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Enantioselective hydrolysis of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated solvents via lipases from *Carica pentagona* Heilborn and *Carica papaya*

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Abstract

A crude lipase prepared from *Carica pentagona* Heilborn latex was explored as an effective enantioselective biocatalyst for the hydrolytic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated organic solvents. Comparisons of the enzyme performance with that from *Carica papaya* lipase indicated that both lipases showed low tolerance to the hydrophilic solvent and were inhibited by (*S*)-naproxen and 2,2,2-trifluoroethanol. Improvements on the enzyme activity and enantioselectivty were demonstrated when both lipases in partially purified forms were employed. By using the thermodynamic analysis, the enantiomeric discrimination was mainly driven by the difference of activation enthalpy for all reaction systems except for employing *Carica papaya* lipase as the biocatalyst for (*R*,*S*)-fenoprofen 2,2,2-trifluoroethyl thioester.

Keywords: Lipases; *Carica papaya*; *Carica pentagona* Heilborn; (*S*)-Naproxen; Hydrolytic resolution

1. Introduction

Lipases are widely used as versatile biocatalysts for the regioselective and enantioselective biotransformation with or without the presence of organic solvents [1,2]. Although plants represent an abundant source of industrial enzymes, lipases are mainly produced from animals or microorganisms. This is attributed to the low lipase content in the post-germination seeds, bran part of grains or wheat germs [3,4]. Recently, this drawback was overcome as lipases, having high levels activity and employed as promising biocatalysts for lipids bioconversion, were available in large quantities from plant latex [4–6].

Lipases aggregated in the non-water-soluble matrix of plant latex, e.g. *Caricaceae*, *Asclepia* or *Euphorbiaceae* latex, are regarded as naturally immobilized enzymes. The sun-, oven- or spray-dried *Carica papaya* latex, with the commercial name of papain, is well known for containing many enzymes like proteases, lysozyme, chitinase and lipase [7]. Recently, a crude *Carica papaya* lipase (CPL) possessing highly enantioselectivity for the hydrolytic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl thioester or ester for producing the desired (*S*)-naproxen was reported [8,9]. Therefore, the added value of crude papain might increase as it may be easily separated and sold in two portions: the water-soluble constitute (mainly containing proteases) and the sedimentable particles (mainly containing lipase). As illustrated in Scheme 1 for the hydrolytic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated organic solvents, an improvement of the enzyme performance is first demonstrated by using a partially purified *Carica papaya* lipase (pCPL), i.e. the sedimentable particles, as the biocatalyst.

Babaco (*Carica pentagona* Heilborn), also known as highland papaya, is a subtropical plant originating in the Ecuado-

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Nomenclature

E_t	enzyme concentration based on total weight (mg/ml)
E	enantiomeric ratio, defined as ratio of initial rates V_S/V_R
$\Delta\Delta G$	difference in activation free energy for the transient states of (*R*)- and (*S*)-substrate (kJ/mol)
$\Delta\Delta H$	difference in activation enthalpy for the transient states of (*R*)- and (*S*)-substrate (kJ/mol)
k_{2R}, k_{2S}	kinetic constant for (*R*)- and (*S*)-ester, respectively (mmol/h g)
K_{mR}, K_{mS}	Michaelis–Menten constants for (*R*)- and (*S*)-ester, respectively (mM)
K_{PS}, K_Q	inhibition constants for (*S*)-naproxen and 2,2,2-trifluoroethanol, respectively (mM)
P_S	(*S*)-naproxen concentration (mM)
R	gas constant (J/mol K)
S_R, S_S	(*R*)- and (*S*)-ester concentrations, respectively (mM)
$\Delta\Delta S$	difference in activation entropy for the transient states of (*R*)- and (*S*)-substrate (J/mol K)
T	absolute temperature (K)
V_R, V_S	reaction rates of (*R*)- and (*S*)-ester, respectively (mM/h)
X_R, X_S	conversions of (*R*)- and (*S*)-ester, respectively

rian Andean mountains. It is a naturally occurring hybrid of *Carica stipulate* Badillo and *Carica pubescens* Lenne and Koch [10]. In order to promote and develop babaco culture, the proteolytic, lipolytic and interesterification activity in *Carica pentagona* Heilborn latex has been reported [5]. As a continuation of promoting babaco culture, the hydrolytic resolution of Scheme 1 was employed as a model system for exploring crude *Carica pentagona* Heilborn lipase (CPHL) and its partially purified preparation (pCPHL) as enantioselective biocatalysts. Moreover, the performance for all lipase preparations was compared and lead to the conclusion that pCPL is the best lipase.

