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Solvent effects on the enantioselectivity of the thermophilic lipase QLM in the resolution of (*R*, *S*)-2-octanol and (*R*, *S*)-2-pentanol

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Abstract

The thermophilic lipase QLM-catalyzed resolution of (*R*, *S*)-2-octanol and (*R*, *S*)-2-pentanol *via* transesterification was carried out in various organic solvents, and the solvent effects on the enzyme's enantioselectivity were investigated. A significant negative correlation between the enantiomeric ratio, *E*, and the size of the solvent molecules was observed. The highest *E* value, 21, was obtained in the small molecular-sized solvent dichloromethane when (*R*, *S*)-2-octanol was resolved with vinyl acetate as acyl donor. Thermodynamic analysis indicated that the difference in activation free energy between the two enantiomers was about 25.5% lower in dichloromethane than in the solvent-free system, and the change in the difference in activation entropy between the enantiomers was the main contributor to the changes in *E* values with the molecular size of solvents.

Keywords: Thermophilic lipase; Resolution; *sec*-Alcohol; Enantiomeric ratio; Thermodynamic analysis

1. Introduction

Lipases (EC 3.1.1.3) are interfacially activated triacylglycerol hydrolases that can function in both aqueous and organic media, and exhibit excellent activity and stereoselectivity towards a wide range of substrates [1,2]. Resolution of racemates with lipases is one of the most efficient biocatalytic strategies for producing enantiomerically pure fine chemicals [3]. Generally, the enantioselectivity of lipases can be modulated by genetic engineering, chemical modification and immobilization of the enzymes [4–6]. In addition, medium engineering has proven to be a very appealing and efficient technique for improving the enzyme enantioselectivity towards various target reactions [3,7–10], and there have been several investigations of correlations between enzymes' enantioselectivity and solvent parameters, such as log *P*, dielectric constant and molecular size [9–11]. However, there is still no general understanding of the solvent effects, due to the diversity of substrates and enzymes employed [12,13].

Thermophilic enzymes are more thermally active and stable in organic solvents than other enzymes, and have wide potential applications in industrial processes [14]. Lipase QLM is an extracellular enzyme from *Alcaligenes* sp. with an optimal temperature of 65–70 °C and molecular weight of 31 kDa [15]. It shows a wide specificity and high activity towards a variety of esters, and is applicable to enantioselective hydrolysis or esterification of racemic compounds [15–18]. However, to the best of our knowledge, there have been no previously published studies of solvent effects on the thermophilic lipase-catalyzed resolution of *sec*-alcohols.

In this study, solvent effects on the enantioselectivity of lipase QLM towards *sec*-alcohols were investigated. Thermodynamic analysis of the temperature dependence of the enzyme's enantioselectivity in the selected organic solvents was also performed to obtain better understanding of the mechanisms underlying the solvent effects.

2. Materials and methods

2.1. Materials

Thermophilic lipase QLM was a gift provided by Meito Sangyo Co., Ltd. (*R*, *S*)-2-octanol, (*R*, *S*)-2-pentanol, vinyl acetate and isopropenyl acetate were purchased from Aldrich, and all the organic solvents used in the enzymatic reactions were

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Table 1
Thermodynamic components of the enantiomeric ratio, *E*, for lipase QLM-catalyzed transesterification of (*R*, *S*)-2-octanol with vinyl acetate as acyl donor in organic solvents and solvent-free system at 298 K

Solvent	Molecular volume (Å^3)	*E*	$\Delta_{R\text{-}S}\Delta G$ (kJ/mol)	$\Delta_{R\text{-}S}\Delta H$ (kJ/mol)	$T\Delta_{R\text{-}S}\Delta S$ (kJ/mol)
Dichloromethane	106.80	21.24	−7.57	−12.79 ± 0.57	−5.22 ± 0.55
Acetone	122.04	14.41	−6.61	−12.16 ± 0.53	−5.55 ± 0.50
THF	134.78	10.37	−5.79	−11.60 ± 0.64	−5.80 ± 0.59
Dioxane	141.47	6.29	−4.56	−10.03 ± 0.32	−5.48 ± 0.30
Cyclohexane	179.11	8.61	−5.33	−13.86 ± 0.31	−8.59 ± 0.29
n-Hexane	216.76	9.19	−5.50	−15.09 ± 0.77	−9.60 ± 0.72
Decaline	260.47	9.17	−5.49	−13.05 ± 0.35	−7.55 ± 0.33
Solvent-free	–	11.42	−6.03	−11.80 ± 0.74	−5.77 ± 0.66

Error values are ±standard errors of the linear regression of ln *E* vs. 1/*T*.

of analytical grade and dried over 5 Å molecular sieve before use.

