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Lipase-Catalyzed Kinetic Resolution of Large Secondary Alcohols Having Tetraphenylporphyrin

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Abstract: Large secondary alcohols, 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin (**1a**) and the zinc complex **1b**, designed on the basis of the transition-state model recently proposed by us, were subjected to the lipase-catalyzed transesterifications. The alcohols were resolved using lipases from *Pseudomonas cepacia* (lipase PS), *Candida antarctica* (CHIRAZYME L-2), *Rhizomucor miehei* (CHIRAZYME L-9), and *Pseudomonas aeruginosa* (lipase LIP) with high enantioselectivities (E >100).

Lipases (EC 3.1.1.3) are useful biocatalysts for the kinetic resolution of racemic alcohols and esters,[1] and hence various attempts have been made to disclose the factors determining the stereoselectivity of lipases.[2–4] Recent kinetic studies by Hirohara and his co-workers[3] and by us[4] have demonstrated that the enantioselectivity in the lipase-catalyzed reactions originates from the transition state and that chiral recognition in the binding step (Michaelis complex) is unimportant for the enantioselectivity. We have also proposed the transition-state model to rationalize the enantioselectivity of lipases toward secondary alcohols, as shown in Figure 1. Figure 1 shows that in the transition state, the larger substituent attached to the stereocenter in the faster-reacting (typically, (*R*)-) enantiomers is directed toward the external solvent, but not accommodated in any binding pocket of the enzyme.[4] Our transition-state model, therefore, predicts that even very large secondary alcohols whose larger substituent cannot be accommodated in any pocket of the enzyme[5] can be efficiently resolved if they are appropriately designed.

In order to substantiate this prediction, we selected tetraphenylporphyrin as a substituent because of its size (edge-to-edge distance of *ca.* 17 Å), rigidity and synthetic accessibility, and designed 5-[4-(1-hydroxyethyl)phenyl]-10,15,20-triphenylporphyrin (**1**) shown in Chart 1.[6] The tetraphenylporphyrin moiety of the (*R*)- and (*S*)-enantiomers of **1** can be represented by R^1 and R^2, respectively, in the transition structure shown in Figure 1. Obviously, (*S*)-**1** seems to be highly disfavored because of severe steric repulsion between the tetraphenylporphyrin moiety (R^2) and the enzyme wall, whereas (*R*)-**1** is likely to be susceptible to the acylation. In this paper, we report the lipase-catalyzed kinetic resolution of the free base porphyrin **1a** and the zinc complex **1b**.

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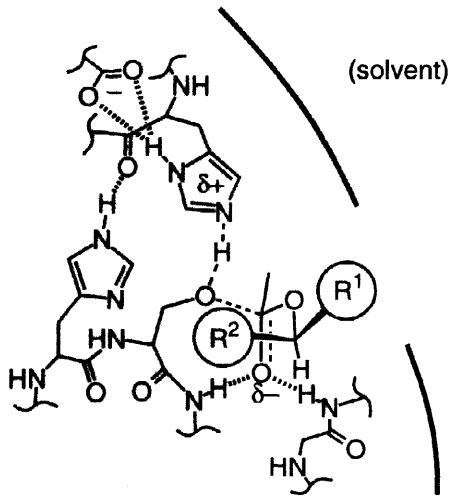


Figure 1. Transition-state model to rationalize the enantioselectivity in the lipase-catalyzed reactions for secondary alcohols and the corresponding esters.

(i) The enantiomer-differentiating transition state in the rate-determining step is shown.

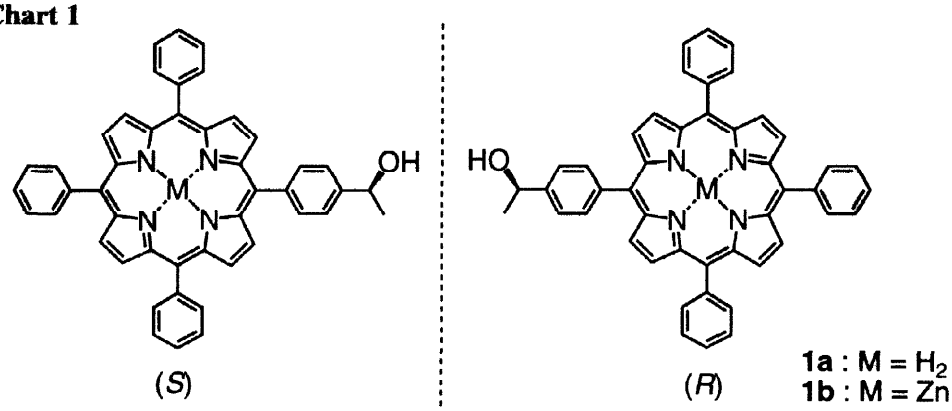
(ii) The absolute configuration of the carbonyl carbon atom where the oxygen atom of the serine of the catalytic triad is leaving/attacking is determined by the spatial arrangement of the catalytic residues.

(iii) The C–O bond of the substrate (secondary alcohol) takes the *gauche* conformation with respect to the breaking/forming C–O bond, which is due to the stereoelectronic effect.

(iv) The hydrogen atom attached to the stereocenter in the substrate is *syn*-oriented toward the carbonyl oxygen atom.

For details, see ref 4.

