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Rational strategies for highly enantioselective lipase-catalyzed kinetic resolutions of very bulky chiral compounds: substrate design and high-temperature biocatalysis

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Abstract—Rational approaches to successful lipase-catalyzed kinetic resolutions of very bulky chiral compounds are briefly reviewed: substrate design and high-temperature biocatalysis. The molecular design based on a stereo-sensing mechanism was the key to the success in the kinetic resolution of 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin. The lipase-catalyzed transesterification of 1,1-diphenyl-2-propanol in *n*-decane proceeded with excellent enantioselectivity at high temperatures up to 120 °C in an autoclave.

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1. Introduction

Chiral functional molecules, such as chiral ligands in asymmetric synthesis and chiral synthetic receptors in host–guest chemistry, are occasionally large and bulky and can often show poor reactivity for lipases. Herein, rational methods for achieving the highly enantioselective kinetic resolution of very bulky chiral compounds are briefly reviewed. 5-[4-(1-Hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin was successfully designed on the basis of a stereo-sensing mechanism shown below. The completely enantioselective acylation of 1,1-diphenyl-2-propanol, which is consistent with the mechanism, proceeded at high temperatures up to 120 °C, with the highest conversion being obtained at 80–90 °C.

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2. Theoretical background

Lipases are currently one of the best biocatalysts for the preparation of a wide range of optically active alcohols.[1] The most unusual but attractive feature of lipases is the simultaneous achievement of high enantioselectivity and broad substrate specificity, in addition to high catalytic activity and thermostability in organic solvents. In fact, hundreds of secondary alcohols have been resolved with high enantioselectivities by a single lipase, for example, a lipase from *Burkholderia cepacia* (formerly named *Pseudomonas cepacia*) called lipase PS (Amano Enzyme). Obviously, it is difficult for the traditional lock-and-key paradigm[2] to account for this unusual behavior of lipases, which are capable of showing high enantioselectivity and broad substrate specificity, simultaneously. Elucidating the origin of this unique feature of lipases has therefore been an important and intriguing subject. Several methods have been used to disclose the

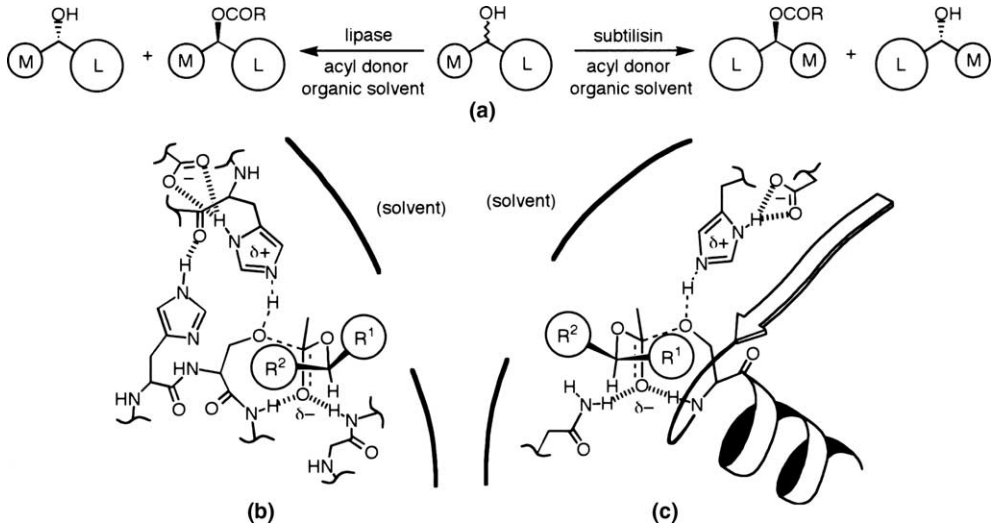
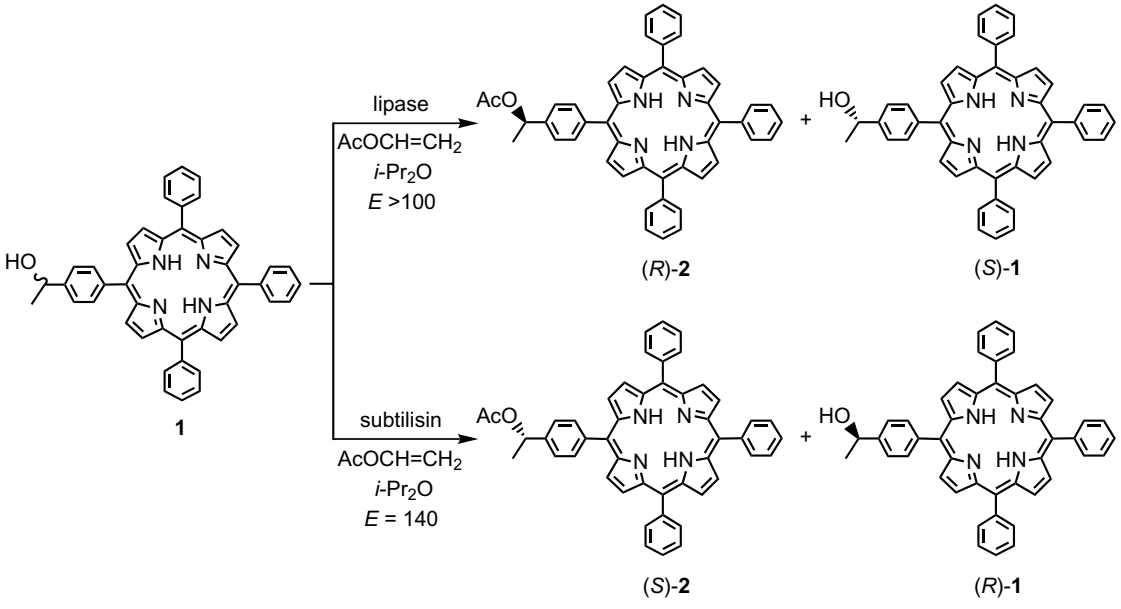


