Enzymatic Kinetic Resolution of Secondary Alcohols by Esterification with FA Under Vacuum

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ABSTRACT: Kinetic resolution of some chiral secondary alcohols [2-octanol, 1-phenylethanol, and 1-(2-naphthyl)ethanol] with high enantioselectivity ($E > 300$) was achieved by direct esterification with FFA catalyzed by immobilized *Candida antarctica* B lipase. The reaction equilibrium was shifted toward the synthetic side by the removal of the water formed under vacuum. Esterification of *rac*-2-octanol at an alcohol/FFA molar ratio of 2:1 was used as a model reaction. The chain length of FFA and their structure influenced the reaction rate but did not have a measurable effect on *E*. The best acyl donor was decanoic acid: >47% conversion at 4 h with virtually perfect *E*. Temperature did not affect *E* in the range studied (15–45°C), but temperatures at the higher end afforded improved reaction rates. The reaction rates and *E* were compared for three different acyl donors. The initial reaction rate increased in the following order: ethyl laurate < lauric acid < vinyl acetate. *E* was high ($E > 300$) for all acyl donors. Racemic 1-phenylethanol and 1-(2-naphthyl)ethanol were also resolved by direct esterification with decanoic acid in short times (3 and 4 h, respectively) with $E > 300$ and excellent conversions. Preparative-scale kinetic resolution of 2-octanol was also performed.

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KEY WORDS: Enantioselectivity, enzymatic kinetic resolution, free fatty acid, lipase, secondary alcohol, vacuum.

Optically pure secondary alcohols are important chiral synthons for industrially and pharmaceutically useful compounds. Enzymatic kinetic resolutions of racemates are often the least expensive and the most practical routes to obtain enantiopure compounds. Lipases are well known as effective stereoselective catalysts for a wide range of reactions including the acylation of secondary alcohols (1). The kinetic resolution of secondary alcohols catalyzed by lipases was studied extensively. Displacement of the reaction equilibrium in favor of product formation is crucial for the achievement of high reaction yields. An impressive number of studies have concentrated on using irreversible acyl transfer reactions, yet only a few of them have reported on the removal of a volatile reaction co-product as a means for displacing the reaction equilibrium (2–5).

An optimal reaction system would be one in which solvent is not necessary as a reaction medium, one which uses an inexpensive and readily available acyl donor, with a facile means of reaction equilibrium displacement, and one which affords simple separation and recovery of both enantiomers.

Direct esterification with FFA has been used less for the kinetic resolution of alcohols due to problems related to water formation. Water resulting from the reaction is more difficult to remove from the reaction system than the more volatile ethanethiol or ethanol resulting from the use of thioethyl esters or ethyl esters as acyl donors (3). Lower pressure applied to the reaction system is required to reach the b.p. of water at a certain temperature. At such low pressure, the water molecules attached to the enzyme (which are essential for maintaining its catalytic activity) are also removed, leading to enzyme inactivation (6). The modified water activity of the system might also affect the biocatalyst's enantioselectivity.

The *Candida antarctica* B lipase (CALB) used in this work is a very versatile and robust enzyme. It is used in a wide range of synthetic applications including enantioselective reactions (5,7). Immobilization on a macroporous resin renders the lipase active at the extremely low water activities of the reaction systems under reduced pressure (5,8,9) without modification of enantioselectivity (10).

FFA offer several advantages for the kinetic resolution of alcohols. FFA are inexpensive, readily available, and chemically stable compounds. They are natural substrates for lipases; hence, substrate inactivation of the enzyme is avoided. Water is the co-product of the reaction, and, unlike other co-products such as acetaldehyde that result from the use of vinyl esters as acyl donors, it is harmless to the enzyme. Also, the unreacted alcohol is easily separated from the resulting esters by distillation due to their large difference in b.p.

The enzymatic kinetic resolution of secondary alcohols by direct esterification with FFA under vacuum was studied in this work. Immobilized CALB was used as catalyst. The effects of FFA chain length and temperature on the esterification of *rac*-2-octanol were studied. The efficiency of FFA, FFA ethyl ester, and vinyl acetate as acyl donors for kinetic resolution of *rac*-2-octanol was investigated. The above method was applied to the kinetic resolution of *rac*-1-phenylethanol and *rac*-1-(2-naphthyl)ethanol.

