Catalytic Activity of Lipid-coated Enzymes in Organic Media

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Abstract: A new way of solubilizing hydrolytic enzymes in organic media was achieved by coating the enzyme with lipid monolayers. Lipid-coated enzymes have been used successfully for a number of types of reactions in organic media or organic-aqueous two-phases, for example enantioselective esterification by lipase, transphosphatidylation of water-insoluble phospholipids by phospholipase D, hydrolysis of a lipophilic substrate by a catalytic antibody, and transglycosylation by $\beta$-D-galactosidase.

Key words: Lipid-coated enzyme; enzyme reaction in organic solvents; lipase; phospholipase D; catalytic antibody; $\beta$-D-galactosidase.

Introduction. In recent years, there has been much interest in the use of enzymes as catalysts in organic solvents. The advantage of using hydrolytic enzymes in organic solvents is to increase the solubility of lipophilic substrates and also to cause reverse reaction such as enantioselective esterification or transesterification. There have been several approaches to using a lipase as a synthetic catalyst in organic solvents. In addition to the water-in-oil emulsion and the reversed micellar system containing a small amount of water, there are two previous reports of the use of both hydrophobic and hydrophilic organic solvents as a reaction medium for lipase: i) Klibanov and co-workers reported the direct dispersion of powdered lipase in organic solvents, to produce an ester exchange catalyst for heterogeneous solutions and ii) Inada and co-workers prepared a poly(ethylene glycol) (PEG)-grafted lipase that is soluble or swelled in hydrophobic organic solvents and catalyses simple ester syntheses from aliphatic alcohols and acids. Of prime importance to the use of enzymes in organic media is the need to avoid enzyme deactivation or denaturation.

In this article, we introduce a new method of solubilizing enzymes in hydrophobic organic solvents by coating the enzyme with a lipid monolayer (Fig. 1). A lipid-coated enzyme was prepared simply by mixing aqueous solutions of both enzyme and lipid, then collecting and lyophilizing the resulting precipitate. The prepared lipid-coated enzyme was insoluble in buffer solution but freely soluble in most organic solvents such as benzene, ethyl acetate, isooctane, isopropyl ether, dimethyl sulfoxide (DMSO) and ethanol. In the lipid-coated enzyme, the hydrophilic head groups of the lipids interact with the hydrophilic surface of the enzyme and the lipophilic alkyl chains extend away from its surface and solubilize the enzyme in hydrophobic organic solvents. The protein content in the complex was estimated to be 8–10 wt%, that is 150±50 lipid molecules per enzyme molecule, both from elemental analysis (C, H and N) and from the UV absorption of the aromatic amino acid residues in the protein. Gel permeation chromatography in organic solvents confirmed that the lipid-coated enzyme molecules do not aggregate with each other and that the coating lipids are stable and are not removed from the enzyme surface in organic media.

Enantioselective esterification catalysed by a lipid-coated lipase in isooctane. Fig. 2 shows typical time courses of ester syntheses from $R$-, $S$-, and racemic 1-phenylethanol and excess lauric acid catalysed by a lipid-coated lipase B (from Pseudomonas fragi 22–39B) in dry isooctane at 40°C. When the racemic alcohol was used, the esterification reached equilibrium near 50% conversion, within 2 h. The $R$-isomer was completely converted to ester, but the $S$-isomer scarcely reacted with lauric acid. This indicates clearly that a lipid-coated lipase can recognize the $R$-1-phenylethanol and convert it to ester, but not the $S$-isomer.

We compared our lipid-coated lipase system with
other systems under the same reaction conditions. The time courses of these reactions are shown in Fig. 3. When the lipid-coated lipase B was used (curve a), R-1-phenylethanol was completely converted to the ester within 3 h with high enantioselectivity \( \nu_R=50 \text{mM s}^{-1} \) (mg of protein)\(^{-1} \), \( \nu_R/\nu_S=250 \). When lipase powder was dispersed directly in isooctane,\(^5\) it gave a very slow reaction rate (1/100 times) compared to that of the lipid-coated lipase \( \nu_R=0.5 \text{mM s}^{-1} \) (mg of protein)\(^{-1} \), \( \nu_R/\nu_S=260 \). In the dispersion method (curve d), the enzyme exists as a suspension in the substrate organic solution, therefore, a large amount of enzyme may be required to achieve a fairly high reaction rate. In the water-in-oil system (curve c),\(^6\) in which 1 mg of native lipase B is solubilized in a buffer solution (pH 5.6) and emulsified in isooctane, the rate of ester synthesis was very slow \( \nu_R<0.1 \text{mM s}^{-1} \) (mg of protein)\(^{-1} \), \( \nu_R/\nu_S=80-120 \) and decreased with increasing reaction time. It seems that the presence of a small amount of water in the water-in-oil emulsion system caused the reverse hydrolysis reaction. When PEG-grafted lipase B was used (curve b),\(^6\) the esterification proceeded at a fair rate with high enantioselectivity \( \nu_R=30 \text{mM s}^{-1} \) (mg of protein)\(^{-1} \), \( \nu_R/\nu_S=280 \) in the initial stages. However, the conversion reached a plateau at 70% yield after 40 h in dry isooctane. This suggests that the water produced in the reaction is retained near the amphiphilic PEG chains and causes the reverse hydrolysis.