Figure 1. (a) Empirical rules for the lipase- and subtilisin-catalyzed kinetic resolutions of secondary alcohols. L and M represent the larger and smaller substituents, respectively. Typically, (*R*)- and (*S*)-enantiomers react faster in the lipase- and subtilisin-catalyzed kinetic resolutions, respectively. (b) (c) Transition-state models for the (b) lipase- and (c) subtilisin-catalyzed kinetic resolutions of secondary alcohols. In both models, (i) the C–O bond of a substrate takes the *gauche* conformation with respect to the breaking C···O bond, which is due to the stereoelectronic effect, and (ii) the H atom attached to the asymmetric C atom of the substrate is *syn*-oriented toward the carbonyl O atom of the acetyl group. When such a locally favorable conformation is taken, the faster-reacting enantiomer can direct the larger substituent [R^1 in (b) and R^2 in (c)] towards the external solvent without severe steric hindrance, whereas the slower-reacting enantiomer directs the larger substituent [R^2 in (b) and R^1 in (c)] toward the protein wall, causing severe steric repulsion. Even if any other conformation is taken, the slower-reacting enantiomer necessarily becomes less stable than the antipodal enantiomer. For details, see Ref. 4.

mechanism of enantioselectivity, such as (i) substrate mapping, (ii) X-ray crystallographic analysis, (iii) computational calculations, (iv) kinetic analysis, (v) thermodynamic analysis, (vi) site-directed or random mutagenesis, and (vii) spectroscopic methods, all of which have been summarized in a previous review.[3]

A stereo-sensing mechanism of lipases toward secondary alcohols has been revealed as shown in the transition-state model in Figure 1b.[4a] In this model, enantioselectivity is explained only by the conformational requirements and repulsive interactions in the transition state, with no binding interaction between the enzyme's pockets and the substrate's substituents involved. Such a new concept can be applied to subtilisins (Fig. 1c).[4b] These transition-state models can rationalize (i) the simultaneous achievement of high enantioselectivity and broad substrate specificity, (ii) the opposite enantiopreferences of lipases and subtilisins for secondary alcohols (Fig. 1a), (iii) the low activity for secondary alcohols having bulky substituents on both sides, and (iv) little or no activity for tertiary alcohols.[4]



Scheme 1. Lipase- and subtilisin-catalyzed kinetic resolutions of **1**.

