Kinetic Studies on the Optical Resolution of 2-Alkanol by *Pseudomonas cepacia* Lipase Catalyzed Transesterification with Vinyl Acetate in Organic Solvent

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Abstract: The optical resolution data were analyzed for *Pseudomonas cepacia* lipase (PCL) catalyzed transesterification between vinyl acetate and 2-alkanol in organic solvent. The plots of log *E* (*E*: the enantiomeric ratio as the reaction is irreversible) against the extent of conversion (*c*) showed the maxima for all systems used. The competitive reaction in the optical resolution was verified by the analysis of the resolution data and an accordance of the enantiomeric excess (*ee*) vs. *c* plots between racemic and optically pure alcohols. The reverse reaction in the optical resolution was experimentally proved by a decrease in optical purity for the reaction of optically pure 1-methylheptyl acetate and 2-octanol in the presence of PCL. The first order plot showed a linearity for the reaction with (*R*)-2-octanol but curvature for the (*S*) counterpart. Based on the above observations, we concluded that the transesterification with vinyl acetate did not obey the reported optical resolution equations because acetylation of the (*S*) isomer in racemic 2-alkanol with the acetyl-enzyme intermediate was suppressed. Thus, *E* increased with *c* in the range of below 0.5.

Key words: *Pseudomonas cepacia* lipase (PCL), transesterification, vinyl acetate, enantioselectivity (*E*), kinetic study

1 Introduction

Vinyl acetate (VA) is a potential and practical acetylating reagent for the optical resolution of enantiomers in the racemate by lipase (triacylglycerol hydrolase, EC 3.1.1.3) catalyzed transesterification in organic solvent: VA irreversibly reacts with the active serine (Ser) residue in lipase to give the acetyl-enzyme intermediate [Enz-Ac] because vinyl alcohol is immediately converted to acetaldehyde by keto-enol tautomerization. This irreversibility is considered to accelerate the reaction

rate. Such a reaction is called "irreversible transesterification" (1) and applied to the optical resolution of numerous secondary alcohols (1-5).

On the other hand, the analyses of optical resolution data are complicated for lipase catalyzed transesterification with VA. It is reported that the enantioselectivity [*E*: the enantiomeric ratio for the homocompetitive reaction (6-9), *i.e.*, as the reaction is irreversible] at low conversion is greater than that at high conversion (>50%) for *Pseudomonas fluorescens* lipase (PFL) catalyzed transesterification with racemic β -methyl-(2-

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Enz : *Pseudomonas cepacia* Lipase (PCL)

2-Alkanol (Abbreviation)

Scheme 1 *Pseudomonas cepacia* Lipase Catalyzed Transesterification between Vinyl Acetate (VA) and 2-Alkanol (1) in Organic Solvent.

thiophene)propanol in chloroform (10), which suggests that the reaction with VA is reversible. The notable observation is that the *E* value increases as a function of substrate conversion (<50%) for *Pseudomonas cepacia* lipase (PCL) catalyzed transesterification with ($\pm$)-6-methyl-5-hepten-2-ol (racemic sulcatol) in chlorinated solvents (11). Furthermore, we previously reported that the *E* value is maximized against the extent of conversion (*c*) for PCL catalyzed transesterification with 2-alkanol (1) in organic solvent (12).

These led us to investigate the kinetic studies on the optical resolution by PCL catalyzed transesterification as shown in **Scheme 1**. The action of VA on the reaction was also compared to that of triacetyl glycerol (TA) whose reaction is a reversible type (**Scheme 2**). In this paper, individual 2-alkanol was abbreviated to X2-OH, where X is carbon number of the alcohol and 2- shows the position of hydroxy group (**Scheme 1**).

2 Experimental

2.1 Materials

Vinyl acetate (VA, Wako Pure Chemical, Osaka) and triacetyl glycerol (TA, Tokyo Chemical Industry, Tokyo) were dried over molecular sieves 3A (1/16, Wako Pure Chemical, Osaka) before use. 2-Alkanol (1) was purchased from Tokyo Chemical Industry, Tokyo [2-heptanol (72-OH) ~ 2-undecanol (112-OH)] and Lancaster, Lancashire [2-dodecanol (122-OH)] and used as

Scheme 2 PCL Catalyzed Transesterification between Triacetyl glycerol (TA) and (1) in Organic Solvent.

received. (*R*)-1-(1-naphthyl)ethyl isocyanate [(*R*)-NEI, *ee*>99%, Tokyo Chemical Industry, Tokyo] and optically pure (*R*)- and (*S*)-2-octanols [(*R*)- and (*S*)-82-OH, *ee*>99%, Chisso Chemical, Tokyo] were used as received. Silica gel (Silica gel 60, 70-230 mesh, Katayama Chemicals, Osaka) was used as received or after heating at 200°C for 2h when the activity was not sufficient for the separation of the product and the unreacted substrate. Organic solvents were purified by the ordinary method (13) and dried over molecular sieves 3A. *Pseudomonas cepacia* lipase (PCL, Lipase PS, Amano, Nagoya, 43 U/mg for hydrolysis) was used as received.

