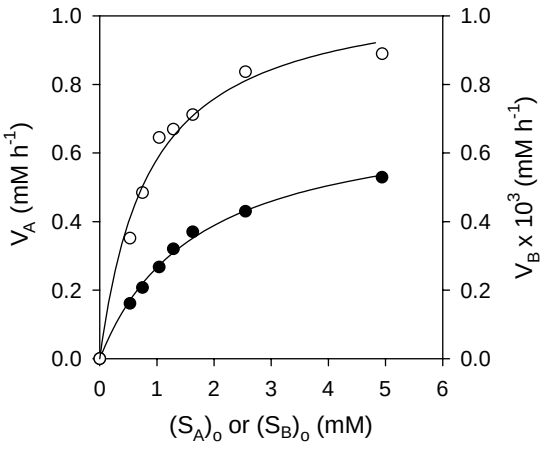

Fig. 2. Variation of V_A (●) with $(S_A)_0$ and V_B (○) with $(S_B)_0$ in the kinetic resolution of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester with $(E)_0 = 5$ mg/ml at 45 °C. Best-fit results (—).

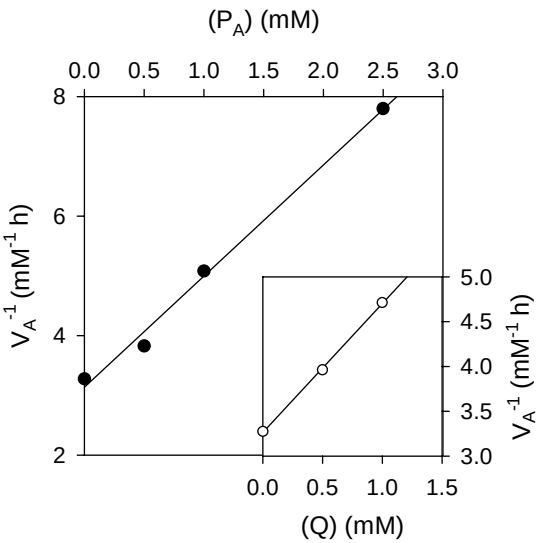

Fig. 3. Variation of V_A^{-1} (●) with (P_A) and V_A^{-1} (○) with (Q) at the initial stage with $(E)_0 = 5$ mg/ml at 45 °C. Best-fit results (—).

Table 1
Kinetic constants obtained from the kinetic resolution and racemization of (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester and (*R*, *S*)-naproxen 2,2,2-trifluoroethyl thioester in isooctane at 45 °C

Substrate	(*R*, *S*)-Ester*	(*R*, *S*)-Thioester**
k_{2A} (mM ml/(h mg))	0.638	9.63×10^{-3}
K_{mA} (mM)	7.56	9.69
k_{2A}/K_{mA} (ml/(h mg))	8.44×10^{-2}	9.94×10^{-4}
k_{2B} (mM ml/(h mg))	5.51×10^{-4}	8.50×10^{-5}
K_{mB} (mM)	2.02	22.3
k_{2B}/K_{mB} (ml/(h mg))	2.73×10^{-4}	3.81×10^{-6}
E^* (i.e. $k_{2A}K_{mB}/k_{2B}\ K_{mA}$)	309	260
K_p (mM)	2.55	Not determined
K_Q (mM)	3.30	Not determined
k_h (h^{-1})	4.10×10^{-3}	$4.06 \times 10^{-6}(B)$
k_{int} (h^{-1})	1.26×10^{-2}	$2.48 \times 10^{-4}(B)$
k_1 (h^{-1})	0.2	Not determined
k_2 (h^{-1})	0.01	Not determined
r	0.1	Not determined

* By adding 3.846 mg/ml (or 10 mM) of p-TBD in racemization experiments.