2.2. Kinetic resolution of (R, S)-2-octanol and (R, S)-2-pentanol

The thermophilic lipase QLM-catalyzed kinetic resolution of *sec*-alcohols through transesterification in each of the organic solvents listed in Table 1 was studied. In each case, racemic *sec*-alcohol (10 mmol) and acyl donor (vinyl acetate or isopropenyl acetate, 15 mmol) were mixed in 8 ml of the organic solvent in a 20 ml reaction vessel. The reaction was also examined in the solvent-free system, in which the substrate and acyl donor were mixed directly. The reaction was initiated by the addition of lipase QLM (15 mg), and the solution was continuously stirred at 298 K to ensure that all of the enzyme particles were homogeneously dispersed in the reaction medium. Samples were taken for analysis at regular intervals between 0 and 50% conversion.

2.3. Determination of enantiomeric ratios

The enantiomeric excess values of the remaining substrate, ee_s, and the produced ester, ee_p, were determined by chiral capillary gas chromatography (GC). Baseline separation was readily obtained using an *HP-Chiralβ* column (30 m × 0.25 mm × 0.25 μm, J&W, USA) and N_2 as carrier gas at an average velocity of 39 cm s^{-1}. The temperature of the injection pool and detector was 300 °C. Following injection, the column oven temperature was held at 65 °C for 40 min, then increased at 10 °C min^{-1} to a final temperature of 95 °C, which was maintained for 10 min. The enantiomeric ratio, *E*, was calculated from the enantiomeric excess of the substrate (ee_s, %) and the product (ee_p, %) at a certain conversion (*c*, %) based on the following equation [19].

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \quad c = \frac{ee_s}{ee_s + ee_p} \tag{1}$$

2.4. Thermodynamic analysis

In an enantioselective enzyme-catalyzed reaction, the enantiomeric ratio, *E*, is related to the difference in activation free energy between the enantiomers ($\Delta_{R\text{-}S}\Delta G^{\#}$) as $-RT\ln E = \Delta_{R\text{-}S}\Delta G^{\#} = \Delta_{R\text{-}S}\Delta H^{\#} - T\Delta_{R\text{-}S}\Delta S^{\#}$. The following equation relates *E* to its enthalpic and entropic components [20]:

$$\ln E = \frac{-\Delta_{R\text{-}S}\Delta H^{\#}}{R} \cdot \frac{1}{T} + \frac{\Delta_{R\text{-}S}\Delta S^{\#}}{R} \tag{2}$$

where $\Delta_{R\text{-}S}\Delta G^{\#}$, $\Delta_{R\text{-}S}\Delta H^{\#}$ and $\Delta_{R\text{-}S}\Delta S^{\#}$ are the differences in activation free energy, activation enthalpy and activation entropy between the reactions converting the two enantiomers, respectively.

The procedures in the thermodynamic experiment were the same as those described above, except that the reactions were carried out at the temperatures indicated in Fig. 1. Thus, by studying the temperature dependence of the *E* value, $\Delta_{R\text{-}S}\Delta H^{\#}$ and $\Delta_{R\text{-}S}\Delta S^{\#}$ values could be determined from the straight line of ln *E* vs. 1/*T*, and the results provided valuable indications regarding the thermodynamic parameters governing the enzyme's enantioselectivity.

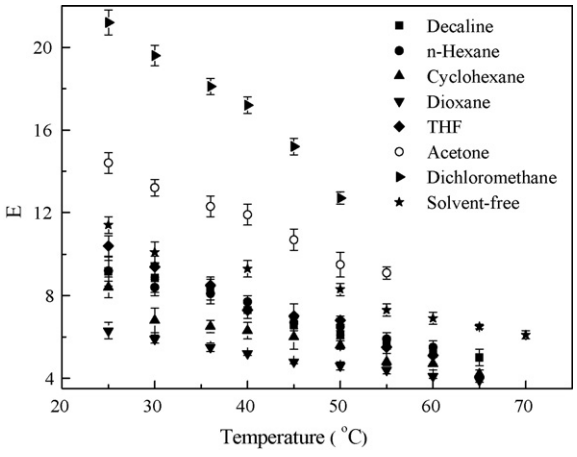

Fig. 1. *E* values as a function of temperature in the resolution of (*R*, *S*)-2-octanol with vinyl acetate as acyl donor in the tested organic solvents and the solvent-free system.