2. Materials and methods

2.1. Materials

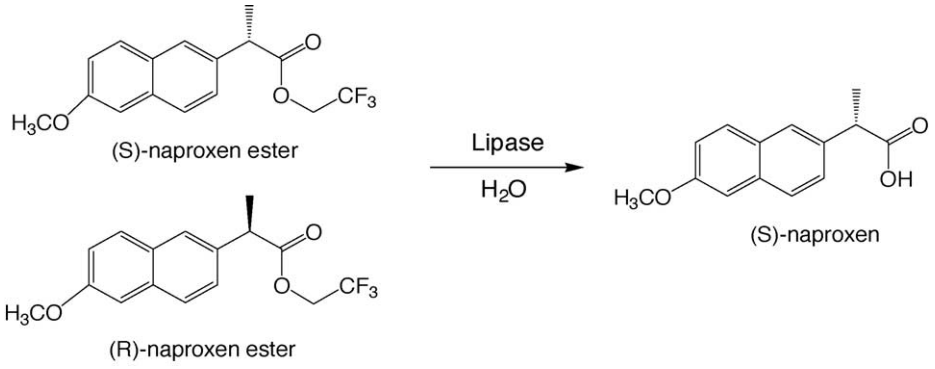
Optically pure (*S*)-naproxen ((*S*)-2-(6-methoxy-2-naphthyl) propionic acid) and crude papain (P-3375, 2.1 units/mg solid, product from Sri Lanka) from *Carica papaya* latex were purchased from Sigma (St. Louis, MO). Fresh *Carica pentagona* Heilborn latex was collected and lyophilized in Quito, Ecuador, as previously described [5]. Other chemicals of analytical grade were commercially available, e.g. 2,2,2-trifluoroethanol from Aldrich (Milwaukee, WI), isooctane, cyclohexane, isopropanol and acetic acid glacial from Tedia (Fairfield, OH); 2-nitrotoluene and *tert*-butyl methyl ether from Fluka (Buchs, Switzerland). Racemic naproxen was obtained by racemizing (*S*)-naproxen at 140 °C in ethylene glycol containing NaOH as previously demonstrated [8].

2.2. Analysis

Hydrolysis of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated organic solvents 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*)-naproxen ester with the retention time of 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 glacial (80/20/0.5, v/v/v) at a flow rate of 1.0 ml/min. UV detection at 270 nm was employed for quantification at the column temperature of 25 °C.

2.3. Preparation of partially purified lipases

To 5 g of the crude papain or dried *Carica pentagona* Heilborn latex was added 25 ml deionized water at 4 °C with gentle stirring for 30 min. The resultant solution was centrifuged at 12,000 rpm for 10 min and the supernatant discarded. The above procedures were repeated once more. The remaining precipitate was then collected and lyophilized for 7 h, giving 15 and 27% recovery (based on total weight) of pCPL and pCPHL, respectively.



Scheme 1.

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

The acid chloride of (*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 resolution was evaporated to dryness under vacuum, 30 ml of benzene containing 2.7 g of 2,2,2-trifluoroethylanol and 1.19 g of pyridine added, and refluxed for 4 h. By cooling down the reaction solution, 50 ml aqueous solution containing 3 mM sodium carbonate and 100 ml deionized water were successively employed four and two times, respectively, to extract the excessive alcohol and remaining (*R*,*S*)-naproxen. The organic layer was separated, dried over anhydrous 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 (*R*,*S*)-naproxen ester in white powder was obtained. The desired racemic naproxen ester was confirmed from the retention time in HPLC. ^{1}H NMR spectra were also recorded at 200 MHz on a Bruker AC-200 spectrometer in deuteriochloroform solutions with tetramethylsilane as an internal standard. Chemical shifts in ppm were reported as: δ 1.58 (3H, d), 3.92 (3H, s), 3.94–4.00 (1H, q), 4.36–4.57 (2H, m), 7.12–7.26 (2H, q), 7.38–7.41 (1H, q), 7.70–7.73 (3H, m), where the abbreviations d, m, q and s were the peak multiplicities of doublet, multiplet, quartet and single, respectively.

