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Enzymatic kinetic resolution of primary alcohols by direct esterification in solvent-free system

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Abstract

Direct enzymatic esterification catalyzed by immobilized *Candida antarctica* lipase B (CALB) and *Rhizomucor miehei* lipase (RML) was evaluated for kinetic resolution of some primary alcohols with a chiral center at the next carbon atom: 2-methoxy-2-phenylethanol (**1**), 2-phenyl-1-propanol (**2**) and 1-phenyl-1,2-ethanediol (**3**). The reactions were performed in solvent-free system with removal of water at low pressure. CALB was superior to RML in both reaction rates and enantioselectivity. The influence of acid species on enantioselectivity of CALB was studied on esterification of **1**. In a series of free fatty acids, the highest enantioselectivity value was obtained for decanoic acid. Among other acid species investigated, 4-oxopentanoic acid gave the best results. The position of the double bond in pentenoic acid affected the reaction rate and enantioselectivity. Enantioselectivity of CALB increased significantly with reducing the reaction temperature. Direct esterification for kinetic resolution of **2** and **3** was also investigated.

RML has a stricter substrate selectivity towards both: the acid and alcohol. Lowering the reaction temperature had no effect on enantioselectivity.

Keywords: Enzymatic kinetic resolution; Esterification; Lipase; Primary alcohol; Solvent-free system

1. Introduction

Homochiral primary alcohols are valuable intermediates in asymmetric synthesis. While lipase-catalyzed asymmetric transformations are very efficient tools for preparation of enantiomerically pure secondary alcohols [1], kinetic resolution of racemates of primary alcohols by the same method is more difficult to achieve. This is due to the much lower enantioselectivity of lipases towards chiral primary alcohols. Various reaction factors affecting enantioselectivity were investigated in previous studies: the solvent [2,3], temperature [4], acyl donor [2,5–7], etc. Also, the nature of lipase and the substrate structure determined the enantioselectivity of a certain transformation [8–10].

To our knowledge, there are no studies to date on kinetic resolution of primary alcohols in solvent-free systems with displacement of reaction equilibrium by the removal of a volatile reaction co-product under reduced pressure. Such systems offer some practical advantages over the systems employing organic solvents as reaction media. Beside the advantage of no need of organic solvents as reaction media, these reaction systems are more compact and have a higher volumetric productivity. Also, lower amount ratio of catalyst to reactants is necessary. Removal of a volatile reaction co-product under reduced pressure (in the present study: water) ensures an efficient way of displacement of reaction equilibrium in favor of ester formation and therefore, allows reducing the excess of the acyl donor employed to only a few percents.

The most employed acylating agents for kinetic resolutions of alcohols are vinyl esters [3,5,7]. The structure of the acyl moiety was shown to affect the observed enantioselectivity very much. As free acids are more stable chemically, less toxic, cheaper and more readily available, it is worthwhile investigating their efficiency as acyl donors in asymmetric esterification of chiral primary alcohols. The enantiomers of some secondary alcohols were very efficiently separated by esterification with free fatty acids

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under low pressure in solvent-free system in one of our recent studies [11], and therefore, we attempted to extend the application of this method to primary alcohols extending also the range of the carboxylic acids investigated.

In the present study, lipase-catalyzed esterification in solvent-free system was evaluated as a method for kinetic resolution of some primary alcohols with a chiral center at the next carbon atom: 2-methoxy-2-phenylethanol (**1**), 2-phenyl-1-propanol (**2**) and 1-phenyl-1,2-ethanediol (**3**). We selected these three compounds because they have been difficult to resolve by lipase-catalyzed reactions at the primary hydroxyl group, and also, because their enantiomers are important intermediates and building blocks for various synthetic applications [2,5,10,12]. A range of carboxylic acids were used as acyl donors. Formed water was removed from the system by evaporation at low pressure pushing the reaction equilibrium towards ester formation. Immobilized *Candida antarctica* lipase B (CALB) and *Rhizomucor miehei* lipase (RML) which can maintain their activity in systems with very low water content under reduced pressure were chosen as catalysts [13,14]. The temperature effect on reaction rate and enantioselectivity was also investigated.

2. Materials and methods

2.1. Materials and enzymes

Decanoic acid (>99%), octanoic acid (>99%), hexanoic acid (>99%), pentanoic acid (>95%), 4-pentenoic acid (>98%) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). *cis*-9-Octadecenoic acid (99%) was from Sigma. *trans*-3-Pentenoic acid (>90% *trans* form, >95% sum of isomers) was obtained from Fluka. *trans*-2-Pentenoic acid was from Fluorochem (Derbys, UK). 4-Oxopentanoic acid was bought from Nakalai Tesque Inc. (Kyoto, Japan). 2-Methoxy-2-phenylethanol was from Aldrich. 2-Phenyl-1-propanol and 1-phenyl-1,2-ethanediol came from Tokyo Kasei Kogyo (Tokyo, Japan). Other chemicals used were of analytical grade.

