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Hydrolysis of ethyl mandelate and esterification of 2-bromopropionic acid in micro-emulsions

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SUMMARY

Lipase from *Candida cylindraceae* possessed higher catalytic activity, both in the hydrolysis of (DL)-ethyl mandelate in sodium dodecyl sulfonate/*n*-butanol/*n*-octane oil-in-water micro-emulsion and in the esterification of α-bromopropionic acid with *n*-butanol in sodium dodecyl sulfate/*n*-butanol/*n*-octane water-in-oil micro-emulsion, than in traditional water and oil biphasic solutions.

INTRODUCTION

When enzyme reactions are carried out in two-phase aqueous-organic systems, use of more hydrophobic organic solvents, having log *P* values >4, are normally required as more hydrophilic solvents tend to strip essential bound water from the enzyme molecule. Pancreatic lipase appears to be an exception to this generalization in that it appears to work in both hydrophilic and hydrophobic solvents [7]. Its stability in hydrophilic solvents may be due to the enzyme's unusually strong binding capacity for essential water. Water is more loosely bound to yeast and fungal lipases and is stripped off by more hydrophilic solvents [17]. Aliphatic hydrocarbons, having seven or more hydrocarbon atoms, including *n*-octane, are suitable hydrophobic solvents for use in enzyme biotransformations. Because the solubility of *n*-octane in water is limited, surfactants may be used to promote micro-emulsion formation in order to increase catalytic activity [12].

Lipases function most effectively in reaction systems containing an aqueous and organic phase. The presence of an interface is an obligatory condition and a prime location for enzymatic activity [5,10]. Micro-emulsions [11] are complex homogeneous systems containing, in most instances, four components: water, oil, surfactant and cosurfactant (usually an alcohol). They are typically divided into two main types, oil-in-water and water-in-oil. Micro-emulsions are useful in triacylglycerol transformations because they present the required large interfacial area [3] and can facilitate control of water content which may be important in determining whether esterification or hydrolysis is favored in the enzymatic reaction.

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Production of optically active compounds represents a major challenge in modern organic chemistry and the enantiospecificity of enzymes can be exploited to implement asymmetric biotransformation processes. In this study, water-in-oil and oil-in-water micro-emulsions were used to examine the lipase-catalyzed enantioselective resolution of α-(DL)-bromopropionic acid and (DL)-ethyl mandelate by esterification and hydrolysis, respectively. The two chemical reactions involved are illustrated in Fig. 1.

MATERIALS AND METHODS

Preparation of micro-emulsions

To ensure that the reactions were carried out in micro-emulsions, phase diagrams were prepared to identify possible regions of micro-emulsion formation. Two pseudo-ternary phase diagrams obtained by the water titration method are illustrated in Figs 2 and 3. The areas of sodium dodecyl sulfonate and sodium dodecyl sulfate micro-emulsions are large and the micellar solutions transferred directly from oil-in-water to water-in-oil micro-emulsions. Therefore, the suitable ratio of oil to water was a key parameter. Sodium dodecyl sulfonate oil-in-water and sodium dodecyl sulfate water-in-oil micro-emulsions were prepared by the following procedures:

Assume (1) surfactant : *n*-butanol = 1 : 2 (w/w)
(2) surfactant + *n*-butanol + *n*-octane = 2 g
(3) $R_{h/s}$ = *n*-octane: (surfactant + *n*-butanol) = 5% (w/w)
($R_{h/s}$ is the ratio of hydrophobic solvent to surfactant + cosurfactant)

Sodium dodecyl sulfonate, *n*-butanol and *n*-octane were mixed quantitatively according to formulas 1, 2 and 3. The mixture was then titrated with 0.1 molar TRIS-HCl, pH 8.0 until it became transparent. The sodium dodecyl sulfonate/*n*-butanol/*n*-octane oil-in-water transparent solution was utilized for hydrolysis of ethyl mandelate. Similarly, using