2.5. Temperature and solvent effects on kinetic resolution

Unless specified, to 15 ml of water-saturated isooctane containing 3 mM (*R*,*S*)-naproxen ester was added 5 mg/ml of crude papain (referred as CPL) or 0.75 mg/ml pCPL, lyophilized *Carica pentagona* Heilborn latex (referred as CPHL) or pCPHL. The resultant solution was stirred with a magnetic stirrer at 45 °C. Samples were removed and injected onto the HPLC system at different time intervals for analysis, from which the time-course conversion, initial rate for each substrate and hence enantiomeric ratio (i.e. *E* value defined as the ratio of initial rates for both substrates) were determined. Similar experiments were performed when temperature was varied from 35 to 70 °C. More experiments were carried out except that water-saturated cyclohexane or *tert*-butyl methyl ether containing 30% saturated water was employed as the solvent.

In order to study the kinetic behaviors, similar experiments were performed in water-saturated isooctane at 45 °C where the concentration of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester was varied in the range of 1–8 mM. More experiments were carried out for 3 mM (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester, in which (*S*)-naproxen concentration varied from 0.2 to 0.9 mM or 2,2,2-trifluoroethanol concentration varied from 1 to 4.5 mM was initially added for investigating the product inhibition.

2.6. Kinetic and thermodynamic analysis

By considering 2,2,2-trifluoroethanol as a good leaving group and an excess of water employed, an irreversible Michaelis–Menten kinetics coupled with the product inhibition leading to the rate equation for (*S*)-naproxen ester, i.e. $V_S = k_{2S}(S_S)(E_t)/[K_{mS}(1 + (P_S)/K_{PS} + (Q)/K_Q + (S_S)]$, was applied for the kinetic analysis. Symbols (E_t), (Q), (P_{AS}) and (S_S) denoted concentrations of enzyme, 2,2,2-trifluoroethanol, (*S*)-naproxen and (*S*)-naproxen ester, respectively. Moreover, k_{2S} and K_{mS} were the kinetic constants; K_{PS} and K_Q are the inhibition constants for (*S*)-naproxen and 2,2,2-trifluoroethanol, respectively. Similar rate equation with the subscript "*S*" replaced by "*R*" for (*R*)-naproxen ester was employed for the kinetic analysis.

The transition state theory relates the microscopic rate constant of an elementary step to the Gibbs free energy difference between reactant's ground state and the activated transition state. By assuming Michaelis–Menten kinetics for each substrate in the acylation step, an equation relating the *E* value to the difference of Gibbs free energies $\Delta\Delta G$ at the transition states for both enantiomers, i.e. $RT\ln(E) = -\Delta\Delta G = -\Delta\Delta H + T\Delta\Delta S$, has been derived [11]. From the variation of ln(*E*) with the inverse of absolute temperature, the difference of activation enthalpy $\Delta\Delta H$ and that of activation entropy $\Delta\Delta S$ can then be estimated.

3. Results and discussion

3.1. Effects of temperature and solvents

Typical time-course conversions of (*R*)- and (*S*)-naproxen 2,2,2-trifluoroethyl ester for various lipase preparations in water-saturated isooctane at 45 °C were illustrated in Fig. 1. From the figure and others at different temperature (not shown there), the initial rates V_R and V_S and hence *E* value (defined

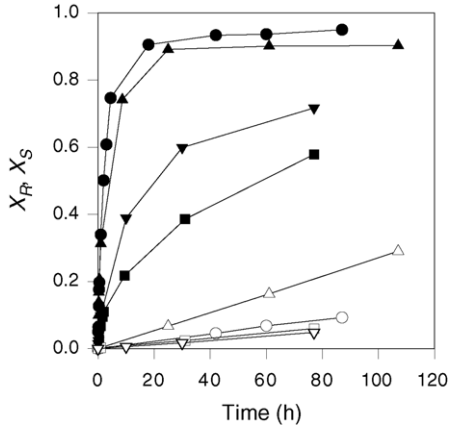


Fig. 1. Time-course conversions X_R (empty) and X_S (filled) for CPL (◯, ●), pCPL (△, ▲), CPHL (□, ■) and pCPHL (▽, ▼). Conditions: 5 mg/ml for CPL and 0.75 mg/ml for pCPL, CPHL and pCPHL, 1 mM (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated isooctane at 45 °C.