Table 1. Lipase- and subtilisin-catalyzed kinetic resolutions of **1** in dry *i*-Pr_2O at 30 °C

Entry	Enzyme	Conv. (%)	% Yield (% ee)				
			2	*R*/*S*	**1**	*R*/*S*	*E*
1	Lipase PS[a]	48	44 (>98)	*R*	47 (89)	*S*	>298
2	Chirazyme L-2[b]	50	45 (>98)	*R*	47 (>98)	*S*	>458
3	Chirazyme L-9[c]	5	8 (>98)	*R*	90 (5)	*S*	>104
4	Lipase LIP[d]	50	46 (>98)	*R*	36 (>98)	*S*	>458
5	ChiroCLEC-BL[e]	42	40 (97)	*S*	57 (71)	*R*	140

[a] *B. cepacia* lipase (Amano Enzyme).
[b] *C. antarctica* lipase (Boehringer Mannheim).
[c] *R. miehei* lipase (Boehringer Mannheim).
[d] *P. aeruginosa* lipase (Toyobo).
[e] Subtilisin Carlsberg (Altus Biologics).

3. Substrate design and limitation of the size of substrate

A gigantic and rigid secondary alcohol, 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin **1**, was designed on the basis of the transition-state models (Scheme 1).[5] The transition-state models indicate that any large substrate, if well designed, should be acceptable because the larger substituent of the faster-reacting enantiomer is directed toward the external solvent. Figure 1b and c suggest that one enantiomer of **1** [(*R*)-**1** for lipases and (*S*)-**1** for subtilisins] is susceptible to the acylation because the tetraphenylporphyrin moiety can be directed toward the external solvent and that the other enantiomer [(*S*)-**1** for lipases and (*R*)-**1** for subtilisins] is highly disfavored because of a severe steric repulsion between the tetraphenylporphyrin moiety and the protein.

The results are listed in Table 1. The transesterifications of **1** were catalyzed by lipases from *Burkholderia cepacia*, *Candida antarctica*, *Rhizomucor miehei*, and *Pseudomonas aeruginosa* and by subtilisin Carlsberg. Excellent enantioselectivities (E >100) were observed in all cases, with 'complete kinetic resolutions' being achieved in entries 2 and 4, where both alcohol **1** and ester **2** were enantiomerically pure (>98% ee). Lipases and subtilisin Carlsberg exhibited the opposite enantiopreferences for **1**, as expected. As shown in Figure 2, the faster-reacting enantiomer of **1** can minimize steric hindrances when the tetraphenylporphyrin moiety is directed toward the external solvent in the specific conformation shown in the transition-state models (Fig. 1). Table 1 and Figure 2 strongly suggest that the accommodation of a larger substituent in a pocket of the enzymes is not necessarily required for attaining high enantioselectivity and that the size of a substrate is unlimited if appropriately designed. Further work is currently underway to convert the enantiomerically pure alcohol **1** to a chiral diporphyrin receptor with C_2 symmetry.

Schemes 2 and 3 show other examples of the lipase-catalyzed acylations of very bulky and rigid compounds. Tamiaki et al. have reported the diastereoselective lipase-catalyzed transesterifications of methyl bacteriopheophorbide-*d* **3** (Scheme 2).[6] Preparation of enantiomerically pure **3** was needed to investigate the supramolecular structure of the light-harvesting antennae of photosynthetic bacteria. Lipases from *P. aeruginosa* and *B. cepacia* showed catalytic activity but with low diastereoselectivity (E = 2.9–2.0), whilst those from *Candida rugosa*, *C. antarctica* and porcine pancreas showed no activity. These results make a sharp contrast with those observed for **1** (Table 1). Chlorin **3** has the OH group at the 3^1 position which is very close to the chlorin skeleton, whereas porphyrin **1** has a phenyl group as a spacer between the OH group and the porphyrin skeleton. The crystal structures of lipases in the Protein Data Bank indicate that such a spacer is necessary for reducing steric hindrances. Tanaka et al. have reported that helicene **5** can be resolved into the enantiomers with high enantioselectivity (Scheme 3).[7] Optically active helicene **5** may be used as a chiral ligand for asymmetric catalysts. To resolve 100 mg of **5**, 2.99 g of lipase PS was used, which indicates that the reaction is very slow despite the primary alcohol. (*M*)-Mono acetate was also produced in 37% yield in 77% ee. Interestingly, lipases from *B. cepacia* and *C. antarctica* showed the opposite enantiopreferences for **5**, although no clear mechanism is available. The enantioselectivity and the reaction rate for **5** or its derivatives could be improved by clarifying the mechanistic aspect for primary alcohols.