3. Results and discussion

3.1. Effects of temperature and solvent characteristics

Generally, lowering the reaction temperature increases the enantioselectivity of lipase-catalyzed resolution of racemates [21]. Therefore, some reactions catalyzed by mesophilic lipases have been carried out at temperatures as low as −40 °C to improve their enantioselectivity [21]. However, under such conditions reaction times are very long, and it is expensive to control the temperature. The optimum temperature for lipase QLM activity is 65–70 °C, therefore it is postulated that it may provide acceptable enantioselectivity and thus substantial further benefits even at room temperature. This assumption was verified by our analysis of the resolution of (*R*, *S*)-2-octanol with vinyl acetate as acyl donor catalyzed by lipase QLM across a wide range of temperatures (25–70 °C). As shown in Fig. 1, the enzyme's enantioselectivity increased as the temperature decreased in the selected organic solvents and solvent-free system. Therefore, the reaction temperature was fixed at 25 °C for the following investigations.

It is widely recognized that enzyme enantioselectivity is strongly affected by the solvents used [8]. Lipase QLM showed moderate enantioselectivity, with an *E* value of 11, in the solvent-free system at 25 °C, whereas it had sufficiently high enantioselectivity for practical purposes, yielding an *E* value over 20 in dichloromethane (Fig. 1). The effects of the log *P*, dielectric constant and molecular size of the solvents on the enantioselectivity of lipase QLM were investigated to explore possible correlations between solvent parameters and enzyme enantioselectivity. No correlations were found between either log *P* or dielectric constant and the *E* values (Fig. 2A and B). These findings are consistent with previous reports regarding the kinetic resolution of *sec*-alcohols [22,23]. Unexpectedly, however, a significant negative correlation between *E* values and the molecular size of the solvents was found (Fig. 2C), and only one of the tested solvents (dioxane) deviated substantially in this respect. The highest *E* value, 21, was obtained in the small molecular-sized solvent dichloromethane. In addition, the differences in *E* values were much stronger among the solvents with molecular sizes below ca. 160 Å^3.

To assess the generality of the correlation observed for lipase QLM, the kinetic resolution of (*R*, *S*)-2-pentanol with the same acyl donor, and (*R*, *S*)-2-octanol with isopropenyl acetate as acyl donor, was examined in the same set of organic solvents. Similar correlations were observed in both of these cases, as shown in Fig. 3. Thus, the size of the solvent molecules appears to be a key factor governing the solvent effects in the thermophilic lipase QLM-catalyzed resolution of *sec*-alcohols.

In an investigation of the effects of solvent molecular size on *Candida antarctica* lipase B enantioselectivity for the resolution of (*R*, *S*)-3-methyl-2-butanol with vinyl octanoate as acyl donor, Hult and co-workers obtained higher *E* values in the large molecular-sized solvents *n*-hexane and decaline than in the smaller molecular-sized solvents they tested [11]. In contrast to their results, we found the opposite trend. This apparent discrepancy may be due to the dependence of *E* values for the resolution