Table 1
Effects of temperature on hydrolytic resolution of 3 mM (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated isooctane

	Temperature							
	25 (°C)	35 (°C)	45 (°C)	50 (°C)	55 (°C)	60 (°C)	70 (°C)	80 (°C)
CPL [8]								
$V_S \times 10^1$ (mM/h)		2.34	3.26	4.07		4.74	2.91	
$V_R \times 10^4$ (mM/h)		3.00	9.00	21.0		39.0	91.5	
E		780	363	194		122	32	
pCPL								
$V_S \times 10^1$ (mM/h)		4.25	5.10			6.53	6.78	6.32
$V_R \times 10^4$ (mM/h)		9.00	21.0			51.0	82.5	94.5
E		472	243			128	82	67
CPHL								
$V_S \times 10^2$ (mM/h)	6.45	6.90	8.25		7.35	5.55		
$V_R \times 10^4$ (mM/h)	4.50	6.00	9.00		10.5	13.5		
E	143	115	92		70	41		
pCPHL								
$V_S \times 10^2$ (mM/h)		9.75	14.2		5.25			
$V_R \times 10^4$ (mM/h)		3.00	6.00		9.00			
E		325	238		58			

Conditions: 5 mg/ml for CPL; 0.75 mg/ml for pCPL, CPHL and pCPHL.

as the ratio of initial rates V_S/V_R) were estimated and represented in Table 1. A change of the temperature from 35 to 60 °C results in an enhancement of V_S and V_R, but decrease of E value. Yet, $E = 122$ at 60 °C indicates that CPL is still very enantioselective in water-saturated isooctane. A great reduction of E value to 32 at 70 °C was found, which was attributed to the enzyme deactivation on decreasing V_S, but not V_R [8,9].

Very similar enzyme enantioselectivty for pCPL and CPL was demonstrated in Table 1. The slight reduction of initial rate V_S, but not V_R, was shown at 80 °C, indicating that the enzyme conformation did not change much such that 93% of initial V_S at 70 °C still remained. A good linear relationship between $\ln(V_R)$ and the inverse of absolute temperature was found in the range of 35–80 °C, implying that the enzyme conformation change on deactivating the lipase was negligible for the unfavorable (*R*)-ester. In comparison with CPL, a 10-fold enhancement of the enzyme specific activity $V_S/(E_t)$ at 45 °C (or 15-fold at 70 °C) for PCPL was estimated from Table 1, implying that pCPL was activated when considering the enzyme purification factor (i.e. 15% recovery from CPL). At temperature greater than 60 °C, the water-soluble proteins stored in the crude papain might deactivate and impede the substrates affinity to the enzyme active site [8]. The purification procedure can remove the contaminant proteins and enhances the lipase specific activity and thermal stability.

Good enantioselectivity of CPHL in water-saturated isooctane, e.g. $E = 92$ at 45 °C, was also demonstrated in Table 1. In general, CPHL has higher specific activity $V_S/(E_t)$ at temperature less than 45 °C when comparing with CPL. Similar behaviors of higher lipolytic and interesterification activity for CPHL at 50 °C have been reported [5]. However, it began to deactivate at 55 °C, leading to the lower $V_S/(E_t)$. Improvements of the enzyme enantioselectivity were shown at temperature less than 45 °C when pCPHL was employed. Moreover, a 1.4-fold enhancement of $V_S/(E_t)$ at 35 °C (or 1.7-fold at 45 °C) for pCPHL was estimated. Unlike pCPL, deterioration of pCPHL activity for (*R*)- and (*S*)-naproxen ester occurred if the enzyme purification factor (i.e. 27% recovery from CPHL) was considered. More deterioration of pCPHL activity and enantioselectivity was shown at 55 °C. Based on the lipase performance for all enzyme preparations (Table 1), the best lipase of pCPL was concluded for obtaining the desired (*S*)-naproxen.

The effect of solvent type on the enzyme performance for various lipase preparations at 45 °C was demonstrated in Table 2. In water-saturated isooctane, improvements of enzyme activity or enantioselectivty for both partially purified lipases were demonstrated. However, 3.2-fold increase of V_R, 2.5-fold decrease of V_S and hence 7.7-fold reduction

Table 2
Effects of solvent type on hydrolytic resolution of 3 mM (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester at 45 °C