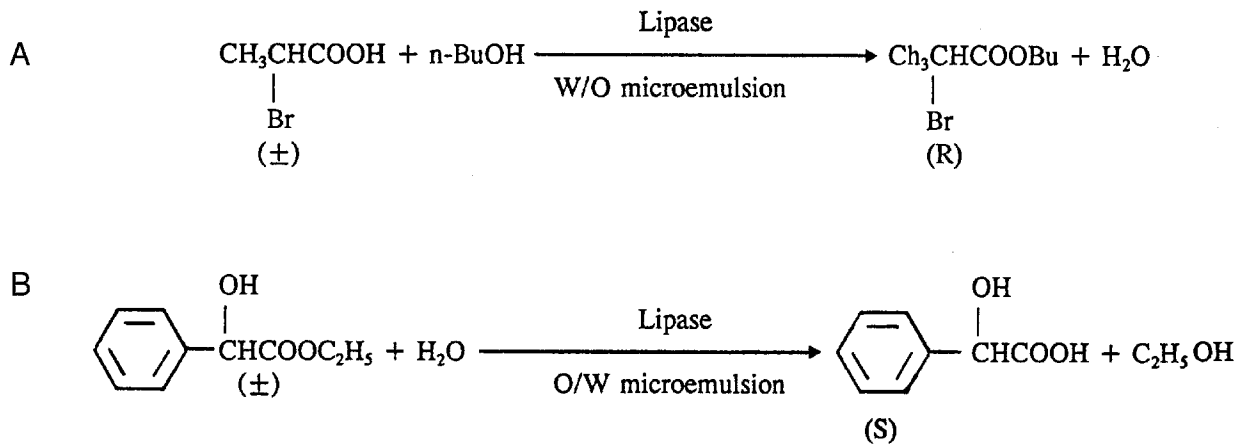

Fig. 1. Lipase enantioselective resolution of (A) α-bromopropionic acid and (B) (DL)-ethyl mandelate. W/O, water-in-oil; O/W, oil-in-water.

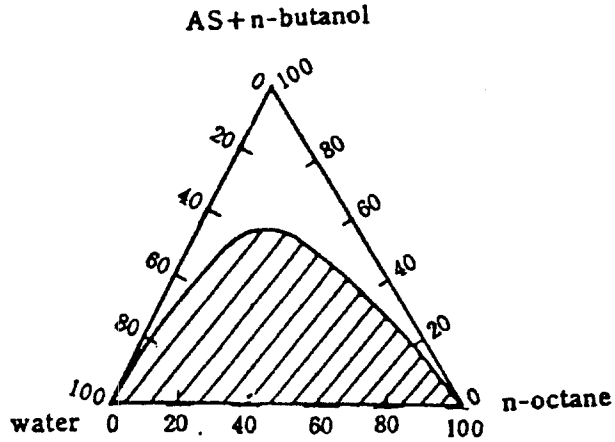

Fig. 2. Pseudo-ternary phase diagram of quaternary system: sodium dodecyl sulfonate/α-butanol/*n*-octane/water. Weight ratio of sodium dodecyl sulfonate (AS) to *n*-butanol is 1/2, T = 25 °C. The shaded area is the micro-emulsion.

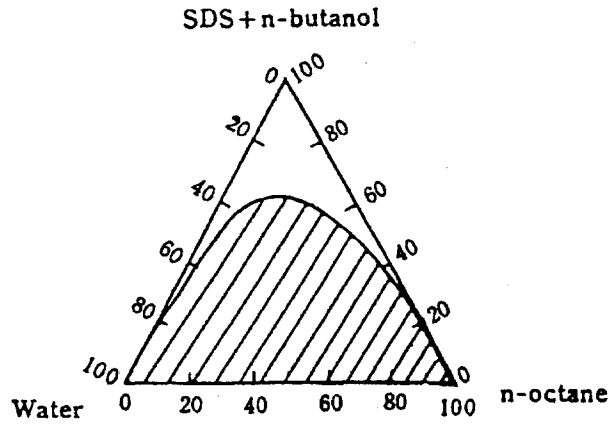

Fig. 3. Pseudo-ternary phase diagram of quaternary system: sodium dodecyl sulfate/*n*-butanol/*n*-octane/water. Weight ratio of sodium dodecyl sulfate (SDS) to *n*-butanol is1/2, T = 25 °C. The shaded area is the micro-emulsion.

$R_{h/s}$ = 90%, sodium dodecyl sulfate/*n*-butanol/*n*-octane water-in-oil micro-emulsion for esterification of α-bromopropionic acid was prepared.