2·2 Analytical Methods

GC analysis was carried out by a Shimadzu 14B Gaschromatograph equipped with a FID detector (for the reaction with VA) and a Hitachi 263-80 Gaschromatograph equipped with a FID detector (for the reaction with TA). Column: G-100 column, 20 m, ϕ 1.2 mm, film thickness 1 μ m (purchased from GL Sciences, Tokyo); Injection and detector temperature: 250°C. For the reaction with VA — Carrier (N_2) pressure: 40 kPa (80°C), Column temperature: 70 $\rightarrow$ 120, 80 $\rightarrow$ 135, 95 $\rightarrow$ 150, 110 $\rightarrow$ 165, 125 $\rightarrow$ 165 and 135 $\rightarrow$ 195°C programmed with an increase of 10°C/min for the reaction mixtures of 72-OH, 82-OH, 92-OH, 102-OH, 112-OH and 122-OH, respectively. For the reaction of TA with 82-OH — Carrier speed (N_2): 30 mL/min; Column temperature: 70 $\rightarrow$ 160°C programmed with an increase of 10°C/min.

HPLC analysis was performed by a JASCO (Japan Spectroscopic Co., Tokyo) Intelligent HPLC System equipped with a UV monitor. Column: combined two columns of Finepak SIL (particle size: 5 μ m), ϕ 4.6 $\times$ 250 mm (JASCO, Tokyo) or of Inertsil SIL (particle size: 5 μ m), ϕ 4.6 $\times$ 250 mm (GL Sciences, Tokyo); Mobile phase: dichloromethane containing 0.05 vol% of methanol; Flow rate: 1 mL/min; Detection wavelength: 254 nm.

The enantiomeric excess (*ee*) of enantiomerically enriched and optically pure alcohols, (*R*)- and (*S*)-**(2)**, was determined as the corresponding diastereomers (carbamates) after derivatization with (*R*)-NEI (**14**). The optical purity of (*R*)-NEI was corrected by using (*R*)- and (*S*)-82-OH (*ee*=0.995) which were purchased or prepared in our laboratory by repeating the transesterification with VA in 1,2-dichloroethane.

The water content was measured by a Mitsubishi MCI Model-CA-02 Moisturemeter: PCL was 1.6-1.9 wt% and the substrate solution was below 0.01 vol%.

2·3 Optical Resolution

2·3·1 Reaction of VA with (**1**)

To a stirred solution (33-40 mL) containing VA (1.53-1.63 M) (1 M=1 mol dm⁻³) and (**1**) (0.39-0.88 M) at 30°C was added PCL (30-100 mg/mL-sub. soln.). The reaction was followed by GC and terminated by filtration when it reached at the desired conversion. The enzyme was washed with two 20 mL portions of the solvent. Except the case of dioxane, the combined filtrates were evaporated. The residue was dissolved with

20-30 mL of benzene and loaded on the silica gel column (Silica gel 80-100 g, ϕ 30 $\times$ 600 mm). The compounds were eluted with benzene (800-1000 mL) and methanol (300 mL). In the case of dioxane, the combined filtrates were diluted with 300 mL of benzene, washed with three 100 mL portions of water, dried over anhydrous sodium sulfate and evaporated. The residue was treated by the same method as described above.

The benzene fractions (300-800 mL), which contained (*R*)-enriched ester [(*R*)-**(2)**], were combined and evaporated. Yield: 65-95%.

Alkaline solution (1 M KOH/10% H₂O-C₂H₅OH, 10 mL/g-ester) was added to the (*R*)-enriched ester and the resulting mixture was left for 18-24 h at room temperature. A 100 mL of saturated sodium chloride solution (sat. NaCl) was added to the mixture and the compounds were extracted with three 30 mL portions of hexane. The combined extracts were washed with three 30 mL portions of sat. NaCl, dried over anhydrous sodium sulfate and evaporated to give the corresponding (*R*)-enriched alcohol [(*R*)-**(1)**]. Yield: 60-90% [based on (*R*)-**(2)**].

The methanol fractions (300 mL), which contained the unreacted alcohol, were concentrated until crystals of silica gel appeared. A 100 mL of sat. NaCl and 5 mL of the alkaline solution were added to the residue and the compounds were taken up by the same method for (*R*)-enriched (**1**), yielding the desired (*S*)-enriched alcohol [(*S*)-**(1)**]. Yield: 50-85%.