EXPERIMENTAL PROCEDURES

Materials. *Rac*-2-octanol (>98%), *rac*-1-phenylethanol (>98%), *rac*-1-(2-naphthyl)ethanol (>99%), caprylic (octanoic)

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acid (>99%), capric (decanoic) acid (>99%), lauric (dodecanoic) acid (>99%), and palmitic (hexadecanoic) acid (>95%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stearic (octadecanoic) acid (99%) was obtained from ICN Biochemicals Inc. (Aurora, OH). Oleic (*cis*-9-octadecenoic) acid (99%) and myristic (tetradecanoic) acid (99%) were from Sigma (St. Louis, MO). Other chemicals used were of analytical grade.

Immobilized CALB (Chirazyme L-2, c.-f, C2, Lyo.) (EC 3.1.1.1.) was purchased from Roche Diagnostics (Mannheim, Germany).

Lipase-catalyzed kinetic resolution of secondary alcohols in a solvent-free system. Secondary alcohol (20 mmol) and FFA (10 mmol) were stirred with a magnetic stirrer (200 rpm) at a specified temperature in a thermostated water bath. CALB (0.115 g) was added and the reaction vessel was connected to a vacuum pump. The pressure was maintained at 5 mm Hg. When different conditions were used, they are specified in text where the results are discussed.

Preparative kinetic resolution of 2-octanol. Rac-2-octanol (13 g, 100 mmol) and decanoic acid (9.5 g, 55 mmol) were stirred with a magnetic stirrer (300 rpm) at 45°C. The reaction was started by addition of CALB (1.15 g) and the vessel was maintained under 5 mm Hg vacuum. The reaction was stopped by filtering the catalyst at 6 h when the conversion of the alcohol had reached about 50%. The catalyst was then washed with ethyl acetate. *S*-2-Octanol was distilled from the filtered reaction mixture at 52–54°C (6 mm Hg) to yield 5.64 g, >99% pure by GC, 97% enantiomeric excess (ee) (84% yield after isolation). The distillation residue was neutralized with a 2 M NaOH solution, and 2-octyldecanoate was extracted twice with hexane. The organic fraction was washed twice with water and dried over anhydrous $MgSO_4$. *R*-2-Octyldecanoate (13.58 g), 98% pure by GC, >99% ee (94% yield after isolation), was obtained.

Analysis. Intermittently, 15 μL samples were withdrawn from the reaction mixture, dissolved in hexane, and applied to an aminopropyl silica separation pack (BondElut® NH_2; Varian, Harbor City, CA). The unreacted alcohol and the ester formed were eluted selectively with hexane and ethyl acetate, respectively. The unreacted FFA were retained in the pack. The solvent was evaporated from both fractions, and the ester fraction was subjected to hydrolysis.

The ester concentrate was dissolved in 2 mL NaOH (0.5 N in methanol) in a 15-mL polypropylene tube with a screw cap. The tube was heated at 80°C for 30 min. Two milliliters of hexane/ethyl acetate at a volumetric ratio of 7:3 was added and shaken well. Five milliliters of saturated NaCl solution was then added. The organic layer was collected, dried with anhydrous $MgSO_4$, and the solvent evaporated.

Both the unreacted alcohol fraction and the alcohol resulting from hydrolysis of the ester fraction formed were derivatized by acetylation. The alcohol was dissolved in a mixture of 0.6 mL pyridine and 0.2 mL acetic anhydride in a 15-mL polypropylene tube with a screw cap and heated for 1 h at 80°C. Two milliliters of water was added and incubated for 10 min at 80°C. The acetyl ester was then extracted with 2 mL hexane. The hexane layer was washed with 5 mL water, dried over anhydrous $MgSO_4$, and used for analysis by GC.

No racemization was observed during hydrolysis or derivatization, as checked with *S*-2-octanol that underwent the procedure for hydrolysis followed by acetylation.