Immobilized *C. antarctica* B lipase (Chirazyme L-2, c.-f, C2, Lyo.) (EC 3.1.1.1.) was purchased from Roche Diagnostics (Mannheim, Germany). Immobilized *R. miehei* lipase (Lipozyme® RM IM) (EC 3.1.1.1.) was kindly donated by Novozymes A/S (Bagsvaerd, Denmark).

2.2. Lipase-catalyzed esterification of primary alcohols in solvent-free system

Primary alcohol (10 mmol) and acid (5.5 mmol) were stirred on a magnetic stirrer (200 rpm) at a specified temperature in a thermostated water bath. Immobilized enzyme (0.23 g) was added and the reaction vessel was connected to a vacuum pump through a liquid nitrogen trap. The pressure was maintained at 5 mmHg. When different conditions were used, they are specified in text where the results are discussed.

2.3. Analysis

Intermittently, 15 μl samples were withdrawn from the reaction mixture, dissolved in hexane/2-propanol = 99/1 and applied to an aminopropyl silica separation pack (BondElut® NH_2) (Varian, Harbor City, USA). The unreacted alcohol and the formed ester were eluted selectively with hexane and ethyl acetate, respectively. The unreacted carboxylic acid was retained in the pack. The solvent was evaporated from both fractions. The concentrates were dissolved in the solvent used for HPLC analysis of each fraction.

For some compounds, the ester fraction was subjected to hydrolysis prior HPLC analysis. The ester concentrate was dissolved in 2 ml NaOH solution (0.5N in methanol) in a 15 ml polypropylene tube with a screw cap. The tube was heated at 80 °C for 30 min. Two milliliter hexane/diethyl ether = 7/3 was added and shook well. Five milliliter saturated NaCl solution was added. The organic layer was collected, dried with anhydrous $MgSO_4$ and the solvent evaporated. The concentrate was dissolved in the solvent used for HPLC analysis.

The enantiomers of **1** and **2** were separated by HPLC analysis on a Chiralpak AD-H column (Daicel Chemical Industries, Tokyo, Japan) with UV detection at 254 nm. The elution was performed with hexane/2-propanol = 95/5 and 99/1, respectively, at 1 ml/min flow rate and 20 °C. The enantiomeric composition of **3** was determined on a Chiralcel OB-H (Daicel Chemical Industries, Tokyo, Japan) column eluted with hexane/2-propanol = 80/20 at 0.5 ml/min and 20 °C.

The enantiomeric composition of the esters resulted from the reaction of **1** with *cis*-9-octadecenoic acid and **2** with 4-pentenoic acid was determined after hydrolysis to alcohol.

Separation of the enantiomers of decanoic, octanoic, hexanoic, pentanoic, 4-pentenoic, 3-pentenoic and 2-pentenoic acid esters of **1**, and octanoic acid ester of **2** was performed on a Chiralpak AD-H column with hexane/2-propanol = 99/1 at 1 ml/min flow rate and 20 °C. All six possible enantiomers of the esters of compound **3** with 4-pentanoic acid (primary and secondary monoesters, and diester), and the enantiomers of 4-oxopentanoic acid ester of **1** and **2** were separated on the same column and conditions with hexane/2-propanol = 95/5.

Conversion (c) and enantioselectivity (E) were calculated according to Rackels et al. [15] with the formulas:

$$c = \frac{ee_A}{ee_A + ee_E} \quad (1)$$

$$E = \frac{\ln[(1 - eeA)/(1 + eeA/eeE)]}{\ln[(1 + eeA)/(1 + eeA/eeE)]} \quad (2)$$

ee_A and ee_E represent the enatiomeric excess of residual alcohol and formed ester, respectively.

The *R*-isomers of alcohol **1** and **3** were preferentially esterified by both CALB and RML. In the case of alcohol **2**, the *S*-isomer was preferred.

3. Results and discussion

3.1. Enantioselective esterification of primary alcohols catalyzed by CALB

It was demonstrated by previous studies that the structure and size of the acyl donor influence considerably enantioselectivity in lipase-catalyzed direct esterification of secondary alcohols in organic solvents. The esterification with longer chain acids such as decanoic or hexanoic acids had better enantioselectivity than that with shorter chain acids [16,17].

The effect of the acid species used as acyl donors on enantioselectivity of CALB were investigated in direct esterification of *rac*-**1** (Table 1). The acyl donors used in reaction systems under reduced pressure should have relatively high boiling points at normal pressure to minimize their losses by evaporation. Therefore, acids with five or more carbon atoms were investigated.