2·3·2 Reaction of TA with 2-octanol (82-OH)

PCL (100 mg/mL-sub. soln.) was added to a stirred mixture (35-40 mL) of TA (0.76-1.33 M) and 82-OH (0.61-0.88 M) dissolved in 1,2-dichloroethane at 30°C. The reaction mixture was filtered at the desired conversion (determined by GC) and the enzyme was washed with two 20 mL portions of 1,2-dichloroethane. The filtrates were combined and evaporated. The residue was dissolved with 30 mL of benzene and loaded on the silica gel column (Silica gel 80-100 g, ϕ 30 $\times$ 600 mm). When the residue exceeded 8 g of weight, it was divided into two equal portions, and each was dissolved with 30 mL of benzene and separately chromatographed. The compounds were eluted with benzene (800 mL) and methanol (300 mL).

The benzene fractions (300-800 mL) were combined and evaporated to give (*R*)-enriched 1-methylheptyl acetate [(*R*)-82-OAc] (Yield: 65-82 %), which was hydrolyzed by the same method as described above (2·

3·1), yielding the corresponding (*R*)-enriched 2-octanol [(*R*)-82-OH]. Yield: 60-88% [Based on (*R*)-82-OAc].

The methanol fractions (300 mL), which contained the unreacted alcohol, TA, diacetyl glycerol (DA) and a small amount of monoacetyl glycerol (MA), were concentrated. A 50 mL of the alkaline solution was added to the residue and the resulting mixture was left for 24 h at room temperature. TA, DA and MA were completely hydrolyzed, which was confirmed by GC. A 200 mL of sat. NaCl was added to the reaction mixture. The compounds were extracted with three 50 mL portions of hexane and the combined extracts were washed with three 50 mL portions of sat. NaCl, dried over anhydrous sodium sulfate and evaporated to give (*S*)-enriched 2-octanol [(*S*)-82-OH]. Yield: 68-85%.

2·4 Products

2·4·1 Optically pure (*R*)-1-methylheptyl acetate

A mixture of optically pure (*R*)-82-OH (*ee*=0.995, 62 mmol), VA (120 mmol), hexane (50 mL) and PCL (7.0 g) was stirred for 24 h at 30°C and filtered (conversion: quantitative). The enzyme was washed with two 25 mL portions of hexane and the combined filtrates were evaporated. The residue was divided two equal portions. Each was dissolved with 30 mL of benzene and separately chromatographed (Silica gel 100g, ϕ 30 × 600 mm). The compounds were eluted with benzene. The ester fractions (300-800 mL) were combined and evaporated to give the desired optically pure (*R*)-1-methylheptyl acetate [(*R*)-82-OAc (*ee*=0.996)]. Yield: 90%.

2·4·2 Optically pure (*S*)-1-methylheptyl acetate

A mixture of optically pure (*S*)-82-OH (*ee*=0.995, 62 mmol), VA (120 mmol), hexane (50 mL) and PCL (7.0 g) was stirred for 4 d at 30°C (conversion: 90%). The reaction mixture was treated with the same method as described above (2·4·1), yielding the desired optically pure ester [(*S*)-82-OAc (*ee*=0.993)]. Yield: 95%.

3 Results and Discussion

3·1 Solvent Effect

According to the optical resolution theory (7-9), the enantioselectivity (*E*: the enantiomeric ratio) is expressed as Eq. (1) when the reaction is reversible:

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

where *K* is the equilibrium constant, *c* is the extent of conversion, and *ee_p* and *ee_s* are the optical purities in enantiomeric excess of the product and the unreacted (recovered) substrate, respectively.

When the reaction is irreversible (*K*=0), Eq. (1) is reduced to Eq. (2):

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]} = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]} \quad (2)$$

If *E* is calculated by Eq. (2) for the reversible reaction, it will essentially decrease as *c* increases (15). However, it is reported that *E* can be determined by Eq. (2) in the range of *c* where the reverse reaction is negligible even though the reaction is reversible (7).

First of all we screened more than a dozen of solvents for PCL catalyzed transesterification between VA and (*RS*)-82-OH at 30°C. The results were summarized in **Table 1**. Since the plots of ln(1-*c*) against the reaction time (*t*) showed a linearity at below 20% of conversion, the relative rate (=100 in hexane) was calculated from the apparent first order rate constant. The *E* values were determined by Eq. (2) using the optical resolution data (*ee_p*, *ee_s* and *c*) around 30 and 70% of conversions.