The enantiomeric composition of the derivatized fractions was determined by a gas chromatograph (GC-17 AAFW V3; Shimadzu, Kyoto, Japan) equipped with an FID and a WCOT fused-silica column (50 m × 0.25 mm) coated with CP-Cyclodex B 236M (df = 0.25 μm; Chrompack, Middleburg, The Netherlands). The carrier gas was helium, and the column temperature was 130°C.

R- and *S*-1-phenylethanol were separated without derivatization by the above-mentioned column at 130°C. The enantiomeric composition of the ester resulting from the reaction with decanoic acid was determined after its hydrolysis to alcohol.

R- and *S*-1-(2-naphthyl)ethanol were separated by HPLC analysis on a Chiracel OJ column (Daicel Chemical Industries, Tokyo, Japan) with UV detection at 254 nm. The elution was performed with hexane/isopropanol with a volumetric ratio of 85:15 at a 1 mL/min flow rate and 30°C. The enantiomeric composition of the ester resulting from the reaction with decanoic acid was determined after its hydrolysis to alcohol.

Conversion (c) and enantioselectivity (E) were calculated according to Rackels *et al*. (11) with the following formulas:

$$c = \frac{ee_A}{ee_A + ee_E} \quad [1]$$

$$E = \frac{\ln\left[\frac{1 - ee_A}{1 + \frac{ee_A}{ee_E}}\right]}{\ln\left[\frac{1 + ee_A}{1 + \frac{ee_A}{ee_E}}\right]} \quad [2]$$

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

RESULTS AND DISCUSSION

Effect of FFA chain length on the kinetic resolution of 2-octanol. The most common and affordable FFA were chosen as acyl donors for the present study. The alcohols and acyl donors used should have relatively high b.p. at normal pressure (over 170°C) to minimize the losses by evaporation at low pressure. Therefore, FFA with six or more carbon atoms were investigated.

A previous study showed that the E of CALB in the transesterification of 3-methylbutanol with vinyl esters was influenced by the chain length of the acyl donor (12). E increased with the

TABLE 1
Effect of FA Chain Length and Temperature on the Kinetic Resolution of 2-Octanol

FA	Temperature (°C)	Initial rate (μmol/min·mg)[a]	Reaction time (min)	Conversion of alcohol (%)	ee_A[b] (%)	ee_E[c] (%)	Enantioselectivity *E*
Hexanoic	25	0.86	360	48.6	94.5	>99	>300
Octanoic	25	0.65	300	47.2	89.4	>99	>300
Decanoic	25	1.12	240	47.7	91.1	>99	>300
Dodecanoic	15	0.46	360	45.0	82.0	>99	>300
Dodecanoic	25	0.94	180	42.9	75.2	>99	>300
Dodecanoic	35	1.37	180	47.1	88.9	>99	>300
Dodecanoic	45	1.73	120	46.0	85.2	>99	>300
Tetradecanoic	45	1.76	180	48.7	95.1	>99	>300
Hexadecanoic	45	1.48	240	48.7	95.1	>99	>300
cis-9-Octadecenoic	25	0.42	300	44.4	79.7	>99	>300
cis-9-Octadecenoic	45	1.01	180	49.0	95.9	>99	>300
Octadecanoic	45	0.91	180	46.6	88.4	>99	>300

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

chain length of the vinyl ester. The longest, vinyl octanoate, afforded the highest *E*.

In direct esterification of *rac*-2-octanol, *E* was very good for all FFA species used (>>300), without significant differences at such high values. Substrate specificity of the lipase (calculated as the initial reaction rates) determined the acylation efficiency of FFA (Table 1). In the series hexanoic, octanoic, decanoic, and dodecanoic acid, the initial reaction rate at 25°C was the lowest for octanoic acid. The remaining three had close values. Starting from tetradecanoic acid, the reaction temperature had to be increased to 45°C to ensure complete dissolution of FFA in the reaction mixture. Dodecanoic and tetradecanoic acid had virtually the same values for the initial reaction rate at 45°C, followed by a gradual decrease correlated with the increase in the chain length of FFA to octadecanoic acid.

cis-9-Octadecenoic acid, which is a common acid in vegetable oil, had a significantly lower reaction rate at 25°C. *cis*-9-Octadecenoic acid performed slightly better than octadecanoic acid at 45°C, although the double bond present in *cis*-9-octadecenoic acid made the molecule bulkier and less flexible and therefore presumably more difficult to access the active site of the lipase. There are probably other favorable effects, such as hydrophobic interactions with the enzyme, that compensate for the steric hindrance.

