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Carbohydrate Research 298 (1997) 65–73

CARBOHYDRATE RESEARCH

Transglycosylation in a two-phase aqueous–organic system with catalysis by a lipid-coated β-D-galactosidase [1]

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Received 22 July 1996; accepted 7 October 1996

Abstract

A lipid-coated β-D-galactosidase 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 enzyme 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. Effects of coating lipid molecules, origins of enzymes, reaction in organic solvents, and chemical structures of acceptor alcohols on the transgalactosylation catalyzed by the lipid-coated enzyme were studied. This system could also be applied in a large-scale synthesis on the 0.1–1 g scale.

Keywords: Transgalactosylation; β-D-Galactosidase; Aqueous-organic two-phase reactions; Lipid-coated enzymes

1. Introduction

In recent years, enzymatic transglycosylations catalyzed by glycosyl transferases or glycoside hydrolases have been the focus of considerable research [1–3]. These enzymatic syntheses have advantages in that reactions occur regioselectively and stereoselectively without using protection and deprotection processes that are required with synthetic chemical methods. In enzymatic transglycosylations, reverse hydrolyses by glycosidases have been more widely used than glycosyl transferase reactions, because glycosidase enzymes are widely applicable and useful for the preparation of a wide variety of transglycosides. In the transglycosylation catalyzed by glycosidase using its reverse hydrolysis, water-miscible organic solvents have been added to increase yields of transglycosylations and decrease hydrolysis reactions [4–12]. However, the transglycosylation yields in these examples have usually been low because the hydrolysis reactions proceeded fast relative to the transglycosylations in homogeneous aqueous–organic media [4–12]. If the reaction could be carried out in

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[1] Enzyme-lipid Complex 13. For part 12, see Y. Okahata and T. Mori, Trends Biotechnol., in press.

0008-6215/97/$17.00
PII S0008-6215(96)00298-4

nonaqueous organic solvents without the denaturation of enzymes, it is deemed likely that the transglycosylation products could be obtained in high yields.

We have reported a lipid-coated enzyme system in which the enzyme surface is covered with dialkyl amphiphiles, and these lipophilic alkyl tails serve to solubilize the enzyme in hydrophobic organic solvents [13–19]. When such a lipid-coated lipase was prepared, it was shown to act as an efficient catalyst for enantioselective esterifications (the reverse hydrolysis) in dry isooctane [15,16]. The catalytic efficiency was large compared with that of other lipase systems in organic media such as poly(ethyleneglycol)-grafted lipase [20–22] and a lipase dispersion system [23–28]. For example, a lipid-coated phospholipase D can catalyze a choline head-group exchange reaction of phosphocholine lipid in a two-phase aqueous–organic system [17]. The lipid-coating system can also be applied to catalytic antibodies in water-miscible organic media [19].

In a previous paper we reported that a lipid coated β-D-galactosidase can catalyze transgalactosylation to hydrophobic alcohols from *p*-nitrophenyl β-D-galactopyranoside (Gal-O-*p*NP) in dry 2-propyl ether. The yields of the transgalactosylated compounds were much higher than those catalyzed by a native galactosidase in water-miscible organic and aqueous buffer media [29], presumably because the lipid-coating system can be used in dry organic media without side hydrolysis reactions. However, this system could not be applied for a large-scale synthesis because of the low solubility of the galactosyl donor (Gal-O-*p*NP) in hydrophobic organic media. Gal-O-*p*NP was prepared by O-glycosylation using skillful protection and deprotection of galactose.

In this study we report a new transgalactosylation system by using a lipid-coated β-D-galactosidase in a two-phase aqueous–organic system in which both the lipid-coated enzyme and hydrophobic acceptor alcohols exist in the organic phase and an excess amount of inexpensive lactose as galactose donor is present in the aqueous buffer solution. A schematic illustration of the transgalactosylation is shown in Fig. 1. It should be noted that the enzyme exists in the organic solvent phase. Although native β-D-galactosidase may initially be considered for the transglycosylation, in reality, it was found not to catalyze the transglycosylation in this system. Synthesis through the glycosylation of useful compounds, such as substrates for assays of the enzyme activity, using the two-phase system was also examined. β-D-Galactosidase was chosen since the glycosidases are widely studied and