Table 1 showed that 1,2-dichloroethane, acetonitrile and acetone gave higher *E* than the other solvents. A similar tendency is reported for PCL catalyzed reversible transesterification between tributylglycerol (TB) and secondary alcohol in organic solvent (16-18). An exciting and significant observation in **Table 1** was that *E* around 30% of conversion was smaller than that around 70% for both transesterifications in chlorinated (carbon tetrachloride) and nonchlorinated solvents (dioxane, tetrahydrofuran, acetone and acetonitrile). This suggests that *E* increases with *c*, which is also supported by the fact that *E* was almost comparable between 30 and 70% of conversions for several solvents (**Table 1**).

Table 1 Solvent Effect on the Relative Rate and the Enantioselectivity (*E*) for PCL Catalyzed Transesterification between VA and Racemic 2-Octanol [(*RS*)-82-OH] in Organic Solvent at 30°C.

Solvent ^{a)}	Rate ^{b)}	<i>t</i> /min ^{d)}	<i>c</i> /° ^{d)}	<i>ee</i> _P /° ^{e)}	<i>E</i> /– ^{f)}	<i>t</i> /h ^{d)}	<i>c</i> /° ^{d)}	<i>ee</i> _S /° ^{g)}	<i>E</i> /– ^{f)}
Hex	100 ^{c)}	70	27	78	11	24	72	97	8
Hep	98	80	26	77	10	24	69	97	10
Oct	100	80	30	77	11	24	71	98	9
i-Oct	109	75	30	73	9	24	73	97	7
c-Hex	110	70	30	70	7	24	77	98	7
PhH	85	90	27	72	8	24	75	97	7
Tol	87	80	29	74	9	24	76	98	7
TCM	85	70	28	68	7	24	71	96	8
DCE	60	120	28	85	17	24	66	99	16
DO	44	150	31	71	8	24	70	98	10
IPE	82	80	31	73	9	24	80	97	6
THF	–	150	30	79	12	24	67	99	13
DMK	–	125	29	81	13	24	67	99	15
MCN	49	135	28	82	13	24	65	99	19

a) Hex: hexane, Hep: heptane, Oct: octane, i-Oct: isooctane (2,2,4-trimethylheptane), c-Hex: cyclohexane, PhH: benzene, Tol: toluene, TCM: carbon tetrachloride, DCE: 1,2-dichloroethane, DO: dioxane, IPE: diisopropyl ether, THF: tetrahydrofuran, DMK: acetone, MCN: acetonitrile.

b) The relative rates were calculated from the initial rate constants at below 20% of conversion under [VA]=1.63M, [82-OH]=0.53M and PCL=30 mg/mL-sub. soln.

c) The initial rate constant was $2.2 \times 10^{-6} \text{ s}^{-1}\text{mg}^{-1}\text{mL}$.

d) The reaction time (*t*) and conversion (*c*/°) under [VA]=1.53M, [82-OH]=0.88M and PCL=50 mg/mL-sub. soln.

e) Configuration of ester (**2**) was (*R*).

f) The *E* value was calculated from Eq. (2).

h) Configuration of the unreacted (recovered) alcohol was (*S*).

3·2 Analyses of Optical Resolution Data

In order to clarify the observation in **Table 1**, we analyzed the optical resolution data with use of Eq. (2) for the transesterification between VA and (*RS*)-82-OH in 1,2-dichloroethane and compared the reaction of VA with that of TA. The results were shown in **Fig. 1**. In the case of TA, *E* was essentially independent of *c* in the range of below 0.45 and sharply decreased with *c* in the range of above 0.55 (**Fig. 1B**). The former observation suggests that *K* in Eq. (1) is small and the latter demonstrates that the reaction is reversible. Analysis of the optical resolution data in **Fig. 1B** by Eq. (1) gave *E*=18 and *K*=0.01 except the plot at *c*=0.73. By contrast, *E* increased at first, maximized around 0.5 of *c* and then decreased with *c* for the transesterification with VA (**Fig. 1A**). This is an interesting fact because such a feature has not been reported for the enzymatic reaction in organic solvent so far. The decrease in *E* at higher *c*

(>0.5) may be caused by the reverse reaction.

Table 1 predicts that *E* is maximized against *c* in all cases (chlorinated and nonchlorinated) for PCL catalyzed transesterification with VA. In order to prove it, we investigated the correlation between log *E* and *c* for the transesterification between VA and 2-alkanol (**1**) using hexane, 1,2-dichloroethane and acetonitrile. The results were shown in **Fig. 2**. The log *E* vs. *c* plots showed the maxima for all cases and their features were different depending on the substrate and solvent. It is remarkable and interesting that *E* increases with *c* in the range of below 0.5 for the reaction even in nonchlorinated solvents such as hexane and acetonitrile. (**Fig. 2**), because it is reported that *E* is independent of *c* (<0.5) for PCL catalyzed transesterification between VA and racemic sulcatol in nonchlorinated solvent (toluene) (11). This may be caused by the substrate structure.