	CPL [8]	pCPL	CPHL	pCPHL
Isooctane				
$V_S/(E_t) \times 10^1$ (mmol/h g)	0.653	6.80	1.09	1.90
$V_R/(E_t) \times 10^3$ (mmol/h g)	0.180	2.80	1.20	0.80
E	363	243	91	237
Cyclohexane				
$V_S/(E_t) \times 10^1$ (mmol/h g)	0.266	1.84	0.204	0.166
$V_R/(E_t) \times 10^3$ (mmol/h g)	0.570	2.40	1.20	0.600
E	47	77	18	28
MTBE[a]				
$V_S/(E_t) \times 10^3$ (mmol/h g)	1.20	9.80	0	0
$V_R/(E_t) \times 10^4$ (mmol/h g)	0.632	4.00	0	0
E	19	25		

Conditions: 5 mg/ml for CPL; 0.75 mg/ml for pCPL, CPHL and pCPHL.
[a] Containing 30% of saturated water in MTBE (*tert*-butyl methyl ether).

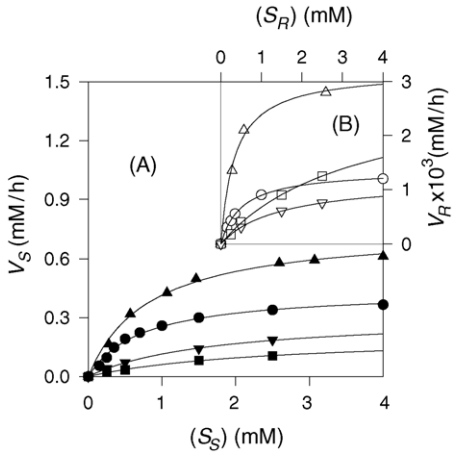


Fig. 2. (A) Variations of V_S with (S_S); (B) variations of V_R with (S_R) in water-saturated isooctane at 45 °C for CPL (◯, ●), pCPL (Δ, ▲), CPHL (□, ■) and pCPHL (▽, ▼). (—) Best-fitted results.

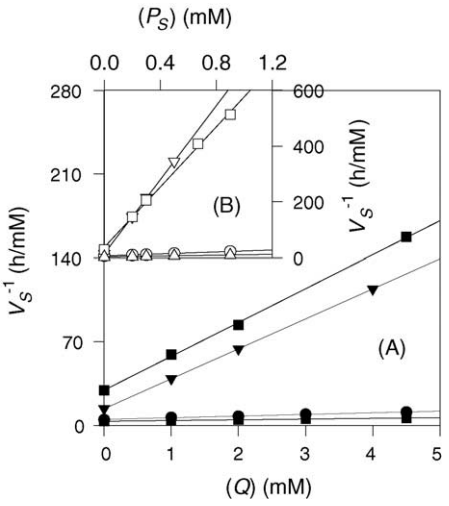


Fig. 3. (A) Variations of $V_S{}^{-1}$ with (Q); (B) variations of $V_S{}^{-1}$ with (P_S) in water-saturated isooctane at 45 °C for CPL (◯, ●), pCPL (Δ, ▲), CPHL (□, ■) and pCPHL (▽, ▼). (—) Best-fitted results.

of E value for CPL in cyclohexane were obtained. Similar behaviors for pCPL were found, implying *Carica papaya* lipase has low tolerance to the hydrophilic solvent. More deterioration of lipase specific activity and enantioselectivty for CPHL or pCPHL was given in Table 2, implying that *Carica pentagona* Heilborn lipase has much lower tolerance to cyclohexane. Very low specific enzyme activity and enantioselectivity for CPL and pCPL, and even complete loss of enzyme activity for CPHL and pCPHL, were obtained when *tert*-butyl methyl ether containing 30% saturated water was employed as the reaction medium.

3.2. Kinetic analysis

Fig. 2 illustrated the variation of initial rate V_R or V_S with substrate concentration in water-saturated isooctane at 45 °C for all enzyme preparations, with which the kinetic constants were estimated and represented in Table 3. The enantioselective discrimination for all lipases was mainly due to the difference of proton transfer among the catalytic triad and leaving alcohol (i.e. k_{2R} and k_{2S}) but not that of substrate affinity (i.e. K_{mR} and K_{mS}) for both enantiomers. A detailed analysis of k_{2R} and k_{2S} for the crude and partially purified lipases indicated that CPL but not CPHL was activated after the purification, when considering the enzyme purification factor. Moreover, both (*R*)- and (*S*)-naproxen esters have higher affinity to *Carica papaya* lipase, when comparing K_{mR} and K_{mS} of CPL (or pCPL) with those of CPHL (or pCPHL).