4. High-temperature biocatalysis

In a series of mechanistic studies, a bulky alcohol, 1,1-diphenyl-2-propanol **7**, was found to show no reactivity for *B. cepacia* lipase immobilized on Celite, lipase PS (Amano Enzyme), at 30 °C.[4d] No reactivity of **7** seemed strange because **1** had been resolved with high enantioselectivity using the same enzyme (Table 1, entry 1). The transition-state model (Fig. 1b) predicts that lipases will show activity for **7** because **7** has a small substituent (Me group) on one side. Careful inspection of the crystal structure of the enzyme suggested a possibility that even (*R*)-**7**, which is predicted to react faster, undergoes severe steric repulsions between the branched diphenylmethyl moiety and a protein part, such as Tyr29, Leu287, and Ile290, which are not shown in the transition-state model.[8]

The simplest way for accelerating the reaction is to increase the reaction temperature. Using *B. cepacia* lipase immobilized on porous ceramic particles called Toyonite (Toyo Denka Kogyo), lipase PS-C II (Amano Enzyme),

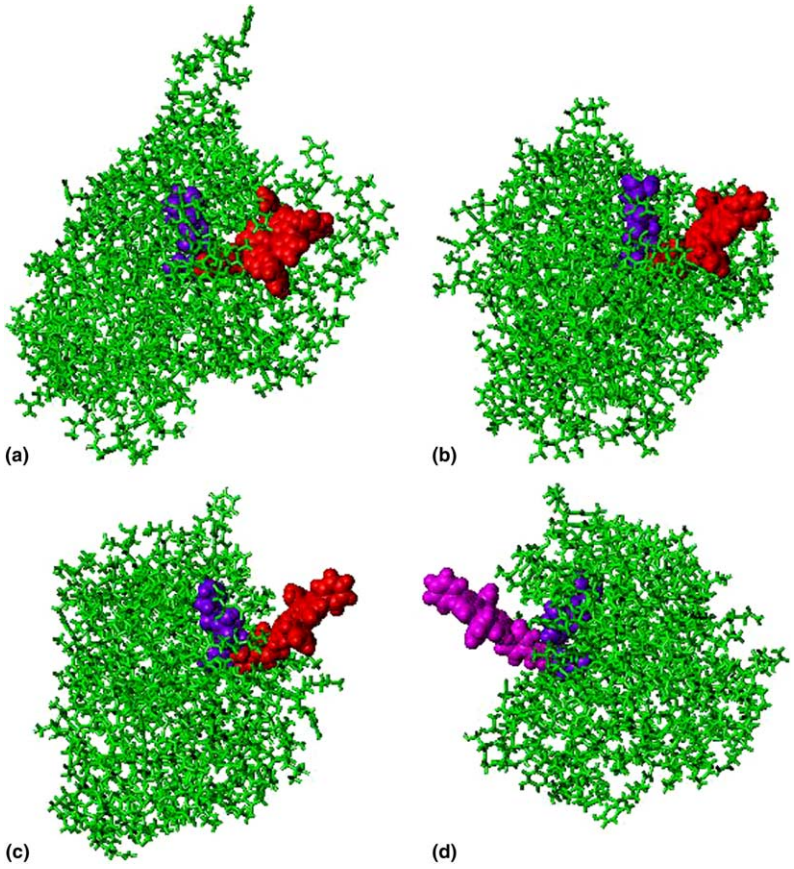
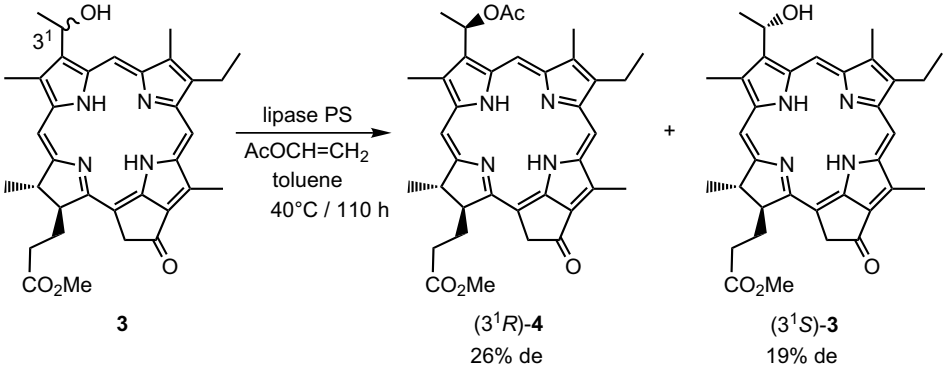
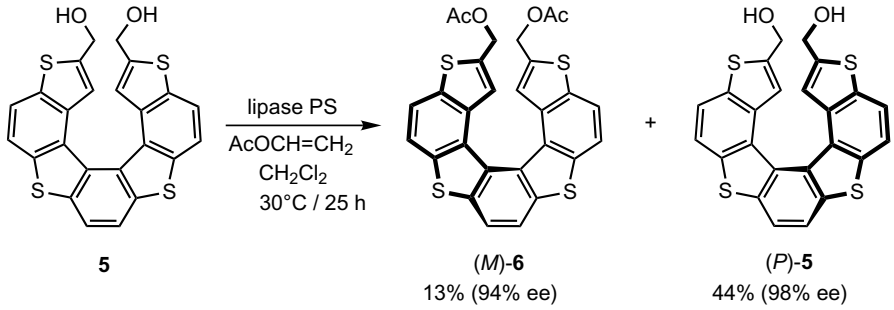