Effect of temperature on the kinetic resolution of 2-octanol with dodecanoic acid. It was previously demonstrated that temperature can play a crucial role in the stereochemical optimization of enzymatic reactions (13). In our study, temperature significantly influenced the reaction rate without any substantial effect on *E* in the range studied (Table 1). The initial reaction rate at 45°C was approximately four times higher than that at 15°C.

Kinetic resolution of 2-octanol with different acyl donors. The performance of four different acyl donors was comparable in their kinetic resolution of *rac*-2-octanol (Fig. 1). *E* was very high for all the acyl donors (>>300). The reaction with ethyl dodecanoate was slower than that with dodecanoic acid, although the ethanol formed was easier to remove from the reaction mixture than water. At 5 mm Hg pressure, both ethanol and water were removed very rapidly from the reaction mixture. Therefore, the denaturing effect of ethanol on the enzyme was minimal and could not account for the difference in the reaction rates. One explanation is that the specificity of lipase is higher for FFA than for the corresponding ethyl ester. This point is supported by the results of a previous work (3), which showed that the specificity constant, V_{max}/K_m, was higher for octanoic acid than for ethyl octanoate in the esterification of *rac*-2-octanol in heptane.

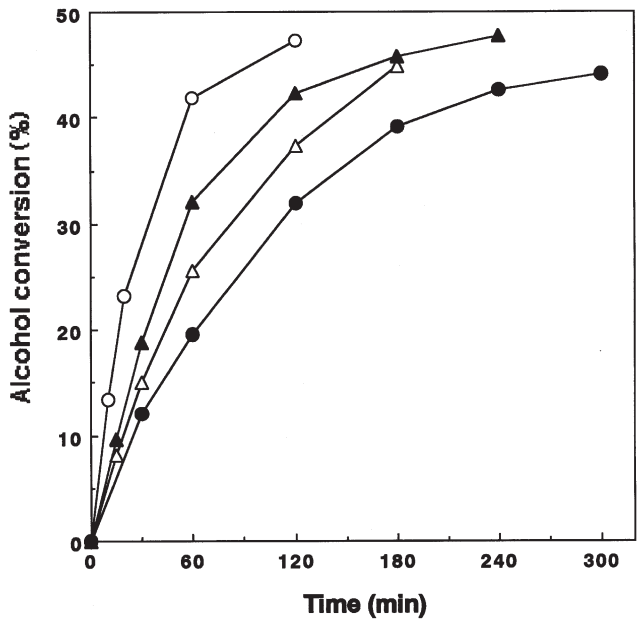

FIG. 1. Kinetic resolution of 2-octanol with different acyl donors: vinyl acetate (○), decanoic acid (▲), dodecanoic acid (△), and ethyl dodecanoate (●). Reactions were performed with *rac*-2-octanol (20 mmol), acyl donor (10 mmol), and 115 mg immobilized enzyme at 25°C and 5 mm Hg. The reaction with vinyl acetate was performed at normal pressure.

TABLE 2
Enzymatic Kinetic Resolution of Some Alcohols by Esterification with Decanoic Acid

Alcohol	Reaction time (min)	Conversion of alcohol (%)	ee_A (%)	ee_E (%)	Enantioselectivity *E*
2-Octanol[a]	120	50	>99	>99	>>300
1-Phenylethanol[a]	180	49.7	98.6	>99	>>300
1-(2-Naphthyl)ethanol[b]	240	50	>99	>99	>>300

[a]Reactions were performed with *rac*-alcohol (20 mmol), acyl donor (11 mmol), and 460 mg immobilized enzyme, at 45°C and 5 mm Hg.
[b]The reaction was performed with *rac*-1-(2-naphthyl)ethanol (20 mmol), decanoic acid (40 mmol), and 460 mg immobilized enzyme, at 45°C and 5 mm Hg. For abbreviations see Table 1.

