DESIGN OF SURFACTANTS SUITABLE FOR SURFACTANT-COATED ENZYMES AS CATALYSTS IN ORGANIC MEDIA

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Introduction

Enzymes are usually stable in water, but their activity rapidly decreases in organic solvents. If the enzyme can catalyze effectively a reaction in organic media, numerous advantages result. One of them is the ability to carry out a synthesis reaction in which substrates are hydrophobic, such as the synthesis of triglycerides, steroid conversions and peptide synthesis. The specificity and reactivity to substrates in an enzyme system are very high compared with those of conventional organic synthesis. Thus an enzymatic reaction in organic media can be used for the production of optically active materials and for the resolution of racemic compounds.

To utilize the enzymatic catalysis in organic media, it is necessary to avoid the deactivation or denaturation of enzymes. At least two approaches to that goal have been successfully developed. In the first approach, enzymes are dissolved in water pools in reversed micelles[6]. In the second approach, enzymes surface-modified with polyethylene glycol[7] or oil-soluble surfactants[1] are directly dissolved in organic solvents. In reversed micelles, inherent catalytic properties of enzymes are generally similar to those in aqueous solutions. In contrast, the latter ones are directly exposed to the solvent and hence exhibit some remarkable novel properties compared to those in water.

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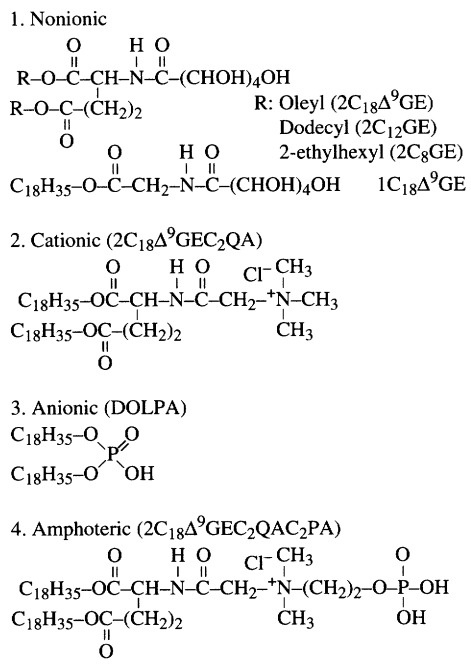

Fig. 1 Molecular structure of surfactants used

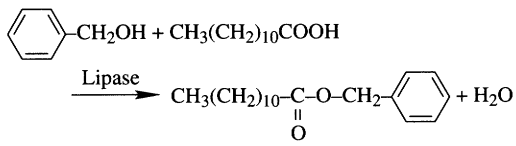

Fig. 2 Overall reaction of esterification catalyzed by surfactant-coated lipase

Recently, Okahata[2] has reported a surfactant-coated lipase which can catalyze an enantioselective esterification of racemic alcohol in various organic solvents. The preparation of the modified enzymes is easy and the enzyme-surfactant complex can effectively catalyze many reactions compared with the other enzyme systems in organic media.

The coating surfactant plays a key role in the enzyme system, because the activity and stability of the enzyme complex depend strongly on the type and structure of the surfactant. The aim of the present study is to find a most suitable surfactant for the preparation of surfactant-coated enzymes which can be utilized in organic media. We have already synthesized many surfactants similar to lipid to use for liquid surfactant membranes[3,4,5]. In this study, the effect of the coating surfactant on the enzyme activity was investigated in detail with newly synthesized surfactants.

1. Experimental

1.1 Reagent

Lipase from *Pseudomonas* sp. was used as the enzyme. The enzyme was provided by Amano Pharmaceutical Co., Ltd. and was used as received. Surfactants such as nonionic $2C_nGE$ (n = 8, 8 (2-ethylhexyl), 10, 12, 14, 18, $18\Delta^9$ (oleyl)) and $1C_{18}\Delta^9GE$, cationic $2C_{18}\Delta^9GEC_2QA$, anionic DOLPA and amphoteric

Table 1. Effect of surfactant type on conversion of esterification

Type	Surfactant	Conv. % (2h)	Conv. % (72h)
Nonionic	$2C_{18}\Delta^9GE$	95	97
Nonionic	$1C_{18}\Delta^9GE$	61	94
Cationic	$2C_{18}\Delta^9GEC_2QA$	40	92
Anionic	DOLPA	–	–
Amphoteric	$2C_{18}\Delta^9GEC_2QAC_2PA$	15	94

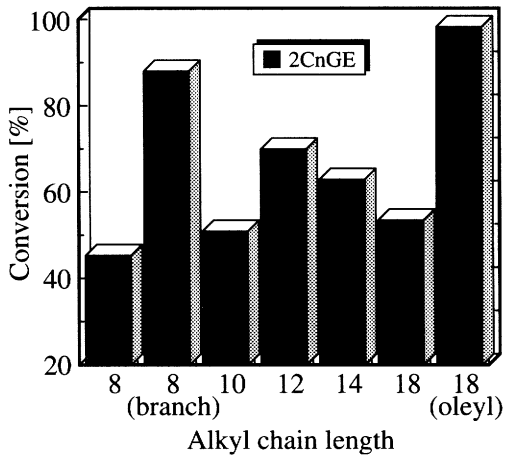

Fig. 3 Effect of alkyl chain length and structure in hydrophobic part of nonionic surfactant on conversion