Figure 2. Computer representations showing plausible transition-state conformations. The enzymes are shown in green, with the catalytic triad residues shown by a purple space-filling representation, and the substrate moieties for (*R*)-**1** and (*S*)-**1** are shown by red and magenta space-filling representations, respectively. The lipases and subtilisin are displayed in orientations similar to those of Figure 1b and c, respectively. The active sites of the lipases are very crowded and obviously even the faster-reacting (*R*)-**1** undergoes steric hindrances to some degree. As shown here, steric hindrances can be minimized when the specific conformation shown in the transition-state model (Fig. 1b) is taken, with the tetraphenylporphyrin moiety directed towards the external solvent. (a) *B. cepacia* lipase (PDB code 1OIL), (b) *C. antarctica* lipase (PDB code 1LBS), (c) *R. miehei* lipase (PDB code 4TGL), and (d) subtilisin Carlsberg (PDB code 2SEC). In each case, **1** was docked after the water molecules and the inhibitor (if any) had been removed from the crystal structures. The structures were drawn with SYBYL 6.4 (Tripos Inc.).

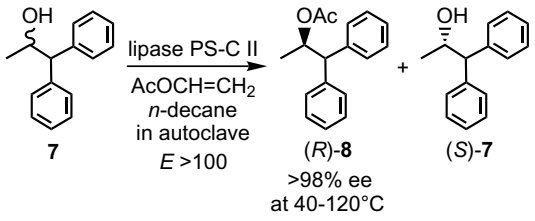


Scheme 2. Lipase-catalyzed acylation of bulky secondary alcohol **3**.

high-temperature biocatalysis was examined. *n*-Decane, which has a high boiling point (174 °C), was used as solvent, while a stainless autoclave was used as a reaction vessel to suppress the evaporation of vinyl acetate. The lipase-catalyzed kinetic resolutions of **7** were conducted with vinyl acetate at 30–120 °C for 3 h (Scheme 4). Although the conversion was only 3% at 30 °C after 3 h, it increased, when increasing the reaction temperature, to reach 39% at 80 and 90 °C. Increasing the reaction temperature further resulted in lower conversions (Fig. 3).[8] The obtained ester **8** was enantiomerically pure (>98% ee) in all cases measured (40–120 °C), while the



Scheme 3. Lipase-catalyzed kinetic resolution of bulky primary alcohol **5**.