Fig. 1 Plots of the Enantioselectivity (E) against the Extent of Conversion (c) for PCL Catalyzed Transesterification with Racemic 2-Octanol (82-OH) in 1,2-Dichloroethane at 30°C.

A: [VA]=1.08-1.63 M, [82-OH]=0.53-0.88 M and PCL=30-50 mg/mL-sub. soln., B: [TA]=0.76-1.33 M, [82-OH]=0.61-0.88 M and PCL=100 mg/mL-sub. soln. The E values were determined by Eq. (2).

The resolution data in **Fig. 1B** fit Eq. (1) with $E=18$ and $K=0.01$ except $c=0.73$.

3.3 Competitive Reaction

According to the optical resolution theory (6-9), enantiomers in the racemate competitively react with the acyl-enzyme intermediate to give the corresponding products for the enzymatic transesterification. This assumption will be proved if the resolution data fit either Eq. (1) or Eq. (2). Unfortunately, PCL catalyzed transesterification with VA fit neither Eq. (1) nor Eq. (2) [Figs. 1A and 2], although the reaction with TA obeyed Eq. (1) with $E=18$ and $K=0.01$ (Fig. 1B). We therefore investigated the competitive reaction in the optical resolution.

The competitive reaction in the enzymatic optical resolution is simply expressed as follows (7):

where A and B are the fast and slow reacting enantiomers in the racemate, and P and Q are the products

Fig. 2 Plots of $\log E$ against c for PCL Catalyzed Transesterification between VA and Secondary Alcohol (1) in Organic Solvent at 30°C.

[VA]=1.63 M, [72-OH]=0.63 M, [82-OH]=0.56 M, [92-OH]=0.51 M, [102-OH]=0.46 M, [112-OH]=0.42 M, [122-OH]=0.39 M and PCL=30-100 mg/mL-sub. soln. The E values were determined by Eq. (2).

from A and B, respectively. From the definitions of ee_P , ee_S and c , one obtains

$$ee_P = \frac{[P] - [Q]}{[P] + [Q]} \quad (3)$$

$$ee_S = \frac{[B] - [A]}{[B] + [A]} \quad (4)$$

and

$$c = \frac{[P] + [Q]}{[A]_0 + [B]_0} \quad (5)$$

where $[A]$, $[B]$, $[P]$ and $[Q]$ are the concentrations of A, B, P and Q at time t and $[A]_0$ and $[B]_0$ are the initial concentrations of A and B, respectively.

From Eqs. (3)-(5), one obtains

$$c_A = c(1 + ee_P) = (1 - c)(1 - ee_S) \quad (6)$$

and

$$c_B = c(1 - ee_P) = (1 - c)(1 + ee_S) \quad (7)$$

where c_A and c_B are the extent of conversion for A and B, respectively. Eqs. (6) and (7) predict that c_A and c_B can be determined from the optical resolution data. In the PCL catalyzed transesterification, A and B are (*R*)- and (*S*)-(1), respectively.

Representative time courses of enantiomers in the racemate were shown in **Fig. 3A** for PCL catalyzed transesterification with VA and compared with those for TA (**Fig. 3B**). These demonstrate that both reactions are competitive with respect to the enantiomers. A similar competitive reaction (time course) was obtained from the data in **Fig. 3A** for the transesterification between VA and 2-alkanols (72-OH~122-OH) in hexane, 1,2-dichloroethane and acetonitrile.

Furthermore we tried to verify the competitive reaction using optically pure (*R*)- and (*S*)-82-OH for the transesterification with VA. From Eqs. (3)-(5) and $[A]_0 = [B]_0$, one obtains

$$ee_P = \frac{([P]/[A]_0) - ([Q]/[B]_0)}{([P]/[A]_0) + ([Q]/[B]_0)} = \frac{c_R - c_S}{c_R + c_S} \quad (8)$$

$$ee_S = \frac{([P]/[A]_0) - ([Q]/[B]_0)}{2 - \{([P]/[A]_0) + ([Q]/[B]_0)\}} = \frac{c_R - c_S}{2 - (c_R + c_S)} \quad (9)$$

Fig. 3 Time Courses of Enantiomers in Racemic 2-Octanol for PCL Catalyzed Transesterification in 1,2-Dichloroethane at 30°C.