Chart 1



Porphyrin **1a** was prepared according to the method of Lindsey.[7] Pyrrole, benzaldehyde and 4-(1-hydroxyethyl)benzaldehyde[8] were allowed to react in the presence of a catalytic amount of $Et_2O{\cdot}BF_3$ in dry CH_2Cl_2 for 2 h at room temperature. Treatment with *o*-chloranil followed by chromatographic separation gave **1a** in 5% yield.[9] The zinc complex **1b** was prepared by the standard method with $Zn(OAc)_2$.[10]

The lipase-catalyzed transesterifications of **1** were carried out with vinyl acetate in dry diisopropyl ether at 30 °C (Scheme 1). Several commercially available lipases were examined. The results are summarized in Table 1. The enantiomeric excess (% ee) values of **1** and **2** obtained by the kinetic resolutions were determined by means of HPLC (Daicel, Chiralcel OD-H column, hexane : 2-propanol = 9 : 1), after they were converted to **1a**. The *E* values were calculated according to the literature.[11] It was confirmed by a control experiment without lipase that the zinc complex **1b** itself cannot function as a catalyst for the transesterification.

The absolute configuration of the remaining alcohols **1** in the lipase-catalyzed transesterifications was determined to be (*S*) by comparison of the HPLC retention time of **1a** with that of (*S*)-**1a** independently prepared from (*S*)-4-(1-hydroxyethyl)benzaldehyde.[12] This fact indicates that **1** is the substrate which obeys the empirical rule.[2a] Four kinds of lipases showed excellent enantioselectivities, which strongly suggests that the accommodation of the larger substituent of substrates in a binding pocket of the enzyme is not necessarily

required to attain high stereoselectivity, and which strongly supports the transition-state model (Figure 1). Interestingly, the zinc complex **1b** was acylated much more slowly than the free base porphyrin **1a** in most cases. We suppose that **1b** is bound to the polar amino-acid residues on the enzyme surface. This coordination may block the active site or may decrease the protein flexibility essential to catalytic activity. The results that even the free base porphyrin **1a** reacted relatively slowly in most cases suggest that unfavorable steric interactions operate between the tetraphenylporphyrin moiety of (*R*)-**1a** and some part of the enzyme such as the lid. The degree of such interactions, which varies with the lipase species, cannot be estimated by the *simplified* transition-state model (Figure 1). The reactivity of **1** may also be inherently lowered by the large size of the molecule.

Scheme 1

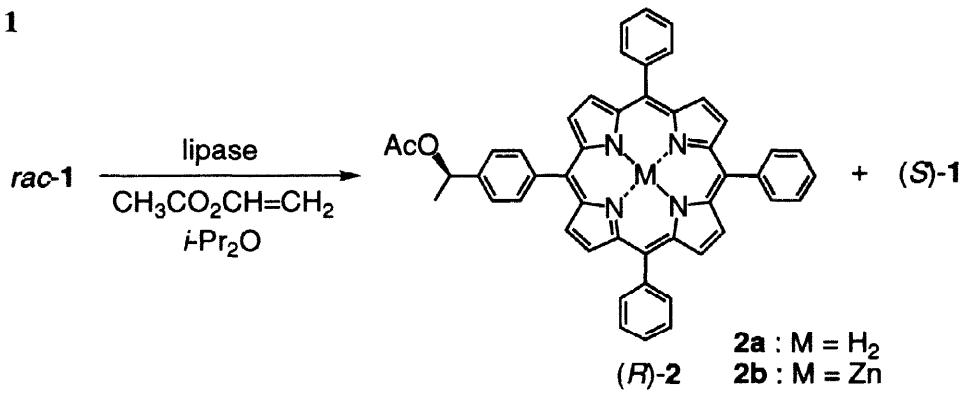


Table 1. Lipase-Catalyzed Enantioselective Transesterifications of **1**.[a]

Lipase	Alcohol	Time (h)	*c* (%)[b]	% Yield[c] (% ee[d])		*E* value[e]
				(*R*)-**2**	(*S*)-**1**	
lipase PS[f]	**1a**	23	48	44 (>98)	47 (89)	>298
lipase PS	**1b**	23	14	12 (>98)	82 (16)	>116
CHIRAZYME L-2[g]	**1a**	9	50	45 (>98)	47 (>98)	>458
CHIRAZYME L-2	**1b**	48	20	24 (>98)	74 (25)	>126
CHIRAZYME L-9[h]	**1a**	48	5	8 (>98)	90 (5)	>104
CHIRAZYME L-9	**1b**	48	8	7 (>98)	78 (8)	>107
lipase LIP[i]	**1a**	5	50	46 (>98)	36 (>98)	>458
lipase LIP	**1b**	5	18	24 (>98)	72 (21)	>122

[a] Conditions; lipase (900 mg), **1** (42 μmol), vinyl acetate (1.3 mmol), dry *i*-Pr_2O (30 mL), 30 °C.
[b] Conversion calculated from ee(**1**)/(ee(**1**) + ee(**2**)) according to ref 11. [c] Isolated yield.
[d] Determined by HPLC (Daicel, Chiralcel OD-H, hexane : 2-propanol = 9 : 1) after conversion to **1a**.
[e] Calculated from $E = \ln [1 - c(1 + ee(\mathbf{2}))]/\ln [1 - c(1 - ee(\mathbf{2}))]$ according to ref 11.
[f] *Pseudomonas cepacia* lipase (Amono Pharmaceutical). [g] *Candida antarctica* lipase (Boehringer Mannheim).
[h] *Rhizomucor miehei* lipase (Boehringer Mannheim). [i] *Pseudomonas aeruginosa* lipase (Toyobo).

In summary, this is the first example of the lipase-catalyzed kinetic resolution of the secondary alcohols rationally designed on the basis of the transition-state model. Enantiomerically pure alcohol **1** thus obtained can be utilized for a variety of purposes such as the building block for chiral diporphyrins useful for the model study of the photosynthetic reaction center and the physicochemical study of circular dichroic spectroscopy.[13] Further work is under way to elaborate the transition-state model.

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