Table 3
Comparison of kinetic and inhibition constants for hydrolytic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated isooctane at 45 °C

	CPL [8]	pCPL	CPHL	pCPHL
$k_{2S} \times 10^4$ (mmol/h mg)	0.838	9.81	2.36	3.79
K_{mS} (mM)	0.602	0.820	1.92	1.46
k_{2S}/K_{mS} (mL/h mg)	0.139	1.20	0.122	0.260
$k_{2R} \times 10^6$ (mmol/h mg)	0.299	5.01	3.91	1.60
K_{mR} (mM)	0.628	0.876	3.37	1.50
$k_{2R}/K_{mR} \times 10^3$ (mL/h mg)	0.476	5.72	1.16	1.07
$K_{PS} \times 10^1$ (mM)	1.68	3.25	0.403	0.155
K_Q (mM)	2.06	3.66	0.768	0.411

Conditions: 5 mg/ml for CPL; 0.75 mg/ml for pCPL, CPHL and pCPHL.

The inhibition constants (Table 3) were furthermore estimated from the inverse of initial rate $V_S{}^{-1}$ varied with the product concentration (P_S) or (Q) in water-saturated isooctane at 45 °C (Fig. 3(A) and (B)). A comparison of K_{PS} and K_Q for *Carica papaya* or *Carica pentagon* Heilborn lipase indicated that (*S*)-naproxen acted as a better enzyme inhibitor than 2,2,2-trifluoroethanol. A furthermore comparison of K_{PS} (or K_Q) for *Carica papaya* and *Carica pentagon* Heilborn lipases lead to the conclusion that both acid and alcohol products have higher affinity to *Carica pentagon* Heilborn lipase. The plateaus of X_S around 0.9 for CPL or pCPL in Fig. 1 was attributed to the product inhibition, implying that in order to increase the (*S*)-naproxen productivity, an in situ separation process such as extraction or adsorption should be combined with the reaction system [16].

3.3. Thermodynamic analysis

The thermodynamic analysis has been proposed to investigate effects of solvent type, acyl donor and acceptor, lipase type and mutant on the temperature dependence of E value in lipase-catalyzed kinetic resolutions [17–21]. The difference in activation free energy $\Delta\Delta G$ for the transient states of fast-reacting enantiomer and the slow-reacting enantiomer can be separated into the differences in activation enthalpy ($\Delta\Delta H$) and activation entropy ($\Delta\Delta S$). Therefore, a clear elucidation on whether the enantiomer discrimination to be either enthalpy-driven or entropy-driven or both equally important is reached.

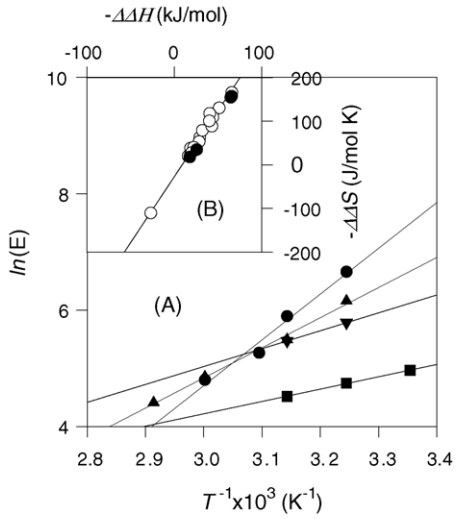


Fig. 4. (A) Variations of ln(E) with the inverse of temperature for CPL (●), pCPL (▲), CPHL (■) and pCPHL (▼); (B) variations of $-\Delta\Delta H$ and $-\Delta\Delta S$ for the present reaction systems (●) and others (○) referred from footnotes of Table 4. (—) Best-fitted results for all data.

By using the data of Table 1, good liner relationships between ln(E) and the inverse of absolute temperature in water-saturated isooctane were illustrated in Fig. 4(A) for all lipases. It stressed that only the data that the lipase was not denatured were employed for estimating $\Delta\Delta H$ and $\Delta\Delta S$. The results were represented in Table 4 and Fig. 4(B) with which a very good linear relationship of $\Delta\Delta S = 37.55 + 2.972\,\Delta\Delta H$ ($r^2 = 0.999$) was obtained. This was attributed to the lipases originating from the same species of *Caricaceae*. When other data represented in Table 4 were furthermore plotted in Fig. 4(B), a good linear relationship of $\Delta\Delta S = 26.87 + 2.951\,\Delta\Delta H$ ($r^2 = 0.979$) was obtained no matter what combination of lipases from various sources, solvents, hydrolysis for (*R*,*S*)-profen 2,2,2-trifluoroethyl ester and thioester or esterification for (*R*,*S*)-naproxen and 2-(4-chloro-phenoxy)propionic acid was made. The good enthalpy–entropy compensation phenomena might be attributed to the narrow range of parameters investigated, i.e. substrates with similar acyl moiety structure, nonpolar solvents and all lipases having similar E values. After comparing the data for each case in Table 4, we concluded that both $-\Delta\Delta H$ and $-\Delta\Delta S$ were important for the enantiomer