A: $[VA] = 1.53$ M, $[82-OH] = 0.88$ M and $PCL = 50$ mg/mL-sub. soln.,

B: $[TA] = 1.33$ M, $[82-OH] = 0.77$ M and $PCL = 100$ mg/mL-sub. soln.

The conversions of the enantiomers were determined by Eqs. (6) and (7) using the optical resolution data (ee_P , ee_S and c).

and

$$c = \frac{c_R + c_S}{2} \quad (10)$$

where c_R and c_S are the extent of conversion for the transesterification with optically pure (*R*)- and (*S*)-82-OH, respectively. If the reaction is competitive with respect to the enantiomers, the plots of ee against c for the racemate should be in accordance with those determined by Eqs. (8)-(10) using the optically pure substrates. In fact, both plots showed an excellent agreement for the transesterification with VA as shown in **Fig. 4**. A similar agreement was also obtained for all transesterifications in **Fig. 2**.

Based on the above observations, we concluded that the transesterification with VA proceeds in a competitive manner as for TA (**Fig. 1A**) and TB (15).

3.4 Reverse Reaction

As shown in **Figs. 1A** and **2**, E decreased with c in the range of above 0.5 due to the reverse reaction. In the transesterification with VA, the reverse reaction is initi-

ated by acetylation of the free active Ser residue in PCL with the product (**2**) to give the corresponding alcohol and the resulting acetyl-enzyme intermediate was attacked by the unreacted alcohol. As a result, the optical purities (ee_P and ee_S) should be diminished. In order to prove it, we examined the change in ee for the reactions between optically pure 1-methylheptyl acetates [(*R*)- and (*S*)-82-OAc] with 2-octanols [(*R*)- and (*S*)-82-OH] in the presence of PCL as the models for the reversibility (**Scheme 3**). They are also the models for the reverse reaction in the transesterification with TA. As shown in **Tables 2** and **3**, the ee values of esters and alcohols were obviously decreased after incubation. This demonstrates that either (*R*)- or (*S*)-82-OH can act on the Ser residue in free PCL and that the former is a stronger acetylating reagent than the latter as expected.

3.5 Kinetic Studies

Our question is why E increased with c in the range of below 0.5 for PCL catalyzed transesterification with VA (**Figs. 1A** and **2**). In order to clarify it, we examined the first order plots [$\ln(1-c)$ vs. t] of enantiomers for the transesterification with VA in 1,2-dichloroethane. Rep-

Fig. 4 Plots of ee vs. c for PCL Catalyzed Transesterification between VA and Racemic and Optically Pure 2-Octanols in Organic Solvent at 30°C.

○ and □: the plots of ee_P vs. c and ee_S vs. c for the reactions with optically pure (*R*)- and (*S*)-82-OH, respectively. [VA]=1.00 M, [(*R*)-82-OH ($ee=0.995$)]=[(*S*)-82-OH ($ee=0.995$)]=0.57 M, PCL=100 mg/mL-sub. soln. The values of ee_P , ee_S and c were determined by Eqs. (8)-(10).

● and ■: the plots of ee_P vs. c and ee_S vs. c for the reaction with racemic 2-octanol, respectively. [VA]=1.53M, [(*RS*)-82-OH]=0.88M, PCL=100 mg/mL-sub. soln.

Scheme 3 PCL Catalyzed Reaction between Optically Pure 1-Methylheptyl Acetate [(*R*)- or (*S*)-82-OAc] and 2-Octanol [(*S*)- or (*R*)-82-OH] in Organic Solvent.

representative results were shown in **Fig. 5A**. The first order plot of (*R*)-82-OH in (*RS*)-82-OH gave a linear correlation but curvature for the (*S*) isomer. A similar result was obtained from the data in **Fig. 2** and their time courses for the transesterification with VA. The first order plots were also examined for the reactions of VA with optically pure 2-octanols. Representative results were shown in **Fig. 5B**, which demonstrates the same tendency as those for the racemate (**Fig. 5A**). A similar result was observed for the reactions with optically pure 2-octanols in all solvents described in **Table 1**.

In the transesterification with VA, the reaction proceeds *via*

and

where k_1 and k_2 are the rate constants. From Eqs. (11) and (12), one obtains

$$\frac{d[2]}{dt} = k_2[\text{Enz} - \text{Ac}][1] = k_a[1] \quad (13)$$

and

$$[\text{Enz}]_0 = [\text{Enz}] + [\text{Enz} - \text{Ac}] \quad (14)$$

where k_a is the apparent first order rate constant with respect to (1) and $[\text{Enz}]_0$ is the concentration of added PCL. The steady-state assumption on Enz-Ac gives

$$\begin{aligned} \frac{d[\text{Enz} - \text{Ac}]}{dt} &= k_1[\text{VA}][\text{Enz}] - k_2[\text{Enz} - \text{Ac}][1] \\ &= 0 \end{aligned} \quad (15)$$

From Eqs. (13)-(15),

$$k_a = \frac{k_1 k_2 [\text{VA}][\text{Enz}]_0}{k_1 [\text{VA}] + k_2 [1]} \quad (16)$$

is introduced.