** With (*B*) as trioctylamine concentration from [21].

to increased activation energy by 11.7 kJ/mol (or 11.2 kJ/mol) at 45 °C in comparison with the corresponding oxa-containing analogue. A similar result of lower thiotransesterification rate by using 4-morpholine ethanol as an acyl acceptor was also found when comparing with the transesterification rate [24]. Yet, different kinetic behaviors were reported for esters and thioesters carrying out non-enzymatic hydrolysis in an alkaline aqueous solution. The effect of "resonance stabilization" and "leaving-group ability" has been employed to predict the reactivity among various carboxylic acid derivatives such as amides, esters, thioesters and acyl chlorides. Therefore, a higher hydrolysis rate for the thioester was expected when considering the lower pK_a of 7.30 for 2,2,2-trifluoroethanethiol than 12.37 for 2,2,2-trifluoroethanol and the weaker resonance effect for the thioester. Indeed, a 1.76-fold increase of the second-order rate constant for the hydrolysis of *p*-nitrothiophenyl acetate compared with that of *p*-nitrophenyl acetate was reported [25].

The formation of two tetrahedron oxyanion intermediates in the acylation and deacylation steps, respectively, for the lipase-catalyzed hydrolysis of esters is a well-accepted kinetic mechanism [26,27]. Obviously, the former was the rate-determining step for the kinetic resolution of (*R*, *S*)-naproxen ester or thioester because an excess of water in comparison with the ester or thioester was employed and a high E^* value obtained. Therefore, a minor change of K_{mA} but not k_{2A} for (*S*)-ester and (*S*)-thioester (Table 1) was attributed to the formation of oxyanion hole in stabilizing the transition state and not in facilitating binding of the substrate. Moreover, a hydrogen bond between the alcoholic moiety of the ester substrate and His449 Nε_2 of the catalytic triad in the acylation step has been proposed to stabilize the transition state [26,27]. It is then very natural to assume that the weak hydrogen bond between the thiolic moiety of thioester substrate and His449 Nε_2 on stabilizing the transition state must play an important role leading to the lower lipase activity for (*S*)-thioester. Similar elucidations are valid for the lower reactivity of (*R*)-thioester compared with (*R*)-ester, although an order of magnitude higher K_{mB} for the former was found. A furthermore study, e.g. using molecular modeling technique to compare the activation energy between the tetrahedron oxyanion intermediate and ground state for each substrate, on confirming of this assumption is still awaiting.

4.2. Racemization of (S)-naproxen ester and non-enzymatic hydrolysis of (R, S)-naproxen ester

Fig. 4(A) illustrates the time-course variations of ln[e.e.$_s$/e.e.$_{s0}$] by using p-TBD concentration as the parameter in isooctane at 45 °C. An induction time of 7.2 h for 0.3846 mg/ml of p-TBD and 10.2 h for other concentrations was estimated by extrapolating the linear part of each curve to the abscissa. This was attributed to the effect of intra-particle diffusion for each enantiomer in the support at the initial stage. The apparent interchange constants varied with the base concentration were estimated from the slope of the linear part for each curve, and represented in Fig. 4(B). An asymptotic value of $1.31 \times 10^{-2}\ h^{-1}$ was obtained when p-TBD concentration was greater than 7.692 mg/ml. This behavior might be attributed to the partitioning of the solute on the support. Therefore, p-TBD concentration of 3.846 mg/ml was employed in the following dynamic kinetic resolution.

A comparison of $k_{int} = 1.26 \times 10^{-2}\ h^{-1}$ (using 3.846 mg/ml of p-TBD) with $k_{2A}/K_{mA} = 8.44 \times 10^{-2}$ ml/(h mg) indicates that unless a very