Thus, the lipid-coated lipase can effectively and completely catalyse ester synthesis in dry organic solutions without changing the enzyme enantioselectivity, because unlike the other enzyme system, the lipid-coated lipase is homogeneously soluble and stable in dry organic solvents.

The lipid-coating method can also be applied to phospholipase D (PLD); the lipid-coated PLD acts as an efficient catalyst for transphosphatidylation of water-insoluble phospholipids in organic solvents.\(^{10}\)

Lipid-coated catalytic antibody in water-miscible organic solvents.\(^{14}\) To date numerous publications have appeared on catalytic antibodies. Various reactions, such as hydrolysis of esters and amides, transesterifications, Diels-Alder reactions, decarboxylations and sigmatropic rearrangements have been reported to be catalysed by catalytic antibodies.\(^{19-23}\) Catalytic
antibodies are induced to compounds (haptens) that mimic the transition states in these chemical reactions. Although substrates in these reactions are generally lipophilic and not very water-soluble, the reactions have been always carried out in aqueous buffer solutions because catalytic antibodies are thought to be soluble and stable only in aqueous solution.

In an effort to make catalytic antibodies more attractive catalysis from a synthetic standpoint, we applied our lipid-coating method to solubilize catalytic antibodies in water-miscible organic solvents. The catalytic hydrolysis of monoester of chloramphenicol was chosen as an example, because the substrate is lipophilic and the catalytic reaction has been studied in aqueous solution.  

Fig. 4 shows the effect of DMSO content in Tris buffer solution (0.05 M, pH 8.0) on the hydrolysis of monoester of Chloramphenicol (55 mM) at 30°C. Catalyzed by a lipid-coated antibody (1 mM, 30 mg of protein) (solid circles), catalyzed by a native antibody (1 mM, 30 mg of protein) (open circles) and spontaneous hydrolysis (squares).

Since catalytic antibodies are designed to catalyse a wide range of organic reactions with lipophilic substrates, it is important to use antibodies in water-miscible or hydrophobic organic solvents. The physical stability of the antibody is also expected to be increased by the coating lipids.

Transgalactosylation catalysed by a lipid-coated β-D-galactosidase in a water/organic two phase system. In recent years, glycoside hydrolases have been applied as transglycosylation catalysts to synthesize glycoside compounds or oligosaccharides in aqueous solution containing water-miscible organic solvents, by making use of the reverse hydrolysis reaction. In contrast to chemical synthesis, enzymatic synthesis has the advantage of providing regio- and stereo-selective products in one-step reaction and without using protection groups. However, in enzymatic synthesis using glycoside hydrolases it has been difficult to obtain high yields of glycosylation products, because in aqueous solution, the hydrolysis reaction predominates over transglycosylation. If the reaction could be carried out in nonaqueous organic solvents without denaturing the enzymes, the high yields of transglycosylation products would be obtained. We have also used our lipid-coating method to solubilize β-D-galactosidase in organic media.

Transgalactosylation from a ten-fold excess of lactose as a galactosyl donor to 5-phenyl-1-pentanol (PhC₅OH) as a galactosyl acceptor in a two-phase system of isopropyl ether and phosphate buffer (10 mM, pH 5.1) at 30°C. When the lipid-coated β-D-galactosidase was solubilized in the organic phase (Fig. 5a), a 66% of yield of transglycosylated Gal-OCSPh was obtained, but no lactose was hydrolysed even after 8 days. It was confirmed from ¹H- and ¹³C-NMR that the chemical structure of the Gal-OCSPh product remained in the β-configuration of D-galactose. As the amount of PhC₅OH consumed corresponds with that of Gal-OCSPh produced, this means that the carbocation intermediate formed in the active site of the enzyme in isopropyl ether was attacked mainly by the alcohol. By contrast, when a native β-D-galactosidase was employed (Fig. 5b), the starting substrate PhC₅OH was completely recovered after 8 days. Since neither the galactosylation nor the hydrolysis reaction proceeded in the two-phase system, a native enzyme may be denatured at the interface between the...
In summary, the lipid-coating system for enzymes is useful for solubilizing enzymes in organic media without denaturation. We have shown that this lipid-coating method can be applied for various enzymes as well as catalytic antibodies. The lipid-coating system has a variety of possibilities for other applications. When the organic solution of the lipid-coated enzyme is cast on an electrode substrate, a thin enzyme film can be obtained as a sensor membrane that is insoluble in aqueous solution.\(^{17,18}\)

Fig. 5. Typical time courses of transgalactosylation from lactose (10 mM) to 5-phenyl-1-pentanol (PhC5OH, 1.0 mM) at 30°C catalysed by (a) a lipid-coated \(\beta\)-D-galactosidase and (b) a native \(\beta\)-D-galactosidase from Escherichia coli in the two-phase of isopropyl ether and 10 mM phosphate buffer (pH 5.1). [Enzyme]=0.1 mg of protein/20 mL. The lower part of the figure shows the transglycosylation catalysed by a lipid-coated enzyme between galactose acceptor alcohols in the organic solution and lactose in the buffer solution.

**References**


aqueous and organic phases.