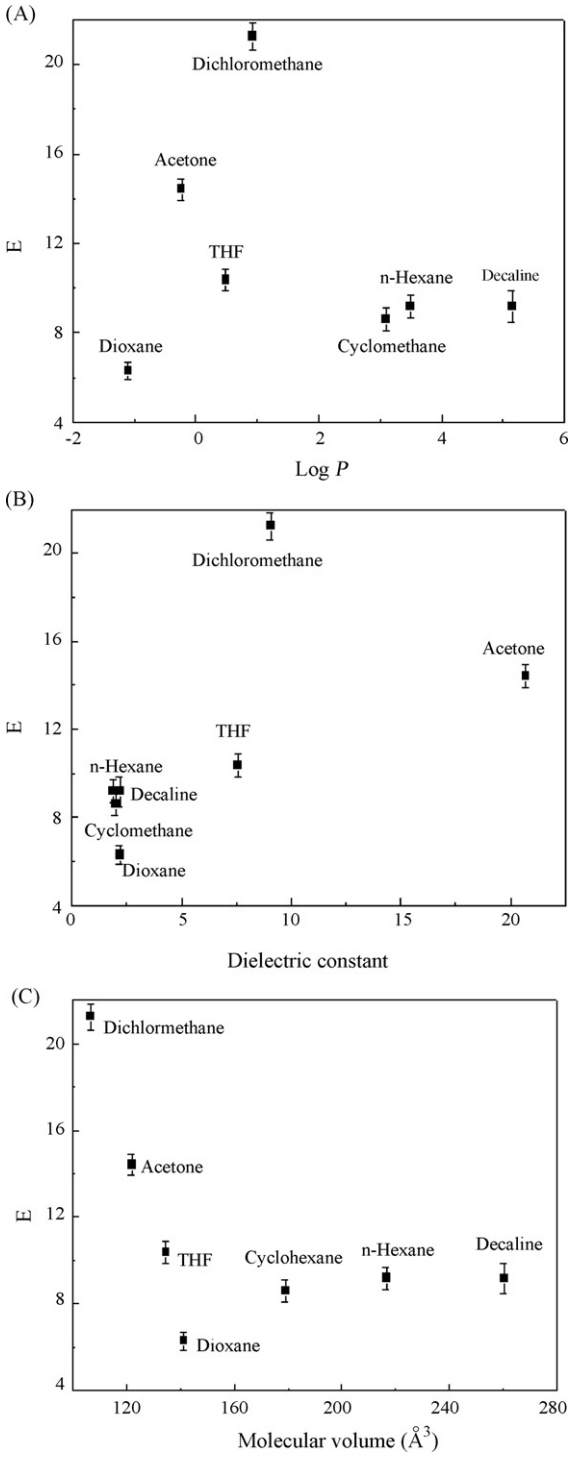

Fig. 2. *E* values at 298 K as a function of solvent characteristics in the resolution of (*R*, *S*)-2-octanol with vinyl acetate as acyl donor in the tested organic solvents: (A) log *P*; (B) dielectric constant; (C) molecular volume.

of racemic compounds on the difference in the stability of the two enantiomers' transition states, which depend in turn on the conformation of the active site of the lipase and the molecular structure of the substrates employed [3]. Therefore, it is not unexpected to find differences in the trends between enzyme enantioselectivity and solvent characteristics amongst different enzymes and substrates, due to the complexity of the reaction

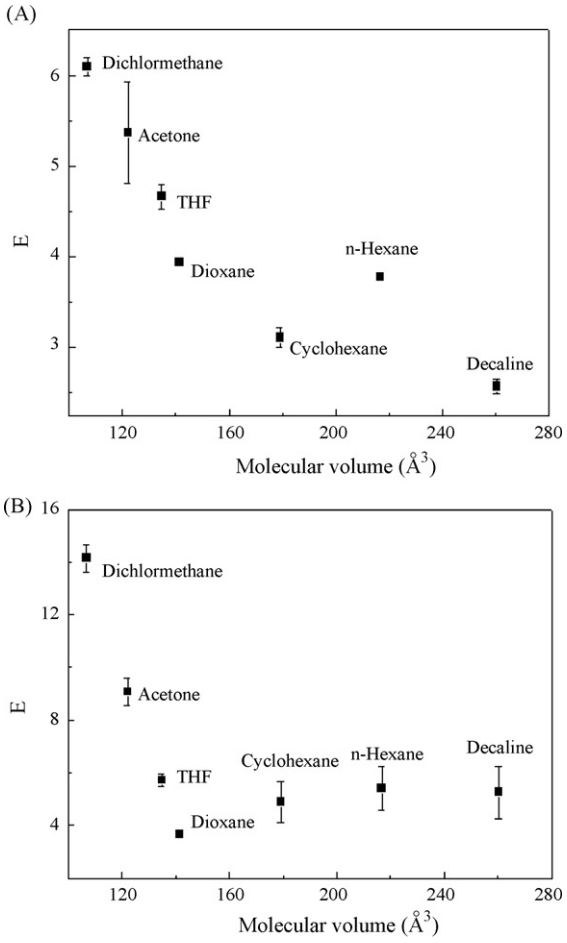

Fig. 3. E values at 298 K as a function of solvent molecular volume: (A) resolution of (*R*, *S*)-2-pentanol with vinyl acetate as acyl donor; (B) resolution of (*R*, *S*)-2-octanol with isopropenyl acetate as acyl donor.

systems. The key finding here was that the thermophilic lipase QLM exhibited high enantioselectivity for chiral *sec*-alcohols in the small molecular-sized solvent dichloromethane.