Table 4
Comparisons of $-\Delta\Delta H$, $-\Delta\Delta S$ and $-T\,\Delta\Delta S$ (at 45 °C) in various reaction systems

Compounds	$-\Delta\Delta H$ (kJ/mol)	$-\Delta\Delta S$ (J/mol L)	$-T\,\Delta\Delta S$ (kJ/mol)	Driven by
CPL				
Naproxen ester [8]	65.4	157.2	50.0	$\Delta\Delta H$
Naproxen thioester [9]	64.6	154.6	49.2	$\Delta\Delta H$
pCPL				
Naproxen thioester	41.8	89.1	28.3	$\Delta\Delta H$
Suprofen thioester	44.1	109.2	34.7	$\Delta\Delta H$
Ibuprofen thioester	32.0	78.9	25.1	$\Delta\Delta H$
2-Phenyl propionic thioester	40.4	117.1	37.2	$\Delta\Delta H$
Ketoprofen thioester	28.9	62.5	19.9	$\Delta\Delta H$
Fenoprofen thioester	−27.0	−110.4	−35.1	$\Delta\Delta S$
Flurbiprofen thioester	15.8	20.2	6.4	$\Delta\Delta H$
Naproxen thioester[a]	51.3	130.4	41.5	$\Delta\Delta H$
Naproxen ester[b]	42.9	88.6	28.2	$\Delta\Delta H$
Fenoprofen ester	18.5	38.1	12.1	$\Delta\Delta H$
Naproxen ester[c]	20.4	31.8	10.1	$\Delta\Delta H$
2-(4-Chloro-phenoxy) propionic acid [12]	19.8	28.0	8.9	$\Delta\Delta H$
CPHL				
Naproxen ester[b]	17.6	17.8	5.7	$\Delta\Delta H$
pCPHL				
Naproxen ester[b]	25.6	34.9	11.1	$\Delta\Delta H$
CRL[c]				
Naproxen thioester	65.7	165.8	52.7	$\Delta\Delta H$
Naproxen ester	18.7	25.3	8.0	$\Delta\Delta H$
Naproxen ester[a]	40.0	100.5	32.0	$\Delta\Delta H$
Naproxen [13]	28.3	53.3	16.9	$\Delta\Delta H$
Ibuprofen [14]	22.2	40.1	12.8	$\Delta\Delta H$

Others without the superscript from [15].
[a] In water-saturated cyclohexane.
[b] Present report.
[c] CRL as *Candida rugosa* lipase.

discrimination. Yet, the former was dominating for all reaction systems except for employing *Carica papaya* lipase as the biocatalyst for (*R*,*S*)-fenoprofen 2,2,2-trifluoroethyl thioester.

4. Conclusions

With the hydrolytic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester in water-saturated solvents as the model system, improvements of the lipase activity (10- and 15-fold enhancements at 45 and 70 °C, respectively) and thermal stability were obtained when employing a partially purified *Carica papaya* lipase as the biocatalyst. The model system was extended for the exploration of a crude *Carica pentagona* Heilborn lipase as an effective enantioselective biocatalyst, e.g. $E = 92$ at 45 °C. Kinetic analysis demonstrated that all lipase preparations showed low tolerance to the hydrophilic solvent and were inhibited by (*S*)-naproxen and 2,2,2-trifluoroethanol. The thermodynamic analysis furthermore indicated that the enantiomer discrimination was driven by both the difference of activation enthalpy and that of activation entropy. Yet, the former was dominating for all reaction systems except for employing *Carica papaya* lipase as the biocatalyst for (*R*,*S*)-fenoprofen 2,2,2-trifluoroethyl thioester. The partially purified *Carica papaya* lipase was selected as the best lipase preparation after comparing the enzyme thermal stability, activity and enantioselectivity.

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