Enantioselectivity increased slightly with the increase of the chain length from hexanoic to decanoic acid. The use of *cis*-9-octadecenoic acid (a common fatty acid in vegetable oil) resulted in both lower enantioselectivity and reaction rate. Pentanoic acid produced the best results (highest enantioselectivity and initial reaction rate) in the series of normal chain saturated acids. The enantioselectivity varied as follows: pentanoic > decanoic > octanoic > hexanoic acid. The reaction rates for pentanoic and hexanoic acid had very close values and were double the rates for octanoic and decanoic acid (which were very close also) (Table 1).

The introduction of a double bond in the molecule of pentanoic acid influenced enantioselectivity and initial reaction rate depending on its position. Both parameters decreased in the following order: pentanoic > 4-pentenoic > *trans*-3-pentenoic > *trans*-2-pentenoic acid. The results suggest that the steric and (or) the electronic effects induced by the presence of a double bond in molecule contribute to the modification of the active site environment resulting in a decrease of both enantioselectivity and the acyl specificity (estimated as the initial reaction rate) of CALB with the double bond closeness to the carboxylic group. 4-Pentenoic acid had very close values of enantioselectivity and initial rate to that of pentanoic acid. An explanation might be that double bound is far enough from the acyl group so that the differences in the steric and electronic effects on the formation of the active acyl–enzyme complex become negligible. The 4-pentenoyl group can be readily cleaved under mild conditions, and therefore, used for protection of labile intermediates in organic syntheses [18,19].

4-Oxopentanoic acid, which was used successfully in the protection of oligonucleotides, is another easily removable group under mild deprotection conditions [20]. In our study, it gave the highest enantioselectivity value for the esterification of **1** (more than double of that for pentanoic acid). The initial reaction rate was half reduced than that for 4-pentenoic acid. Some improvement of enantioselectivity was observed also for esterification of alcohol **2** (Table 2), and also, the reaction rate was lower than when 4-pentenoic acid was used.

Reduced temperature improved significantly the enantioselectivity of CALB (Table 2). Enantioselectivity increased three folds in esterification of **1** with 4-pentenoic acid by lowering the temperature from 25 to 0 °C. An increase of enantioselectivity was observed also for the reaction of **2** with the same acid.

The three primary alcohols used in this study differ only by one substituent at the chiral carbon atom. In the reaction with 4-pentenoic acid, the enantioselectivity decreased with the volume of the substituent at the chiral center in the order methoxy- for **1** > methyl- for **2** > hydroxy- for **3** (Table 2). The lipase specificity (expressed as the initial reaction rate) was the best for alcohol **2**, decreased markedly for **1** and was low for **3**.

The esterification of diol **3** with 4-pentenoic acid was almost not enantioselective ($E = 1.5$). No detectable reaction

Table 1
Effect of acid species on esterification of 2-methoxy-2-phenylethanol by CALB at 25 °C

Acid species	Initial rate (μmol/min mg)[a]	Reaction time (min)	Conversion alcohol (%)	ee_A[b] (%)	ee_E[c] (%)	*E*
4-Oxopentanoic	0.36	30	25.2	30.9	91.8	31
4-Pentenoic	0.85	15	29.2	34.0	82.4	14
trans-3-Pentenoic	0.53	30	30.4	34.1	78.2	11
trans-2-Pentenoic	0.10	120	27.0	19.2	52.1	4
Pentanoic	0.93	15	32.2	38.9	82.1	15
Hexanoic	0.81	15	27.9	29.6	76.4	10
Octanoic	0.35	30	22.2	22.8	79.0	11
Decanoic	0.38	30	25.5	27.4	80.0	12
cis-9-Octadecenoic	0.19	60	26.3	26.3	73.8	9

[a] Amount of alcohol (μmol) esterified per minute and milligram of immobilized enzyme.
[b] Enantiomeric excess of residual *S*-alcohol.
[c] Enantiomeric excess of formed *R*-ester.

Table 2
Direct esterification of primary alcohols catalyzed by CALB

Acid species	Alcohol species	Temperature (°C)	Initial rate (μmol/min mg)[a]	Reaction time (min)	Conversion alcohol (%)	ee_A[b] (%)	ee_E[c] (%)	*E*
4-Oxopentanoic	2-Methoxy-2-phenylethanol	25	0.36	30	25.2	30.9	91.8	31
4-Pentenoic	2-Methoxy-2-phenylethanol	25	0.85	15	29.2	34.0	82.4	14
4-Pentenoic	2-Methoxy-2-phenylethanol	0	0.17	90	28.1	36.5	93.6	43
4-Oxopentanoic	2-Phenyl-1-propanol	0	0.12	120	33.1	39.5	80.0	13
4-Pentenoic	2-Phenyl-1-propanol	0	0.20	60	26.9	27.5	74.7	9
4-Pentenoic	2-Phenyl-1-propanol	25	1.32	10	30.3	22.2	50.1	4
4-Pentenoic	1-Phenyl-1,2-ethanediol[d]	25	0.19	120	25.2	6.0	17.6	1.5

