A Facile Transgalactosylation catalyzed by a Lipid-Coated β-*D*-Galactosidase in the Water-Organic Two Phases

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A lipid-coated β-*D*-galactosidase could act as an efficient catalyst for transgalactosylation in the water-organic two phases: both the hydrophobic lipid-coated enzyme and alcohols were solubilized in isopropyl ether and mixed with an aqueous solution of lactose (a galactosyl donor). When a native β-*D*-galactosidase was employed for the same reaction, neither the transgalactosylation nor the hydrolysis reaction proceeded due to the deactivation of the enzyme at the interface.

In recent years, glycoside hydrolases have been applied as a transglycosylation catalyst to synthesize glycoside compounds or oligosaccharides in aqueous solution containing water-miscible organic solvents by using the reversed hydrolysis reaction.[1] Enzymatic synthesis has advantages to provide regio- and stereo-selective products without using protection groups in one step reaction, in comparison with the chemical synthesis. In enzymatic synthesis using glycoside hydrolases, however, it has been difficult to obtain glycosylation products in a high yield. Because the hydrolysis reaction proceeds fast relative to the transglycosylation due to the presence of aqueous solution.[1] If the reaction can be carried out in organic solvents without the denaturation of enzymes, the transglycosylation products would be obtained in a high yield.

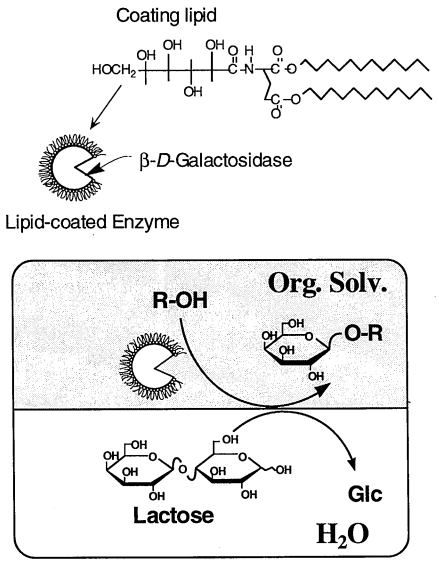

Figure 1. Schematic illustrations of transgalactosylation catalyzed by a lipid-coated β-*D*-galactosidase in aqueous-organic two phases.

We have recently reported a lipid-coated enzyme system, in which hydrophilic head groups of lipids interact with the enzyme surface and two long lipophilic alkyl chains extend away from its surface to solubilize the enzyme in hydrophobic organic solvents.[2-4] The lipid-coated lipase,[2] phospholipase,[3] and catalytic antibody[4] showed high catalytic activities such as enantioselective esterifications in isooctane or benzene.

In this paper, we apply this lipid-coating technique to transglycosylation by using a glycoside hydrolase in aqueous-organic two phases: a lipid-coated β-*D*-galactosidase exists in the organic phase with hydrophobic acceptor alcohols and shows an efficient galactosyl transfer from hydrophilic lactose in the aqueous phase (see Figure 1).

A lipid-coated β-*D*-galactosidase was prepared similarly according to our previous works.[2-4] An aqueous buffer solution (50 mL, 10 mM phosphate, pH 5.1) of the β-*D*-galactosidase (EC 3.2.1.23 from *Escherichia coli*, TOYOBO Co., 50 mg) was mixed with an aqueous dispersion (50 mL) of synthetic glycolipids (50 mg, shown in Figure 1) and stirred for 1 day. The precipitate was gathered by centrifugation, washed with buffer solution and distilled water repeatedly, and then lyophilized. The resulting white powder was soluble in most organic solvents such as chloroform, acetonitrile, benzene and isopropyl ether, but insoluble in aqueous buffer solution. The protein content of the lipid-enzyme complex was determined from both the elemental analysis (C, H, and N) and the UV absorption by aromatic amino acid residues of proteins at 280 nm in chloroform solution. The protein content of the lipid-enzyme complex was 7-8 wt%.

Figure 2 shows typical time courses of transgalactosylation from the 10-fold excess of lactose as a galactosyl donor to 5-phenyl-1-pentanol (PhC_5OH) as a galactosyl acceptor in two phases 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 (Figure 2a), the transglycosylated Gal-OC_5Ph was obtained in 66% yield, but the hydrolyzed galactose did not produce even after 8 days. It was confirmed from ^1H- and ^{13}C-NMR that the stereochemistry of the obtained Gal-OC_5Ph kept the β-configuration of *D*-galactose. As the amount of the consumed PhC_5OH corresponded to that of Gal-OC_5Ph, this means that the formed carbocation intermediate in the active site of the enzyme in isopropyl ether existed in the organic phase and attacked mainly by the alcohol in the organic phase, but not H_2O in the aqueous phase.