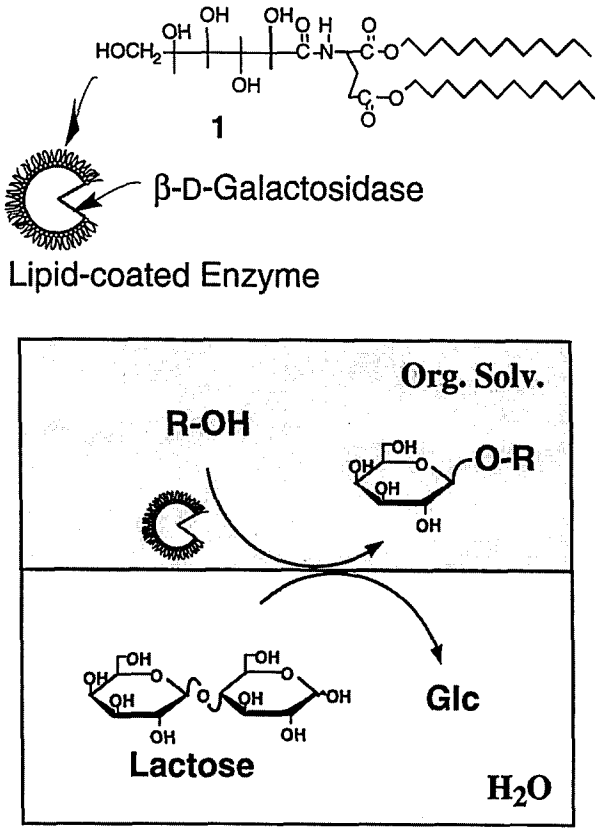


Fig. 1. Schematic illustrations of transgalactosylation in the two-phase organic–aqueous system catalyzed by a lipid **1**-coated β-D-galactosidase.

easily obtained [4–12]. Enzymatic activity was studied under various conditions of coating lipids, enzyme origin, organic reaction solvent, and substrate structures. These reactions are also compared with two-phase aqueous–organic system using a conventional native enzyme.

2. Results and discussion

Fig. 2 shows typical time courses of transgalactosylation from a 10-fold excess of lactose as a galactosyl donor in the aqueous phase to 5-phenyl-1-pentanol (PhC_5OH) as a galactosyl acceptor in the organic phase at 30 °C. When a lipid-coated β-D-galactosidase was solubilized in the organic phase, the transglycosylated product (5-phenyl-1-pentyl β-D-galactopyranoside, Gal-O-C_5Ph) was obtained in 66% yield after eight days as the only product in the organic phase. Galactose, the hydrolyzed product of lactose, was not obtained even after a week in the aqueous phase (Fig. 2a). It was confirmed from ^1H and ^{13}C NMR spectra that the chemical structure of Gal-O-

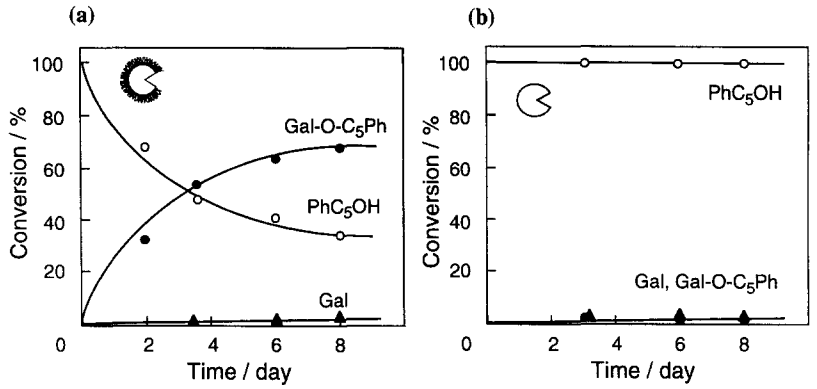


Fig. 2. Typical time-courses of transgalactosylation from lactose (10 mM) to 5-phenyl-1-pentanol (PhC_5OH, 1.0 mM) catalyzed by (a) a lipid **1**-coated β-D-galactosidase and (b) a native β-D-galactosidase from *E. coli*, in a 2-propyl ether–aqueous buffer two-phase system: 30 °C, 10 mL of 2-propyl ether and 10 mL of buffer solution (0.01 M phosphate, pH 5.1), and [Enzyme] = 0.1 mg of protein.