Preparation of biphasic solutions

n-Octane/TRIS-HCl biphasic solution was prepared by mixing *n*-octane and 0.1 molar TRIS-HCl, pH 8.0, in the ratio 10 : 90 v/v. Water/*n*-octane biphasic solution was prepared by mixing these components in the ratio 10 : 90 v/v.

Enzyme reactions

Enzyme reactions were carried out in magnetically stirred reaction mixtures at 30 °C for up to 48 h.

Product extraction and separation

Following biocatalysis, the reaction mixture was adjusted to pH 2 with 1 N HCl to terminate the reaction. The solution was extracted twice with 15-ml portions of ethyl acetate. The organic phases were combined, dried with anhydrous sodium sulfate and evaporated to dryness. The residue was redissolved in ethyl acetate and injected into liquid or gas chromatographs. Products of the (DL)-ethyl mandelate hydrolysis were separated by high performance liquid chromatography (HPLC). Products of α-bromopropionic acid esterification were separated by HPLC and gas chromatography.

Chromatographic analysis of products used

Mandelic acid was analyzed by HPLC using a Shimadzu LC-6A instrument (Shimadzu, Kyoto, Japan) containing a Shimpack CLC-SIL column at 30 °C. The solvent system was *n*-hexane : isopropanol (20 : 1 v/v) and the flow rate was 1 ml min^{-1}. Products were detected by UV absorption at 254 nm. 2-Bromopropionic acid and butyl-α-bromopropionic acid were determined with a Shimadzu LC-6A HPLC containing a Shimpack CLC-ODS column at 30 °C. The mobile phase was methanol : water (19 : 1 v/v) and the flow rate was 1 ml min^{-1}. Products were detected by UV absorption at 226 nm. Butyl α-bromopropionic ester was also analyzed by GC using Hewlett-Packard instrument (Hewlett Packard, London, Ontario, Canada), model 5880A, equipped with a

50 m × 0.2 mm (i.d.) SE-54 column and flame ionization detector. Oven temperature was programmed from 120 °C to 180 °C at 5 °C min^{-1}. Products were quantified by comparison of peak sizes with authentic standards.

Assignment of optical activities

Optical rotations of products were determined and optical activities assigned by comparison with values and published data for the authentic compounds:

(*S*)-mandelic acid, $[\alpha]_D^{20}$ = 156.57 (H_2O), ee = 100% [1]

(*R*)-butyl-α-bromopropionate,

$[\alpha]_b^{25}$ = 18.4 (c = 1, $CHCl_3$), ee 96% [6]

RESULTS AND DISCUSSION

Hydrolysis of (DL)-ethyl mandelate in sodium dodecyl sulfonate oil-in-water micro-emulsion

(DL)-ethyl mandelate (150 mg) and 2 ml of a 10 mg ml^{-1} *Candida cylindraceae* lipase (Sigma, St Louis, MO, USA) aqueous solution were added into 5 ml of sodium dodecyl sulfonate oil-in-water micro-emulsion or 5 ml of *n*-octane/TRIS-HCl biphasic solution. Reaction mixtures were incubated at 30 °C for different time periods and then extracted and analyzed. The time courses of hydrolysis of (DL)-ethyl mandelate are illustrated in Fig. 4. *C. cylindraceae* lipase manifested higher activity in sodium dodecyl sulfonate/*n*-butanol/*n*-octane oil-in-water micro-emulsion than in *n*-octane/water biphasic solution.

