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SHORT COMMUNICATION

Lipase-Catalyzed Production of Optically Active Acids via Asymmetric Hydrolysis of Esters

Effect of the Alcohol Moiety

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ABSTRACT

It has been found that lipase from *Candida cylindracea* hydrolyzes octyl *R*(+)- but not *S*(−)-2-chloropropionate. At the same time, the enzyme exhibits no appreciable stereoselectivity in the hydrolysis of the methyl ester of the same acid. Solubility determination experiments showed that at the concentrations used, methyl 2-chloropropionate was completely dissolved in water, whereas the octyl ester existed as an emulsion in water. It is therefore speculated that in order to express its stereoselectivity the lipase needs to adsorb on the substrate—water interface.

R,S-2-chloropropionic acid was preparatively resolved via yeast lipase-catalyzed asymmetric hydrolysis of its octyl ester. Gram quantities of *R*(+)-chloropropionic acid and octyl *S*(−)-2-chloropropionate of high optical purity were readily prepared.

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INTRODUCTION

Lipases have been used for the preparative resolution of racemic alcohols and carboxylic acids (*1–10*). This approach involves the chemical conversion of a racemic alcohol or acid to the corresponding ester, followed by a lipase-catalyzed asymmetric hydrolysis of the latter. The usefulness and generality of this method is severely restricted by the fact that only a few of the many lipases tested exhibit stereospecificity in the hydrolysis of esters (*2,3,6,8–10*).

In our ongoing studies on the use of enzymes for the production of chiral compounds of practical significance, we were prompted to develop a method for preparing optically active 2-chloropropionic acid and its esters. 2-Chloropropionic acid is used for the synthesis of a number of derivatives of 2-phenoxypropionic acid having wide applications as potent herbicides (*11*). Since only the *R*(+) isomer of 2-phenoxypropionic acids has a herbicidal activity (*12*), it would be advantageous to use only this isomer in agriculture instead of the racemic mixture (because the other isomer, although inactive as a herbicide, displays a high general toxicity). The simplest way to produce optically active 2-phenoxypropionic acid is to start with optically active 2-chloropropionic acid.

We have attempted to employ commercially available yeast lipase for the resolution of racemic 2-chloropropionic acid via enzymatic hydrolysis of its esters. Lipase from *Candida cylindracea* was found to be *nonstereospecific* in the hydrolysis of methyl 2-chloropropionate; at the same time we discovered that the enzyme displayed *absolute stereoselectivity* in the hydrolysis of the octyl ester of the same acid. The present paper deals with investigation of this phenomenon and with its use for production of gram amounts of optically pure 2-chloropropionic acid and its ester.

MATERIALS AND METHODS

Materials

Lipase from *Candida cylindracea* (EC 3.1.1.3) was purchased from Sigma Chemical Co. and had a specific activity of 640 U/mg solid. Racemic methyl 2-chloropropionate, 2-chloropropionic acid, and its chloroanhydride were obtained from Aldrich. Octyl 2-chloropropionate was synthesized by us from 2-chloropropionyl chloride and octanol following the general procedure of Morris and Green (*13*). All other reagents used were of reagent grade.

Measurements

Concentrations of octanol, 2-chloropropionic acid, and its methyl and octyl esters were measured gas chromatographically using a 6-ft glass column packed with Analab's "Super Pak 20M" (N_2 carrier gas, 10 mL/min; detector and injector port temperature, 250°C). The temperature of the column was increased from 50 to 250°C at 16°/min. The retention times observed were 6.1 min for octanol, 7.5 min for 2-chloropropionic acid, 2.3 min for methyl 2-chloropropionate, and 8.2 min for octyl 2-chloropropionate.

Optical rotations of 2-chloropropionic acid and its octyl ester were measured at 589 nm (sodium line) and 25°C using a Perkin-Elmer 243 B polarimeter.