If $k_1[\text{VA}] > k_2[1]$, *i.e.*, Eq. (12) is rate-determining, Eq. (16) will be reduced to,

$$k_a = k_2[\text{Enz}]_0 \quad (17)$$

which suggests that the first order plot should be a lin-

Table 2 PCL Catalyzed Reaction between Optically Pure (*R*)-1-Methylheptyl Acetate [(*R*)-82-OAc] with (*S*)-2-Octanol [(*S*)-82-OH] in Organic Solvent at 30°C^a.

Solvent ^b	<i>t</i> /h	(<i>R</i>)-82-OAc	(<i>S</i>)-82-OH
		<i>ee</i>	<i>ee</i>
Hex	72	0.912	0.755
DCE	72	0.979	0.865
MCN	72	0.956	0.807

a) [(*R*)-82-OAc (*ee*=0.996)]=0.14M, [(*S*)-82-OH (*ee*=0.995)]=0.19M, PCL=100 mg/mL-sub. soln.

b) Abbreviations were described in **Table 1**.

Table 3 PCL Catalyzed Reaction between Optically Pure (*S*)-1-Methylheptyl Acetate [(*S*)-82-OAc] with (*R*)-2-Octanol [(*R*)-82-OH] in Organic Solvent at 30°C^a.

Solvent ^b	<i>t</i> /h	(<i>S</i>)-82-OAc	(<i>R</i>)-82-OH
		<i>ee</i>	<i>ee</i>
Hex	72	0.944	0.948
DCE	72	0.989	0.981
MCN	72	0.982	0.976

a) [(*S*)-82-OAc (*ee*=0.993)]=0.14M, [(*R*)-82-OH (*ee*=0.995)]=0.19M, PCL=100 mg/mL-sub. soln.

b) Abbreviations were described in **Table 1**.

Fig. 5 The First Order Plots for PCL Catalyzed Transesterification between VA and Racemic and Optically Pure 2-Octanols in 1,2-Dichloroethane at 30°C.

A: the plots for enantiomers in (*RS*)-82-OH. [VA]=1.53 M, [(*RS*)-82-OH]=0.88 M and PLC=50 mg/mL-sub. soln.

B: the plots for optically (*R*)- and (*S*)-82-OH. [VA]=1.00 M, [(*R*)-82-OH (*ee*=0.995)]=[(*S*)-82-OH (*ee*=0.995)]=0.57 M and PLC=100 mg/mL-sub. soln.

ear correlation. This is the case for (*R*)-82-OH as shown in **Fig. 5**. The assumption of $k_1[\text{VA}] > k_2[\text{1}]$ is reasonable because the rate for the reaction of VA with 1-octanol was much faster than that with (*R*)-82-OH. These excluded the assumption of $k_1[\text{VA}] < k_2[\text{1}]$.

If $k_2[\text{1}]$ is not negligible,

$$\frac{1}{k_a} = \frac{1}{k_2[\text{Enz}]_0} + \frac{1}{k_1[\text{Enz}]_0} \cdot \frac{[\text{1}]}{[\text{VA}]} \quad (18)$$

is obtained from Eq. (17). In this paper, the reaction was conducted under $[\text{VA}] > [\text{1}]$. Thus, $[\text{1}]/[\text{VA}]$ decreases as the reaction proceeds and $1/k_a$ should be decreased with t because $k_1[\text{Enz}]_0 > 0$. This suggests that the time course should be a sigmoid, which was contrary to the results in **Fig. 3**.

Finally we examined the effects of additives on the transesterification rates for (*R*)- and (*S*)-82-OH in 1,2-dichloroethane. The results were summarized in **Table 4**. In this table, ethyl acetate and acetone were the model compounds of VA and acetaldehyde, and (*R*)- and (*S*)-82-OAc were the products. Unfortunately no change in the rates was observed. A similar tendency is reported for PCL catalyzed reaction of VA with racemic sulcatol (11).

Based on the above observations, we concluded that acetylation of (*S*) isomer in the racemate with the

Table 4 Effect of Additives on the Relative Rate for PCL Catalyzed Transesterification between VA and Optically Pure 2-Octanols [(*R*)- and (*S*)-82-OH] in 1,2-Dichloroethane at 30°C^a).

