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Transglycosylation catalyzed by a lipid-coated β-D-galactosidase in a two-phase aqueous–organic system

Yoshio Okahata *, Toshiaki Mori

Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501, Japan

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Abstract

A lipid-coated enzyme was prepared in which the enzyme surface is covered with a lipid monolayer and two long alkyl lipophilic tails serve to solubilize the enzyme in organic solvents. In a two-phase aqueous–organic system, a lipid-coated β-D-galactosidase exists in the organic (2-propyl ether) phase and acts as an efficient transgalactosylation catalyst for various hydrophobic alcohols with lactose in the aqueous buffer solution. When a native β-D-galactosidase was employed in the two-phase system, neither the transgalactosylation nor the hydrolysis reaction proceeded due to denaturation of the enzyme at the interface.

Keywords: Lipid-coated enzyme; Transgalactosylation; Two-phase system; Organic solvents

1. Introduction

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 [1–17]. Enzymatic synthesis has advantages to provide regio- and stereoselective 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 [1–17]. 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.

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 [18–22]. The lipid-coated lipase [18–20], phospholipase [21], and catalytic antibody [22] 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 gly-

* Corresponding author. Fax: +81-45-924-5836; E-mail: yokahata@bio.titech.ac.jp

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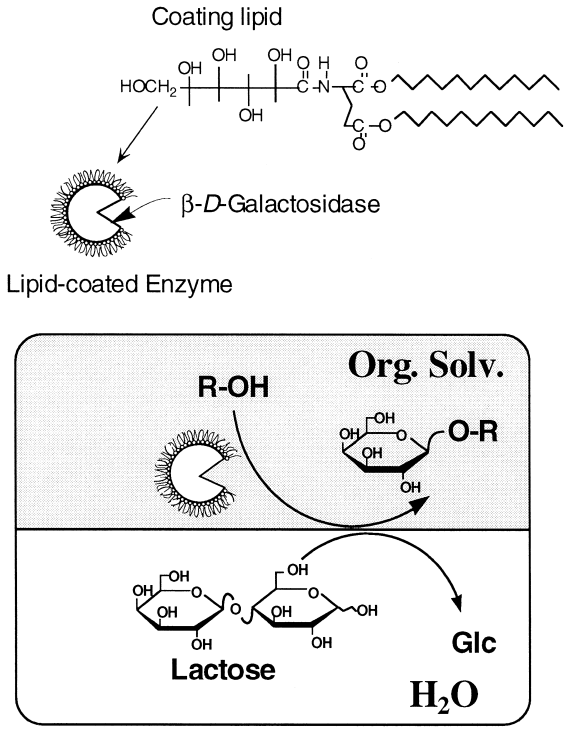

Fig. 1. Schematic illustrations of transgalactosylation catalyzed by a lipid-coated β-D-galactosidase in aqueous–organic two phases.

coside 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 Fig. 1).

2. Experimental

A lipid-coated β-D-galactosidase was prepared similarly according to our previous works [18–22]. 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, 50 mg) was mixed with an aqueous dispersion (50 ml) of synthetic glycolipids (50 mg, shown in Fig. 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.%.

3. Results and discussion

Fig. 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 (Fig. 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 (Fig. 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.

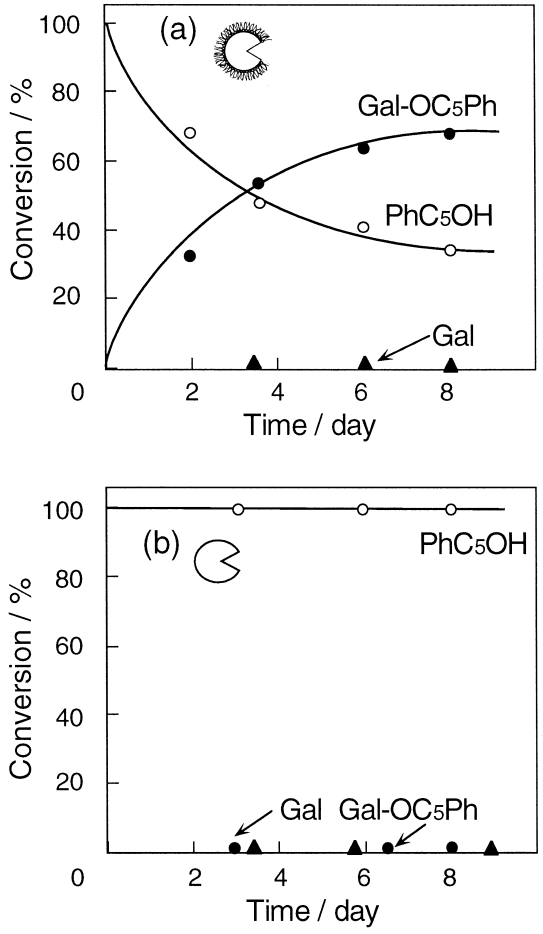

Fig. 2. β-D-Galactosidase from *E. coli* in 10 ml isopropyl ether and 10 mM in 10 ml phosphate buffer (pH 5.1). Enzyme, 0.1 mg of protein/20 ml. Identification and quantification of the substrates and the products were made by comparison of the HPLC retention time and the HPLC peak area to those of the 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].