Kinetics of Enzymatic Hydrolysis of 2-Chloropropionates

The time courses of lipase-catalyzed hydrolysis of methyl and octyl esters of *R,S*-2-chloropropionic acid were determined potentiometrically using a RTS-812 Radiometer recording pH-stat system. In a typical experiment, 40 mL of an 0.1*M* ester solution in 0.5 m*M* sodium tetraborate buffer containing 3 m*M* $CaCl_2$ and 0.2*M* NaCl were placed in the thermostated cuvet of a pH-stat, equilibrated at 37°C, and the pH was adjusted to 8.0. Then 10 mg of the yeast lipase were added, and the acid liberated as a result of the enzymatic hydrolysis was automatically titrated with 4*M* KOH.

The time course of the large-scale enzymatic hydrolysis of octyl *R,S*-2-chloropropionate was followed by periodically withdrawing 2 mL samples, extracting them with 1 mL of ether, and assaying the ether solution both for octanol and octyl 2-chloropropionate by gas chromatography.

Solubility of Esters

We have determined the solubility of both methyl and octyl *R,S*-2-chloropropionates in the aqueous solution used for the aforedescribed potentiometric experiments (0.5 m*M* $Na_2B_4O_7$, 3 m*M* $CaCl_2$, and 0.2*M* NaCl, pH 8.0). A 2 mL volume of either ester was added to 20 mL of the buffer and the mixture was vigorously stirred at 37°C for about 1 h. Then the organic layer was separated by centrifugation, and the aqueous phase was extracted with several portions of ether. The latter were combined, partly evaporated, and the concentration of the ester in them was determined by gas chromatography.

RESULTS AND DISCUSSION

Curve *a* in Fig. 1 illustrates the time course of the hydrolysis of methyl *R,S*-2-chloropropionate catalyzed by lipase from *Candida cylin-*

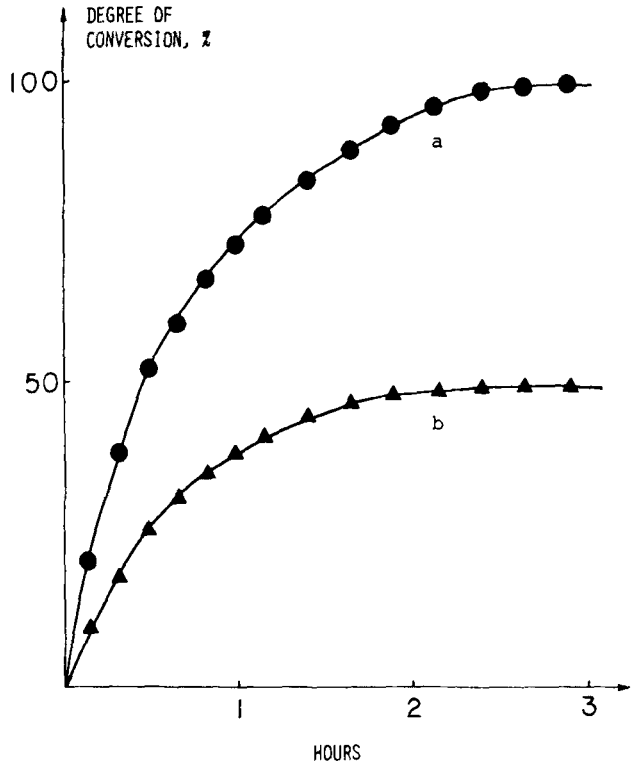

Fig. 1. The time course of the yeast lipase-catalyzed hydrolysis of (*a*) methyl *R,S*-2-chloropropionate and (*b*) octyl *R,S*-2-chloropropionate. Conditions: 0.1*M* substrate, 10 mg of *Candida cylindracea* lipase, pH 8.0, 37°C; reaction mixture contained 0.5 m*M* $Na_2B_4O_7$, 3 m*M* $CaCl_2$, and 0.2*M* NaCl.

dracea. One can see that the racemic ester is completely hydrolyzed by the enzyme; furthermore, the shape of the kinetic curve does not suggest any considerable stereospecificity of lipase (i.e., that one optical isomer is hydrolyzed much faster than another). Hence, it would seem that 2-chloropropionic acid cannot be resolved via hydrolysis of its racemic ester catalyzed by yeast lipase.