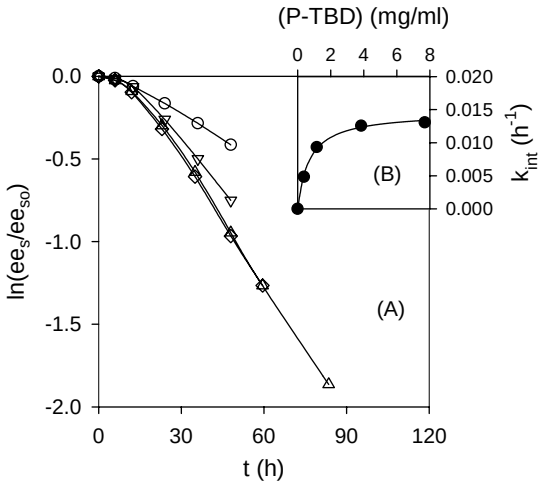

Fig. 4. (A) Time-course variations of $\ln[e.e._s/e.e._{s0}]$ for (*S*)-naproxen ester at 45 °C: For p-TBD concentration of 0.3846 mg/ml (○), 1.154 mg/ml (▽), 3.846 mg/ml (◇) and 7.692 mg/ml (△). (B) Variations of k_{int} with p-TBD concentration.

low lipase concentration, say 0.2 mg/ml, is employed, the racemization rate should be improved in order to develop an effective dynamic kinetic resolution process. The racemization of (*S*)-naproxen ester in isooctane at 45 °C at various trioctylamine concentrations has been carried out (data not shown here), giving $k_{int} = 7.46 \times 10^{-7}(B)$ with (*B*) as trioctylamine concentration. By comparing with $k_{int} = 2.48 \times 10^{-4}(B)$ for (*S*)-naproxen thioester (Table 1), a 332-fold enhancement of the racemization for the (*S*)-thioester was found. This implied that 2,2,2-trifluoroethanethiol moiety must play an important role on increasing the α-proton acidity and hence the formation of α-carbanion during racemization [18]. Therefore, a stronger organic base like p-TBD but not trioctylamine was employed for giving better racemization rate for the remaining (*R*)-naproxen ester.

Similar kinetic behaviors as Fig. 4 (data not shown here) were obtained when performing non-enzymatic hydrolysis of (*R*, *S*)-naproxen ester by adding 3.846 mg/ml of p-TBD in the reaction medium. Thus, an apparent rate constant $k_h = 4.10 \times 10^{-3}\,h^{-1}$ was estimated from the slope of the linear part of the time-course curve. After comparing this value with k_{2A}/K_{mA}, the undesired non-enzymatic hydrolysis in obtaining a high $e.e._p$ value was not negligible when a low lipase concentration, say 0.1 mg/ml, was employed.

4.3. Dynamic kinetic resolution of (*R, S*)-naproxen ester

Experiments of using the immobilized lipase ranging from 1 to 3 mg/ml for the dynamic kinetic resolution of 1 mM of (*R, S*)-naproxen ester were carried out. The time-course variations of the racemate conversion X_t, $e.e._p$ and $e.e._s$ were illustrated in Fig. 5. In comparison with Fig. 1 for a standard kinetic resolution, the addition of p-TBD has a profound effect on improving the $e.e._p$ value when X_t is greater than 50%. In general, increasing the enzyme concentration results in an enhancement of X_t but decrease of the optical purity of (*S*)-naproxen. The limitation of maximum 50% yield for the desired product in a kinetic resolution process is overcome in the dynamic kinetic resolution. For example in Fig. 5(A), one obtained 75.5% yield of (*S*)-naproxen from $X_t = 95.5\%$ and $e.e. = 58.1\%$ at 600 h.

Large deviations between the experimental and theoretical predictions were found if the kinetic constants listed in Table 1 were employed to solve Eqs. (3)–(6). Inspections of the time-course variation of $e.e._s$ in Fig. 5(A) and (C) revealed that the racemization of (*R*)-naproxen ester at any specific time decreased by increasing the lipase concentration. This implied that the racemization capacity of the base decreased when adding more lipases. Indeed, the more was the lipase concentration, the higher was the water in the reaction medium as a certain amount of water initially adsorbed on the lipase. This might enhance the water content dissolving in the solution, leading to more ion-pairs formation with p-TBD, which certainly decreased the base concentration available for performing racemization and non-enzymatic hydrolysis. Similar behaviors of decreasing racemization rates were found if (*S*)-naproxen or 2,2,2-trifluoroethanol was initially added to carry out racemization experiments (data not shown here). On the other hand, increasing the water content might also enhance the non-enzymatic hydrolysis for a fixed p-TBD concentration. Therefore, more studies on assessing the cross-interference among p-TBD, water, acid and alcohol products are needed in order to successfully simulate the present process. Yet, a simple and practical way to consider