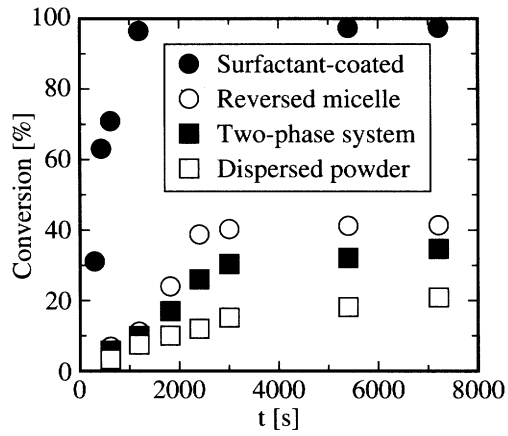

Fig. 4 Comparison of various enzymatic reaction methods

$2C_{18}\Delta^9GEC_2QAC_2PA$ were synthesized according to the procedure described in previous papers[3, 4, 5]. **Figure 1** shows the structure of typical surfactants used in this work along with the abbreviations of these surfactants. Six kinds of organic solvent were used but the aliphatic solvents showed higher activity than did the alcohol, aromatic and chloric solvents. Therefore, iso-octane of analytical grade was mainly used as an organic solvent. As substrates, benzyl alcohol and lauric acid of analytical grade were used.

1.2 Experimental procedure

A typical preparation of surfactant-coated enzymes is as follows. An aqueous solution (250 ml, phosphate buffer (pH 6.8)) of lipase (500 mg) and an aqueous solution (250

ml) of a surfactant (500 mg) were mixed in an ultrasonic cleaner (SHARP UT-204) for 20 minutes, and the solution was incubated in a refrigerator for one day. The precipitates were collected by centrifugation and dried over in vacuum. White powder was obtained and the yield was about 20%. The surfactant-enzyme complex was insoluble in water but almost entirely soluble in organic solvents. Hydrophobic tails of a surfactant solubilize the enzyme complex in organic media. The enzyme content of the complex was determined from an elemental analysis. The content of an enzyme was from 7 to 38 wt% and depended greatly on the surfactant used. The enzymatic activity of the surfactant-coated lipase was investigated in the esterification of lauric acid (3 mM) with an excess amount of benzyl alcohol (6 mM) in iso-octane at 35°C. **Figure 2** shows the overall reaction of the esterification.

The content of the lipase is constant (0.2 g/l) in all experiments. The disappearance of benzyl alcohol and the production of ester compound were followed by gas chromatography (HP 5890) with a capillary column of 15 m (J & W DB1).

2. Results and Discussion

The esterification was carried out by using five kinds of surfactant-coated lipase. The conversions of lauric acid at 2 hours and 3 days are summarized in **Table 1**. At the equilibrium state the conversions are almost same for all complexes, but the complex prepared by the cationic or amphoteric surfactant shows a low reaction rate compared with that of nonionic surfactant $2C_{18}\Delta^9GE$. The strong interaction between the cationic head group of the surfactant and the negatively charged lipase denatured a part of the lipase structure. Further, the nonionic surfactant, which has two oleyl chains in the hydrophobic part, gave a better result than did the nonionic surfactant having only one oleyl chain. When an anionic surfactant was used, the enzyme complex could not be prepared by an electrostatic repulsion for a lipase, because the PI (isoelectric point) of a lipase is relatively low (usually about 5).

Using the complexes prepared by nonionic surfactants, the effect of the alkyl chain length and the structures on the conversion of lauric acid at 2 h was studied, with the results shown in **Fig. 3**. They show a strong link between the structure in the hydrophobic part of a nonionic surfactant and enzyme activity. When the carbon number was the same, surfactants having a branch or double bond showed higher activity than that of a surfactant having a straight chain, because the solubility of the enzyme complex in an organic solvent increases due to such a hydrophobic group. It was found that the surfactant-coated enzyme prepared by the surfactant having two oleyl chains is most suitable as a catalyst in the synthesis of lauric acid benzyl ester in iso-octane. From the elemental analysis of the complex, it was found that the complex prepared by $2C_{18}\Delta^9GE$ contains 27 wt% lipase; that is, one molecule of lipase is coated by 170 molecules of the surfactant.

Furthermore, to confirm the advantage of the enzyme complex prepared by the best surfactant having two oleyl chains as a catalyst in the esterification, four reaction systems were compared. They were two homogeneous reaction systems: (1) the lipase-surfactant complex system, and (2) an AOT-iso-octane reversed micellar system; and two heterogeneous reaction systems: (3) a liquid-liquid (iso-octane-water) system, and (4) a liquid-solid (iso-octane- powder lipase) system. **Figure 4** shows the time courses of conversion of the four enzymatic systems. It is found that the reaction system of the surfactant-coated enzyme is most suitable for the esterification from the viewpoint of reaction rate and final conversion. Final conversion in the reaction systems of (2) or (3) is below 50% even in the equilibrium state, because the ester produced is hydrolyzed again by an excess of water. In the reaction system of (4), the reaction rate was very low because the lipase powder is insoluble in iso-octane.

Conclusions

Surfactant-coated enzymes have been prepared with newly synthesized surfactants. Using lipase-surfactant complexes, the esterification of alcohol and carboxylic acid was investigated in organic media. The activity of enzyme complexes depends strongly on the coating surfactants. The complex prepared by glutamic acid dioleyl ester ribitol, $2C_{18}\Delta^9GE$, was most suitable as a catalyst of esterification in iso-octane.

Acknowledgment

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