Scheme 4. Lipase-catalyzed kinetic resolution of **7** at high temperatures.

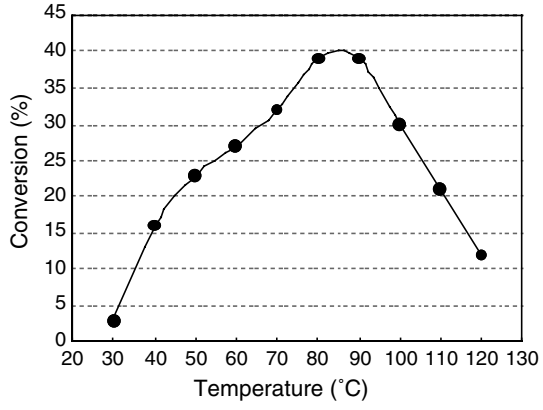


Figure 3. Conversions plotted against the reaction temperature.

enzymatic catalysis proceeded completely enantioselectively even at 120 °C, a sterilization temperature for microorganisms. The transition-state model (Fig. 1b) suggests that the reactivity of the unfavorable enantiomer, (*S*)-**7**, is completely suppressed by a severe steric repulsion between the diphenylmethyl moiety of (*S*)-**7** and the protein wall and/or by a conformational strain, which are caused in the transition state, which in turn leads to the perfect enantiomeric purity of (*R*)-**8** even at 120 °C. Thus, although an increase in the temperature usually decreases enantioselectivity,[4d,9] high enantioselectivity was attained even at high temperatures if the reactivity of the unfavorable enantiomer is sterically suppressed.

Thermostability of the lipase in the Toyonite pores (mean pore size: 60 nm) was found to be much higher than that of the same enzyme immobilized on Celite (lipase PS), the latter of which showed 0% conversion for **7** at 100 °C for 3 h. The thermostability of the lipase immobilized on Toyonite was examined by measuring the remaining activity. The remaining activity of the lipase treated at 80, 100, 120, and 130 °C for 3 h in *n*-decane in an autoclave was 66%, 63%, 31%, and 6%, respectively. These results indicate that although the lipase on Toyonite is denatured gradually at elevated temperatures, it is very robust.[8]

Triggered by the concept represented by the transition-state model, it was found that the abilities of the mesophilic enzyme are comparable to those of (hyper)thermophilic enzymes. Since the same enzyme preparation was confirmed to show catalytic activity for 2-hydroxymethyl-1,4-benzodioxane at −40 °C ($E = 27$), it follows that the single enzyme can show the catalytic function at a very wide range of temperatures from −40 to 120 °C.[8] The low-temperature method has recently emerged as a very effective method for a dramatic improvement in enantioselectivity for unhindered primary alcohols:[9] decreasing the reaction temperature increases enantioselectivity at the expense of the reaction rate. The decreased rate at low temperatures can be improved upon in various ways.[9c,d] As the low-temperature method is a rational strategy for unhindered chiral compounds, details are not described herein.[9]

5. Conclusion

The results of the kinetic resolution of **1** and other bulky chiral compounds **3** and **5** indicate that the scope of the substrate specificity of lipases is much wider than generally believed. Substrate design based on the mechanism was the key to the success, while the potential abilities of lipases for bulky substrates were developed with the aid of the mechanism. In a case where a bulky substrate **7** showed very poor reactivity due to steric hindrances, elevating the reaction temperature with a thermostable lipase was effective for accelerating the reaction. Although increasing the temperature usually decreased the enantioselectivity,[4d,9] high enantioselectivity can be expected even at high temperatures if the structure of the substrate is ideal from the mechanistic viewpoint. These rational strategies are important and useful for the kinetic resolution of very bulky chiral compounds useful for chiral ligands and chiral synthetic receptors.

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