3.2. Thermodynamic analysis

To obtain more detailed information about the effects of solvent molecular size on the enantioselectivity of lipase QLM, the resolution of (*R*, *S*)-2-octanol with vinyl acetate as acyl donor in the selected organic solvents was thermodynamically analyzed.

The contributions of thermodynamic components to the E values, calculated from the data acquired from the experiments in which the effects of temperature on the enantioselectivity of lipase QLM was evaluated, are presented in Table 1. Due to the correlation between E and $\Delta_{R\text{-}S}\Delta G^{\#}$ values, the $\Delta_{R\text{-}S}\Delta G^{\#}$ values markedly decreased in solvents with small molecular size. Most notably, the $\Delta_{R\text{-}S}\Delta G^{\#}$ value was about 25.5% lower when the reaction was performed in dichloromethane than in the solvent-free system, leading to an increase in the E value to 21. Further analysis of the contributions of $\Delta_{R\text{-}S}\Delta H^{\#}$ and $T\Delta_{R\text{-}S}\Delta S^{\#}$ to the E values showed an intriguing phenomenon. In almost all of the solvents (except dioxane), $\Delta_{R\text{-}S}\Delta H^{\#}$ values were lower than that in the solvent-free system, promoting increases in E values. However, the absolute values of $T\Delta_{R\text{-}S}\Delta S^{\#}$ varied highly, depending on the molecular size. In solvents with large molecules (>ca. 160 Å^3), the absolute values of $T\Delta_{R\text{-}S}\Delta S^{\#}$ were larger than in solvents with small molecules and in the solvent-free system. Therefore, differences in $T\Delta_{R\text{-}S}\Delta S^{\#}$ values tended to compensate for differences in $\Delta_{R\text{-}S}\Delta H^{\#}$ values in solvents with large molecules, accounting for the small variations in E values for these solvents. In contrast, the absolute values of $T\Delta_{R\text{-}S}\Delta S^{\#}$ in small molecular-sized solvents (<ca. 160 Å^3) were clearly lower than that in the solvent-free system, and decreased with reductions in the size of the solvent molecules. Thus, changes in E values associated with differences in solvent molecular size appear to have been mainly due to changes in the entropic component.

The $\Delta_{R\text{-}S}\Delta S^{\#}$ parameter of enzymatic resolution is affected by differences between the enantiomers in the conformational degrees of freedom of the protein, losses in conformational entropy of the substrates and solvation [3]. Thus, the mechanisms underlying relationships between the molecular size of solvents and $\Delta_{R\text{-}S}\Delta S^{\#}$ values are complex. Here, the dependence of the $\Delta_{R\text{-}S}\Delta S^{\#}$ value on the solvent molecular size was probably mainly due to differences in the number of solvent molecules involved in the solvation of the enzyme's active site between the enantiomers. However, regardless of whether or not this is the major contributor to the effect of solvent molecular size, the data acquired clearly show that entropic components should be considered when solvent effects on enantioselectivity are being addressed, and the observed correlation may provide a useful guideline for optimizing lipase-catalyzed kinetic resolution reactions, and thus their enantioselectivity.

4. Conclusions

The solvent effects on the enantioselectivity of thermophilic lipase QLM during the resolution of (*R*, *S*)-2-octanol and (*R*, *S*)-2-pentanol were investigated. The size of the solvent molecules was found to be a key factor governing solvent effects on the E values. Choosing a small molecular-sized solvent would improve the enzyme's enantioselectivity. Thermodynamic analysis revealed that changes in E value with the molecular size of the solvents were mainly due to changes in the entropic component. Our research may help to provide further insights regarding the mechanism of solvent effects on enantioselectivity, and an effective tool to optimize the enantioselectivity of enzyme-catalyzed reactions.

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