Additive	Relative Rate/%	
	(<i>R</i>)-82-OH ^b)	(<i>S</i>)-82-OH ^c)
None	100	100
Acetone	100 ± 2	101 ± 5
Ethyl acetate	98 ± 2	101 ± 2
(<i>R</i>)-82-OAc	99 ± 2	97 ± 4
(<i>S</i>)-82-OAc	99 ± 2	100 ± 5

a) [VA]=0.46 M, [(*R*)-82-OH (*ee*=0.995)]=0.14 M, [(*S*)-82-OH (*ee*=0.995)]=0.14 M, [(*R*)-82-OAc (*ee*=0.996)]=28 mM, [(*S*)-82-OAc (*ee*=0.993)]=28 mM, [Acetone]=[Ethyl acetate]=0.042 vol%, PCL=50 mg/mL-sub.soln.

b) The relative rates were calculated from the apparent first order rate constants.

c) The relative rates were calculated from the conversion at $t=1, 2, 3, 4, 5, 6, 7, 8$ h because the first order plots showed curvature (see **Fig. 5**).

acetyl-enzyme intermediate [Eq. (12)] was suppressed during the reaction and thus E increased with c in the range of below 0.5 for the transesterification with VA. As a result, the optical resolution data did not fit the reported resolution equations [Eqs. (1) and (2)] as shown in **Figs. 1A** and **2**.

References

1. Y.-F. WANG, J.J. LALONDE, M. MOMONGAN, D.E. BERGBREITER and C.-H. WONG, *J. Am. Chem. Soc.*, Vol. **110**, 7200-7205 (1988).
2. E. SANTANIELLO, P. FERRABOSCHI, G. GRISENTI and A. MANZOCCHI, *Chem. Rev.*, Vol. **92**, 1071-1140 (1992).
3. K. NAEMURA, *J. Synth. Org. Chem. Jpn.*, Vol. **52**, 49-57 (1994).
4. K. NAKAMURA and Y. HIROSE, *J. Synth. Org. Chem. Jpn.*, Vol. **53**, 668-677 (1995).
5. The recent papers in *Tetrahedron:Asymmetry*.
6. C.-S. CHEN, Y. FUJIMOTO, G. GIRDAUKAS and C.J. SIH, *J. Am. Chem. Soc.*, Vol. **104**, 7294-7299 (1982).
7. C.-S. CHEN, S.-H. WU, G. GIRDAUKAS and C.J. SIH, *J. Am. Chem. Soc.*, Vol. **109**, 2812-2817 (1987).
8. C.-S. CHEN and C.J. SIH, *Angew. Chem. Int. Ed. Engl.*, Vol. **28**, 695-707 (1989).
9. C.-S. CHEN and Y.-C. LIU, *J. Jpn. Oil Chem. Soc.*, Vol. **41**, 724-733 (1992).
10. M. LUNDH, O. NORDIN, E. HEDENSTÖRM and H.-E. HÖGBERG, *Tetrahedron:Asymmetry*, Vol. **6**, 2237-2244 (1995).
11. F. SECUNDO, G. OTTOLINA, S. RIVA and G. CARREA, *Tetrahedron:Asymmetry*, Vol. **8**, 2167-2173 (1997).
12. H. HIRATA, T. KITAMOTO, K. SEKIZAKI, N. INADA, K. YAMADA, M. MAYAMA and H. YANAGISHITA, *Annual Meeting of Jpn. Oil Chem. Soc.*, (Nagoya) 2B-19 (1995).
13. J.A. RIDDICK and W.B. BUNGER, *Organic Solvents*. IV, Wiley-Interscience, New York, London, Sydney, Tronto (1970).
14. H. HIRATA, T. YAMASHINA, K. HIGUCHI, K. SAKAKI and I. IIDA, *J. Jpn. Oil Chem. Soc.*, Vol. **40**, 995-1001 (1991).
15. H. HIRATA, M. MAYAMA, N. INADA, K. YAMADA, H. YANAGISHITA and M. SUGIURA, *J. Jpn. Oil Chem. Soc.*, Vol. **44**, 1067-1074 (1995).
16. H. HIRATA, M. MIYAGISHI and M. MAYAMA, *Nippon Kagaku Kaishi*, Vol. **1998**, 113-118 (1998).
17. H. HIRATA, M. MAYAMA, H. YANAGISHITA and M. SUGIURA, *J. Jpn. Oil Chem. Soc.*, Vol. **47**, 1217-1223 (1998).
18. H. HIRATA, M. MAYAMA, M. OHNO, N. KAMO and H. YANAGISHITA, *Nippon Kagaku Kaishi*, Vol. **2002**, 147-154 (2002).