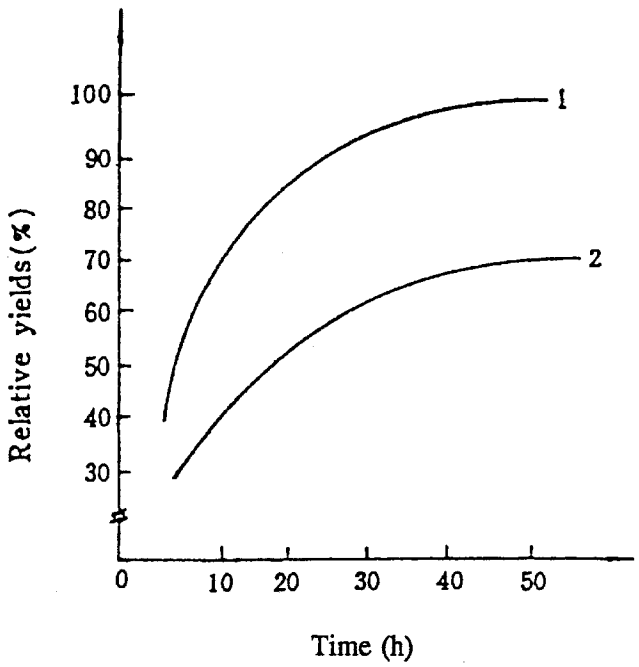

Fig. 4. Time courses of hydrolysis of ethyl mandelate catalyzed by CCL. Relative yield: 68 mg of ethyl mandelate converted to product represents 100% yield. (1) Sodium dodecyl sulfonate/*n*-butanol/*n*-octane oil-in-water micro-emulsion. (2) *n*-octane/TRIS-HCl buffer biphasic system.

TABLE 1

Optical activity of product after hydrolysis of racemic ethyl mandelate for 48 h

Reaction medium	Optical purity $[\alpha]_D$ (C10,H_2O)	ee%
Sodium dodecyl sulfonate/*n*-octane oil-in-water micro-emulsuion	−149.8	96.7
n-Octane/TRIS buffer biphasic solution	−151.3	95.7

After a 48-h hydrolysis period, mandelic acid was separated by column chromatography on silica gel with ether : ethyl acetate (5 : 1 v/v) as eluent and its optical activity determined (Table 1). The hydrolysis product, (*S*)-mandelic acid, was found to have an enantiomeric excess of greater than 95% in both reaction media.

Esterification of α-bromopropionic acid in sodium dodecyl sulfate water-in-oil micro-emulsion

α-Bromopropionic acid (100 mg), 0.5 ml of *n*-butanol and 30 mg *C. cylindraceae* lipase were added into 5 ml of sodium dodecyl sulfate water-in-oil micro-emulsion and into 5 ml of water/*n*-octane biphasic solution. Reaction mixtures were incubated for various time periods followed by product extraction and analysis. Maximum rates of esterification are illustrated in Table 2. Maximum reaction rates were 24 times higher in sodium dodecyl sulfate/*n*-butanol/*n*-octane water-in-oil micro-emulsion as compared with *n*-octane biphasic solution. When α-bromopropionic acid was esterified with *n*-butanol in the water-in-oil emulsion for 6 h, 44 mg of (*R*)-α-bromopropionic acid butyl ester was recovered by silica column chromatography. The optical rotation was $[\alpha]_D^{25}$ 18.2 (Cl, $CHCl_3$) representing an optical purity of ca. 95%. Thus, both catalytic activity and enantioselectivity of *C. cylindraceae* lipase in the sodium dodecyl sulfate water-in-oil micro-emulsion were high.

Therefore, both sodium dodecyl sulfonate/*n*-butanol/*n*-octane oil-in-water micro-emulsions and sodium dodecyl sulfate water-in-oil micro-emulsions provide excellent media for *C. cylindraceae* lipase catalysis to prepare chiral acids and esters. Micelle formation in biocatalytic micro-emulsions is

TABLE 2

Maximum reaction rates of esterification catalyzed by *Candida cylindraceae* lipase in sodium dodecyl sulfate micro-emulsion and biphasic solution

Reaction medium	Sodium dodecyl sulfate/*n*-butanol/*n*-octane water-in-oil micro-emulsion	*n*-octane/water biphasic solution
Maximum reaction rate (mol h^{-1})	4.82×10^{-3}	2.03×10^{-4}

generated by use of surfactants which, on the one hand, promote formation of a high interfacial area but, on the other hand, do not inhibit substrate enzyme interaction. The surfactant should not hinder subsequent separation of reaction products and enzyme recovery [9]. Where purification problems are encountered through using surfactants in micro-emulsions, these may be overcome by use of detergentless micro-emulsions [8,14,15]. These have been applied in biocatalysis with enzymes such as trypsin and chymotrypsin with high retention of catalytic activity and stability.

Candida lipase has become recognized as an important biocatalyst in asymmetric reactions. The enzyme has been used in the resolution of esters to 95–>99% enantiomeric excess in synthesis of bioactive compounds, thiazesin and α-tocopherol [2,16]. A rule to predict the selectivity of the resolution by this enzyme has been proposed [4]. More data on the kinetics of these reactions, combined with enzyme structural data, will provide a greater insight into the mechanisms influencing enantioselectivity of these reactions [13].

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