Vinyl acetate was the most potent acyl donor. The instant removal of the co-product by enolization to acetaldehyde made the acyl transfer reaction irreversible and therefore very effective. Although no studies are known on the deactivation of CALB by acetaldehyde, a previous work showed that the acetaldehyde accumulating in the reaction mixture had a deactivating effect on *C. rugosa* lipase by alkylating the amino residues of the enzyme. This led to a significant decrease in both activity and *E* in the long-term use of the catalyst (14).

The rate of the reaction with decanoic acid, for which the lipase had the highest selectivity, was half of that with vinyl acetate but still afforded high yields in relatively short reaction times.

Kinetic resolution of some secondary alcohols with decanoic acid. The kinetic resolution of *rac*-2-octanol, *rac*-1-phenylethanol, and *rac*-1-(2-naphthyl)ethanol was performed with an excess of decanoic acid to ensure higher reaction yields and better reaction rates (Table 2). Virtually perfect resolution was achieved for all three compounds in short reaction times. The *R*-enantiomer of the alcohol was esterified and the *S*-enantiomer remained unreacted in all cases.

Discussion. Only a few previous studies have been dedicated to direct esterification with FFA as a method for the kinetic resolution of secondary alcohols (15–17). The reactions were performed in organic solvents and used a very high excess of FA (15), removal of the water formed by the addition of molecular sieves, or azeotropic distillation (16) as a means to displace the reaction equilibrium to the synthetic side. In the first case, the reaction times were very long (several weeks). Azeotropic distillation was more efficient than the addition of molecular sieves (16), but the reaction times were still long (48 h). *E* of immmobilized *C. antarctica* lipase in the esterification of secondary alcohols with decanoic acid was very high and not influenced by the water activity in organic media (17).

FFA were considered less potent acyl donors for the kinetic resolution of secondary alcohols than their derivatives, i.e., thioethyl-, vinyl-, and ethyl esters (3). The poorer results are due to the inefficient removal of the water formed under the reaction conditions used. We demonstrated that direct esterification with FFA under low pressure is a highly efficient method for the kinetic resolution of secondary alcohols. High conversions and enantiopurity of both *R*-alcohol and the *S*-ester formed were achieved in much shorter reaction times.

The volumetric productivity of the solvent-free reaction system under reduced pressure was also much better than that of solvent systems. As a comparison, in a previous investigation, resolution of 2-octanol with dodecanoic acid was performed using 4 mL heptane for each millimole of 2-octanol in 96 h at 40°C (16). Applied to our amount of reactants, this ratio would mean roughly a 20-fold increase in the reaction mixture. If we approximate that the same conversion and yields were obtained at the end of both reactions (3 h at 35°C in our case), the volumetric productivity defined as the amount of product obtained per 1 mL reaction mixture in 1 h was more than 600-fold higher for our reaction system.

Lipase selectivity for different FFA species influences only the reaction rates, but all FFA species tested in this work were practical to use. The reaction rates were comparable to that of vinyl acetate (the most frequently used acylating agent for the chemical resolution of alcohols), but FFA have the advantage of being less toxic, more stable, and less harmful to the enzyme. FFA are also at least one order less expensive than the corresponding ethyl-, thioethyl-, or vinyl esters.

The downstream purification of the residual alcohol and ester formed is easier in the case of FFA esters compared to acetyl esters. The difference in b.p. between the alcohol and ester is higher; therefore, the two can be easily separated by distillation. The yields of *S*-2-octanol and *R*-2-octyldecanoate after purification for the preparative-scale kinetic resolution of *rac*-2-octanol were similar to that of a previous investigation using *S*-ethyl thiooctanoate as the acyl donor in a solvent-free system and product separation by distillation (2).

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