3.1. Substrate selectivity

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. 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 Fig. 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 or-

Table 1
Effect of chemical structures of acceptor alcohols on galactosylation catalyzed by a lipid-coated β-D-galactosidase in a two-phase system at 30°C[a]

Acceptor alcohols R-OH	Initial rate, 10^{-3} μM 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	3 ± 2
	0.08	3 ± 2
	2.8[b]	66[b]
	1.8[b] 2.2[c]	40[b] 45[c]
	2.0[c]	42[c]
	1.0[c]	35[c]
	0.07[c]	6.1[c]

[a][Lactose] = 10 mM in 10 ml phosphate buffer solution, [R–OH] = 1.0 mM in 10 ml of isopropyl ether, and a lipid-coated enzyme = 0.1 mg of protein.
[b]β-D-Galactosidase from *E. coli* was used as a lipid-coated enzyme.
[c]β-D-Galactosidase from *B. circulans* was used as a lipid-coated enzymes.

Table 2
Effect of organic solvents in the two-phase system on galactosylations catalyzed by a lipid-coated β-D-galactosidase at 30°C[a]

Solvents	Initial rates, 10^{-3} μM s^{-1} (mg of protein)$^{-1}$	Conversion after 8 days, %
2-propyl ether	2.9	66
isooctane	1.6	39
toluene	1.8	37
diethyl ether	1.1	28
benzene	0.4	8
ethyl acetate	0	–

[a][Lactose] = 10 mM, [PhC_5–OH] = 1.0 mM, [β-D-galactosidase from *E. coli*] = 0.1 mg of protein (10 ml phosphate buffer solution and 10 ml of organic solvent in the two-phase system).

ganic 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 that the combination of enzymatic and chemical syntheses is useful for regio- and stereo-selective oligosaccharide preparations.

Transgalactosylation to β-hydroxyethyl methacrylate (HEMA) having an unstable ester linkage was successful in fair yield. It is generally difficult to carry out the chemical synthesis of *O*-glycosides of ester compounds, because the ester linkage is easily hydrolyzed during deprotection. Transgalactosylation to *p*-nitrophenol, which has a low nucleophilicity, was also successful. The *p*-nitrophenyl β-D-galactopyranoside (Gal-*O*-*p*NP) was useful as a marker substrate for enzymatic hydrolysis. Gal-*O*-*p*NP could be obtained and isolated on a large scale (0.1–2 g) in fair yield (52%). The reaction products were purified by recrystallization and HPLC from the organic layer. Transgalactosylation to cholesterol was also successful although the yield was low due to the secondary OH group. Glycoside derivatives of steroids are known as cardiac drugs.

3.2. Effect of organic solvents

Transgalactosylations catalyzed by lipid-coated β-D-galactosidase (from *E. coli*) were carried out in the two-phase system using various organic solvents. Initial rates and the equilibrium conversions after a week are shown in Table 2. The lipid-coated β-D-galactosidase was soluble in all organic solvents listed in Table 2. The lipid-coated β-D-galactosidase showed fairly high initial rates and conversions in nonpolar solvents such as 2-propyl ether and isooctane. The enzyme activity disappeared in a relatively polar solvent such as ethyl acetate. In water-miscible solvents such as tetrahydrofuran, *N*,*N*-dimethylformamide, acetone, and acetonitrile, the lipid-coated β-D-galactosidase did not show any catalytic activity (data not shown). It might be easily denatured in these systems due to the removal of essential water from the enzyme to the bulk solution. Similar tendencies were observed in the activities of the lipid-coated lipase or phospholipase [18–21].

3.3. Effect of origin of β-D-galactosidase

An advantage to using glycoside hydrolases in glycosylation is that many of those kinds of enzymes are commercially available. Lipid-coated β-D-galactosidases from different origins were prepared and the results of galactosylation in the two-phase system are summarized in Table 3. The enzyme activity for the galactosylation was found to largely depend on the origin of enzymes. In the case of β-D-galactosidase from *bovine liver*, no galactoside was formed.

Table 3
Effects of origin of β-D-galactosidase on transgalactosylations to *n*-, *sec*-, and *tert*-butanols

Origin of β-D-Galactosidase	Conversion after 8 days,[a] % 		
Escherichia coli	67	53	23
Aspergillus oryzae	38	31	0
Bacillus circulans	29	40	0
Bovine liver	0	0	0

[a]Transgalactosylation from lactose (300 mM) in phosphate buffer solution (0.1 ml) and butanol (5.0 mM) in 2-propyl ether (2 ml) catalyzed by a lipid-coated β-D-galactosidase ([enzyme] = 0.1 mg of protein) at 30°C.

The lipid-coated β-D-galactosidase from *Aspergillus oryzae*, as well as from *E. coli*, could mainly catalyze transglycosylation to the primary alcohol, to a lesser extent to the secondary alcohol, but not to the tertiary alcohol. On the other hand, the enzyme from *Bacillus circulans* showed much selectivity to the secondary alcohol over the primary alcohol. These selectivities corresponded with examples of native enzymes in the conventional aqueous solutions [23].

4. Summary

Lipid-coated β-D-galactosidase can efficiently catalyze transglycosylation to alcohols without producing hydrolysis products in aqueous–organic two-phase system. A variety of alcohols was found to be accommodated as the galactosyl acceptors. The enzyme activity for the galactosylation depended on the reaction solvents and the origin of enzymes. This technique is obviously suitable for the transglycosylation compared with the conventional method using a native enzyme in aqueous–organic solvents [24–29].

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