C_5Ph was in the β configuration (see Experimental section). The amount of the consumed PhC_5OH corresponds with the production of Gal-O-C_5Ph. On the other hand, when a native β-D-galactosidase was employed in the same two-phase system, the transgalactosylation (the reduction of PhC_5OH) and the hydrolysis of lactose (the production of glucose) were both hardly observed (Fig. 2b). This result shows that the native enzyme seems to be easily denatured at the aqueous–organic interface. These results indicate that the lipid-coated β-D-galactosidase can act as the efficient glycosylation catalyst in the 2-propyl ether–water two-phase system.

It should be mentioned that the yield of the Gal-O-C_5Ph product plateaued around 70%, even after a week as shown in Fig. 2a. Furthermore, when the lipid-coated enzyme was added to the reaction mixture after the reaction reached the equilibrium, the yield did not increase. When the lipid-coated enzyme had been soaked in 2-propyl ether for two days in advance and both substrates were then added, the transgalactosylation proceeded in 65% yield in eight days and reached the equilibrium as observed for the conventional reaction. These results indicate that the lipid-coated enzyme was not deactivated during the reaction in the organic solvents. When the product, Gal-O-C_5Ph, was added to the organic phase in advance, the yield of the transglycosylation product decreased with an increase in the amount of the added Gal-O-C_5Ph. For example, when the equivalent amount of Gal-O-C_5Ph was added to the substrate, the reaction was completely stopped. The maximizing yield near 65% is explained by the product inhibition and not by the deactivation of the lipid-coated enzyme.

Effect of coating lipids.—Lipid-coated enzymes were prepared from β-D-galactosidase (from *E. coli*) and several kinds of dialkyl amphiphiles having non-

Table 1
Effect of coating lipid structures on preparation of a lipid-coated β-D-galactosidase and its enzymatic activity

Lipids	Preparation		Enzymatic activity
	Yields [a] (mg)	Protein content [b] (wt%)	Conversion after 8 days (%) [c]
Nonionic **1**	32.6	8.8	66
Anionic **2**	40.5	7.0	20
Cationic **3**	5.7	32	17
Zwitterionic **4**	0	–	–

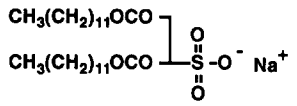
[a] Both aqueous solutions of β-D-galactosidase from *Escherichia coli* (50 mg) and lipids (50 mg) were mixed and precipitates were lyophilized.
[b] Obtained from UV absorption of aromatic amino acid residues in the protein, which was consistent with the protein content obtained from C:N ratio of elemental analyses.
[c] Transgalactosylation was carried out under the same conditions as Fig. 2.

Table 2
Effect of organic solvents in the two-phase system on galactosylations catalyzed by a lipid **1**-coated β-D-galactosidase at 30 °C [a]

Solvents	Initial rates [10^{-3} mM 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 *Escherichia coli*] = 0.1 mg of protein (10 mL phosphate buffer solution and 10 mL of organic solvent in the two-phase system).

ionic, anionic, cationic, and zwitterionic head groups. The results are summarized in Table 1. Chemical structures of the lipid molecules are shown in Fig. 1 and in structures **2**–**4**. Lipid-coated β-D-galactosidases were obtained as precipitates in a fair yield when the nonionic **1** and anionic **2** amphiphiles were employed, but not when the cationic **3** and zwitterionic **4** amphiphiles were employed. Although the complexes were produced in fair yield using the anionic amphiphiles **2**, transgalactosylation activity was very low compared to those of complexes with the nonionic amphiphiles **1**. This is probably because the strong electrostatic interaction between the anionic head groups of amphiphiles and the hydrophilic surface of the β-D-galactosidase denatures the protein structures. These results corresponded to preparations of lipid-coated enzymes such as lipases [13–16,18]. The nonionic dialkyl amphiphile **1** was chosen as the standard coating lipid for β-D-galactosidase in the following experiments.