[a] Amount of alcohol (μmol) esterified per minute and milligram of immobilized enzyme.
[b] Enantiomeric excess of residual alcohol.
[c] Enantiomeric excess of formed ester.
[d] The reaction was performed with 10 mmol 1-phenyl-1,2-ethanediol and 20 mmol 4-pentenoic acid.

at the chiral secondary hydroxyl group was observed at the specified reaction time (2 h).

3.2. Enantioselective esterification of primary alcohols catalyzed by RML

RML behaved very differently from CALB (Table 3). It displayed lower enantioselectivity, and stricter substrate selectivity for both: alcohol and acid. The results in Table 3 indicate that octanoic acid is the best acyl donor. These results are in accordance with a previous report showing that octanoic acid (a natural substrate for RML) was an especially good acyl donor for enantioselective esterification of secondary alcohols in organic solvents [16]. Alcohol **1** reacted only with octanoic acid at a markedly reduced reaction rate than **2**, but the enantioselectivity was better. The reaction rate was drastically reduced for esterification of **2** with 4-pentenoic acid. 4-Oxopentanoic acid was unreactive in esterification of both **1** and **2**. Reduced temperature did not improve the enantioselectivity of RML. No reaction was observed when the diol **3** was reacted with 4-pentenoic acid.

3.3. Discussion

In the enzymatic resolution of a specific chiral compound, the choice of enzyme is a crucial step for achieving the desired separation of enantiomers. Lipase catalyzed asymmetric reactions are widely used in organic chemistry today. Among the lipases employed, CALB is one of the most useful. However, it was thought that it had a too low or no enantioselectivity for primary alcohols [1]. Therefore, it was often not taken into consideration. In our study, it actually displayed a higher enantioselectivity for compound **2** than RML, and the previously reported results with *Pseudomonas lipases* (considered the best for resolution of primary alcohols) for acylation with vinyl acetate in organic solvents [4,10].

The choice of acyl donor is also of great importance. 4-Oxopentanoic acid produced the best results for resolution of **1** and **2**. 4-Pentenoic acid is a good acyl donor also. These acyl groups are easily removable at mild conditions, and therefore, might be used in enzymatic protection of hydroxyl groups in organic syntheses.

Very much improved enantioselectivity for CALB was obtained at reduced temperature in direct esterification at low pressure. The enantioselectivity for the esterification of **1** with 4-pentenoic acid at 0 °C had a similar value to the acylation by CALB with vinyl acetate—the best result for enzymatic resolution of **1** to date [2]. The influence of the reaction temperature on enantioselectivity appears to depend on the nature of the reaction involved. Enantioselectivity of acylation with vinyl acetate by CALB did not improved at reduced temperature [2].

Table 3
Direct esterification of primary alcohols catalyzed by RML

Acid species	Alcohol species	Temperature (°C)	Initial rate (μmol/min mg)[a]	Reaction time (h)	Conversion alcohol (%)	ee_A[b] (%)	ee_E[c] (%)	*E*
Octanoic	2-Methoxy-2-phenylethanol	0	0.03	4	15.4	10.8	59.5	4.4
Octanoic	2-Methoxy-2-phenylethanol	25	0.07	3	29.3	23.6	57.2	4.6
4-Pentenoic	2-Methoxy-2-phenylethanol	25	–	24	0	–	–	–
4-Oxopentanoic	2-Methoxy-2-phenylethanol	25	–	24	0	–	–	–
Octanoic	2-Phenyl-1-propanol	25	0.26	1	36.17	12.7	8.3	1.3
4-Pentenoic	2-Phenyl-1-propanol	25	0.002	24	5.1	1.7	32.5	2
4-Oxopentanoic	2-Phenyl-1-propanol	40	–	24	0	–	–	–
4-Pentenoic	1-Phenyl-1,2-ethanediol	25	–	24	0	–	–	–

[a] Amount of alcohol (μmol) esterified per minute and milligram of immobilized enzyme.
[b] Enantiomeric excess of residual alcohol.
[c] Enantiomeric excess of formed ester.

In conclusion, direct enzymatic esterification under reduced pressure has good potential applicability for kinetic resolution of primary alcohols. Good *E* can be achieved by an appropriate selection of the reaction parameters. The simplicity of this reaction system, high volumetric productivity, the ease of scale-up and down-stream processes, give this method a great potential for industrial applications.

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