Curve *b* in Fig. 1 shows the time course of the lipase-catalyzed hydrolysis of octyl ester of *R,S*-2-chloropropionic acid. In this case only half of the substrate is hydrolyzed by the enzyme. Addition of more lipase does not increase the degree of hydrolysis; therefore, inactivation of the enzyme during the reaction can be ruled out. Base-catalyzed hydrolysis of octyl *R,S*-2-chloropropionate (pH 12.5) afforded a complete hydrolysis of the ester. On the basis of these results one can conclude that yeast lipase hydrolyzes the racemic octyl ester asymmetrically reacting only with one optical isomer (this conclusion was confirmed in the preparative enzymatic hydrolysis described below).

To understand why lipase from *Candida cylindracea* displays absolute stereoselectivity in the hydrolysis of octyl *R,S*-2-chloropropionate, but not in the hydrolysis of methyl *R,S*-2-chloropropionate, we have endeav-

ored to relate this to the physical state of the system. We experimentally determined that the solubility of the methyl ester in the reaction buffer was 130 m*M*, whereas that of the octyl ester was only 1.2 m*M*. The hydrolysis experiments reported in Fig. 1 were carried out at 100 m*M* ester concentration. Since this is lower than the solubility of the methyl ester but much higher than the solubility of the octyl ester, the 100 m*M* former was a true solution, whereas the 100 m*M* latter was an emulsion. It is thought (*14*) that reversible adsorption of the enzyme on the substrate–water interface may play an important role in the lipase catalysis. Obviously, such an interface exists in the case of octyl 2-chloropropionate, but not in the case of the methyl ester. Therefore, it is plausible to assume that, in order to express its stereoselectivity, lipase needs to adsorb on the interface (which presumably triggers necessary conformational changes).

We have decided to preparatively resolve *R,S*-2-chloropropionic acid using asymmetric hydrolysis of its octyl ester catalyzed by *Candida cylindracea* lipase. A 14.3 g quantity of octyl *R,S*-2-chloropropionate (60 mmol) were added to 585 mL of 0.25*M* phosphate buffer (pH 8.0). The emulsion was cooled to 4°C (to prevent racemization of the enzymatically produced 2-chloropropionic acid), and 300 mg of lipase were added. The reaction mixture was vigorously stirred at 4°C for 2 h, after which time the degree of conversion reached 50% and the hydrolysis stopped (as judged by gas chromatography, *see* Methods). Then the unreacted ester and octanol were extracted with three 30 mL portions of ether; the latter was dried with anhydrous $MgSO_4$ and evaporated in a rotary evaporator. The remainder was separated by fractional distillation at 4 mm Hg. At 63–66°C, 6.36 g of octyl 2-chloropropionate (89% yield) were obtained; the ester was 96% pure (by gas chromatography) and had a specific optical rotation $[\alpha]_D = -8.72°$ ($c = 90$, ether). The aqueous solution remaining after ether extraction was adjusted to pH 1.0 with concentrated HCl, followed by extraction of 2-chloropropionic acid with five 50 mL portions of ether. The ether fractions were combined and the solvent evaporated. As a result, 2.54 g of 2-chloropropionic acid (92% yield, 95% purity by GC) with a specific optical rotation $[\alpha]_D = +9.34°$ ($c = 30$, ether) were obtained. Using the literature data concerning optically pure 2-chloropropionic acid (*15*), we have both determined the optical purity of our enzymatically prepared acid (enantiomeric excess = 96%) and assigned to it an absolute configuration (*R*). Thus, yeast lipase hydrolyzes the *R* but not the *S* isomer of octyl 2-chloropropionate, resulting in a mixture of *R*(+)-acid and *S*(−)-ester.

In conclusion, we have succeeded in preparative enzymatic resolution of racemic 2-chloropropionic acid. This was achieved because of the absolute stereospecificity of yeast lipase in the hydrolysis of the *octyl ester* of 2-chloropropionic acid, although the same enzyme exhibited no appreciable stereoselectivity in the hydrolysis of the *methyl ester* of the same acid. That phenomenon points to the important role of the achiral moiety

in the lipase-catalyzed asymmetric hydrolysis of chiral esters. In other words, even if a given lipase is nonstereospecific in the hydrolysis of a particular ester, it may acquire stereospecificity of the achiral moiety is replaced with a more suitable one.

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