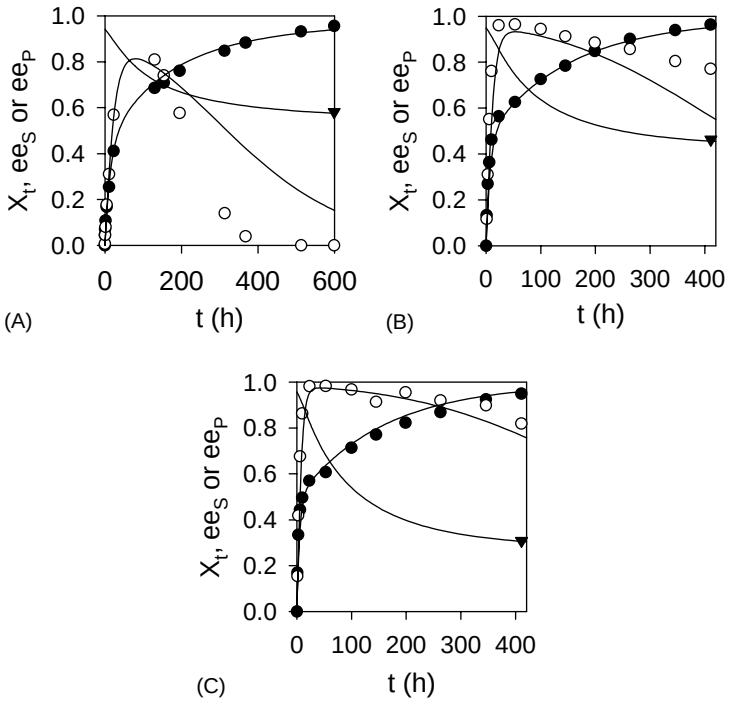

Fig. 5. Effects of enzyme concentration on the time-course variations of X_t (●), e.e.$_p$ (▼) and e.e.$_s$ (○) with p-TBD concentration of 3.846 mg/ml at 45 °C: (A) $(E)_0 = 1$ mg/ml, (B) $(E)_0 = 2$ mg/ml, (C) $(E)_0 = 3$ mg/ml. Best-fit results (—).

this cross-interference is to modify the kinetic constants k_{int} and k_h. By employing 30, 25 and 15% of k_{int} as well as 45, 75 and 95% of k_h for adding 1, 2 and 3 mg/ml of the lipase, respectively, in solving Eqs. (3)–(6), good agreements between the experimental data and best-fit results were illustrated in Fig. 5.

5. Conclusions

A dynamic kinetic resolution process was developed for the (*S*)-naproxen production from (*R*, *S*)-naproxen 2,2,2-trifluoroethyl ester by using lipase-catalyzed hydrolysis with continuous in situ racemization of the remaining (*R*)-ester by p-TBD in isooctane. An irreversible Michaelis–Menten mechanism coupled with the product inhibition and lipase stability for (*S*)-ester could be employed to describe the enantioselective hydrolysis of the racemate. Comparisons of the kinetic constants between (*R*, *S*)-ester and their corresponding sulfur-containing analogue indicated that the lipase has higher enantioselectivity and activity for the ester substrate. An asymptotic value for the apparent interconversion constant was obtained when increasing the p-TBD concentration. The limitation of maximum 50% yield for the desired product in a standard kinetic resolution was overcome in the present resolution process, e.g. 75.5% yield of (*S*)-naproxen obtained, yet, with a disappointing e.e.$_p$ of 58.1%.

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