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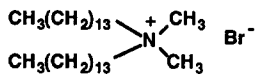

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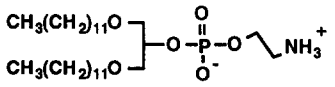

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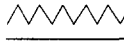
Effect of organic solvents.—Transgalactosylations catalyzed by lipid **1**-coated β-D-galactosidase (from *E. coli*) were carried out in the two-phase system using various organic solvents. Initial rates and the equilibrium conversions after 8 days are shown in Table 2. The lipid-coated β-D-galactosidase 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 [14–19]. 2-Propyl ether was chosen as the organic phase in the two-phase system in the following experiments.

Substrate selectivity.—Table 3 shows the effect of chemical structures of acceptor alcohols on the transgalactosylation from lactose catalyzed by the lipid **1**-coated β-D-galactosidase in the 2-propyl ether–phosphate buffer two-phase system. The enzymatic activity is shown as the initial rate, and the equilibrium yield is that measured after 8 days. Both the initial rate and the yield depended largely on the shape and the chain length of the alkyl alcohols. When the primary alcohols (C_4OH and C_8OH) were employed, the galactoside yields were nearly as high as the case of PhC_5OH as shown in Fig. 2a. The reactivity, however, decreased with increasing alkyl chain length ($C_{10}OH$ and $C_{12}OH$). When the primary alcohol was changed to the secondary or the tertiary alcohol, the transglycosylation activity was depressed in the order of 1° > 2° > 3°. Even when the transgly-

Table 3
Effect of chemical structures of acceptor alcohols on galactosylation catalyzed by a lipid **1**-coated β-D-galactosidase in a two-phase system at 30 °C [a]

Acceptor alcohols 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	3±2
	0.08	3±2

[a] [Lactose] = 10 mM, [R–OH] = 1.0 mM, [β-D-galactosidase from *Escherichia coli*] = 0.1 mg of protein in a two-phase system (10 mL phosphate buffer solution and 10 mL of 2-propyl ether).

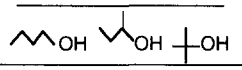
cosylation yield was low, the galactosides were the only product devoid of hydrolyzed galactose.

Effect of origin of β-D-galactosidase.—An advantage of using glycoside hydrolases in glycosylation is that many of these kinds of enzymes are commercially available. Lipid-coated β-D-galactosidases from different origins were prepared with the nonionic amphiphile **1**, and the results of galactosylation in the two-phase system are summarized in Table 4. Whenever the nonionic amphiphile **1** was used as a coating lipid, the lipid-coated β-D-galactosidase was obtained in fair yield and in nearly constant protein content independent of the origin (30 ± 5 mg in yield and 9 ± 3 wt% in protein content). However, the enzyme activity for the galactosylation was found to depend largely on the origin of enzymes. In the case of β-D-galactosidase from bovine liver, no galactoside was formed. 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 [30].

Practical preparations of useful galactoside derivatives.—We have applied this system to produce some useful glycoside derivatives. The result of the galactosylation to various acceptor alcohols is summarized in Table 5. In chemical syntheses of oligosaccharides or carbohydrate derivatives, the reactive hydroxyl groups are often protected by acetyl groups. Since these compounds are soluble in organic solvents but not in aqueous solutions, tetra-*O*-acetyl-D-glucoses are suitable as lipophilic acceptors in the two-phase system. Transgalactosylations to 1,2,3,4-tetra-*O*-acetyl- or 2,3,4,6-tetra-*O*-acetyl-D-glucose were successful in 40–66% yields. Transgalactosylation yields to the primary OH group of 1,2,3,4-tetra-*O*-acetyl-D-glucose were higher than those to the anomeric OH group of 2,3,4,6-tetra-*O*-acetyl-D-glucose. This is the same tendency as shown in Table 3. The yield for 2,3,4,6-tetra-*O*-acetyl-D-glucose was slightly improved by using β-D-galactosidase from *Bacillus circulans*, which shows high activity to the anomeric alcohols.

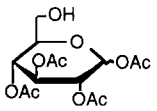
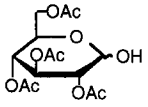
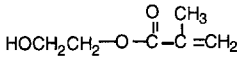
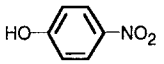
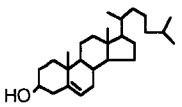
Transgalactosylation to β-hydroxyethyl methacrylate (HEMA) having an unstable ester linkage was successful in fair yield. It is generally difficult to carry out the chemical synthesis of O-glycosides of ester compounds, because the ester linkage is easily hydrolyzed during deprotection. Transgalactosylation to *p*-nitrophenol, which has a low nucleophilicity, was also successful. The *p*-nitrophenyl β-D-galacto-

Table 4
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 **1**-coated β-D-galactosidase ([enzyme] = 0.1 mg of protein) at 30 °C.