On the contrary, when a native β-*D*-galactosidase was employed (Figure 2b), both starting substrates of PhC_5OH and lactose were completely recovered even after 8 days. Since both galactosylation and hydrolysis reaction did not proceed in the two-phase system, a native enzyme may be denatured at the interface between aqueous and organic phases. The lipid-coated β-*D*-galactosidase was found to act as the efficient glycosylation catalyst even in the aqueous-organic two phases.

Table 1 shows the effect of structures of acceptor alcohols on the transgalactosylation from lactose catalyzed by the lipid-coated β-*D*-galactosidase in aqueous-organic two phases. The enzymatic activity is shown by use of the initial rate and the yield at equilibrium after 8 days. Both the initial rate and the yield depended largely on the shape and chain length of alkanols.

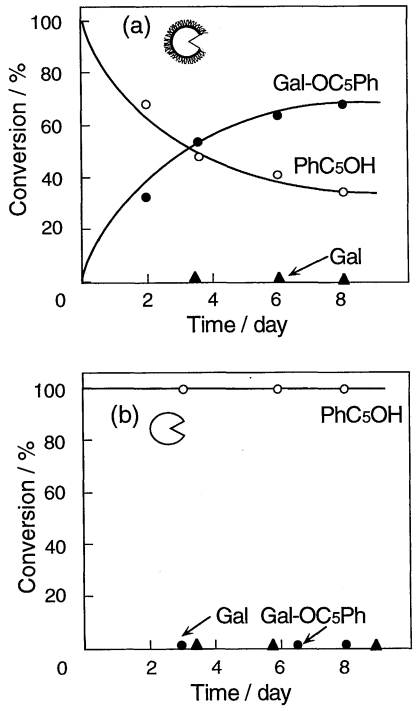

Figure 2. β-*D*-galactosidase from *Escherichia coli* in 10 mL isopropyl ether and 10 mM in 10 mL phosphate buffer (pH 5.1). Enzyme, 0.1 mg of protein/20 mL. Identification and quantification of the substrates and the products were made by comparison of the HPLC retention time and the HPLC peak area to those of the authentic samples, respectively. Production of *D*-galactose was followed with an enzymatic detection using *D*-galactose dehydrogenase [*D*-galactose: NAD^+ 1-oxido-reductase, EC 1.1.1.48].

When the primary alcohols such as 1-butanol and 1-octanol were employed, the yields of galactosides were as high as the case of 5-phenyl-1-pentanol shown in Figure 2a. The reactivity, however, decreased with increasing the alkyl chain length to 1-decanol and 1-dodecanol. When the primary alcohol was changed to the secondary or tertiary alcohol, the transglycosylation yield was depressed in the order of 1° > 2 °> 3°. Even when the yield of transglycosylated products was low, the galactosides were the only products devoid of hydrolyzed galactose.

Saccharide derivatives whose hydroxy groups were partially protected became soluble in organic solutions. When 1,2,3,4-tetra-*O*-acetyl-*D*-glucose or 2,3,4,6-tetra-*O*-acetyl-*D*-glucose was used as acceptor alcohols, the trangalactosylation occurred in fair yield at the 1- or 6-position of glucose. This means the combination of enzymatic and chemical syntheses is useful for regio- and stereo-selective oligosaccharide preparations.

Table 1. Effect of chemical structures of acceptor alcohols on galactosylation catalyzed by a lipid-coated β-*D*-galactosidase in aqueous-organic two phases at 30 °C[a]

acceptor R-OH	initial rate /10^{-3} mM s^{-1} (mg of protein)$^{-1}$	conversion after 8 days/%
	3.2	67
	2.8	53
	0.4	23
	2.9	66
	3.5	82
	0.1	5>
	0.08	5>
	2.8	66
	1.8	40

[a] R-OH, 1.0 mM and β-*D*-galactosidase from *Escherichia coli*, 0.1 mg of protein were solubilized in 10 mL isopropyl ether and lactose, 10 mM in 10 mL phosphate buffer solution (pH 5.1).

In conclusion, the lipid-coated β-*D*-galactosidase can catalyze efficiently the transglycosylation without resulting in the hydrolysis products in aqueous-organic two phases. A variety of alcohols were found to be used as the galactosyl acceptors. This method is currently applying to other glycosidases such as β-*D*-glucosidase and α- or β-*D*-mannosidase.

References and Notes

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