Table 5
Practical preparations of useful galactoside derivatives catalyzed by a lipid **1**-coated β-D-galactosidase in a two-phase system at 30 °C [a]

Acceptor alcohols R–OH	Initial rate [10^{-3} mM s^{-1}(mg of protein)$^{-1}$]	Conversion after 8 days (%)
	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 *Escherichia coli* was used as a lipid-coated enzyme.
[c] β-D-galactosidase from *Bacillus circulans* was used as a lipid-coated enzymes.

pyranoside (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 recrystalyzation 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.

It is meaningful to compare enzyme activities of a native and a lipid-coated one in the same reaction conditions. However, a native β-D-galactosidase denatured in a two-phase aqueous–organic system (see Fig. 2). Therefore, transgalactosylations from lactose to various alcohols catalyzed by a native β-D-galactosidase were studied in homogeneous 70:30 aqueous–acetonitrile solution at 30 °C, in which acetonitrile was added to increase the yield of transgalactosylation and the solubility of acceptor alcohols [4–12]. Yields of transglycosylation to butanols (primary, secondary, and tertiary) and octanol were in the range of 10–30% after eight days. The transgalactosylation yield for cholesterol and *p*-nitrophenol was less than 5% due to the low solubility and the low nucleophilicity, respectively. In the reaction of the homogeneous aqueous–organic solution using a native enzyme, the main products (60–70%) were glucose and galactose that were the hydrolyzed products of lactose.

3. Conclusions

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 largely depended on the coating of the lipid molecules 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. If the two-phase aqueous–organic system can be widely applicable to other glycoside hydrolases, it would be very useful, because one does not need to use the expensive glycoside as the glycosyl donor.

4. Experimental procedures

Materials.—A fine grade of β-D-galactosidases [EC 3.2.1.23] from *Escherichia coli* (TOYOBO Co.,

Tokyo), *Aspergillus oryzae* (Sigma), bovine liver (Sigma), and *Bacillus circulans* (Daiwa Kasei, Osaka) were used without further purification. Preparations of dialkyl amphiphiles; didodecyl *N*-D-gluconoL-glutamate (**1**) [31], sodium 1,2-bis(dodecyloxycarbonyl)ethanesulfonate (**2**) [32], and 1,3-dihexadecyl-*rac*-glycero-2-phosphocholine (**4**) [33] have been reported elsewhere. Dioctadecyldimethylammonium bromide (**3**) was purchased as the finest grade from Sogo Pharmaceutical, Tokyo. The chemical structures of these amphiphiles (**1–4**) are shown in Fig. 1 and in structures **2–4**. Acceptor alcohols of 1,2,3,4-tetra-*O*-acetyl-D-glucose and 2,3,4,6-tetra-*O*-acetyl-D-glucose were synthesized according to conventional methods [34,35]. Other chemicals and organic solvents were purchased from Tokyo Kasei, Tokyo, Nacalai Tesque, Kyoto, and Kanto Chemicals, Tokyo.

Preparation of a lipid-coated β-D-galactosidase. —A lipid-coated β-D-galactosidase was prepared in a manner similar to that reported in our previous papers [13–19,29]. An aqueous buffer solution (50 mL, 0.01 M phosphate, pH 5.1) of the β-D-galactosidase (50 mg) was mixed with an aqueous dispersion (50 mL) of dialkyl amphiphiles (50 mg) at 4 °C and stirred for 1 day at 4 °C. Precipitates were gathered by centrifugation at 4 °C (5000 rpm, 15 min) and repeatedly washed with buffer solution and distilled water, and then lyophilized. The resulting white powder was soluble in most organic solvents such as acetonitrile, benzene, and 2-propyl 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 [13–19,29]. Results are summarized in Table 1.

Catalytic activity of a lipid-coated β-D-galactosidase in a two-phase system.—A typical procedure is as follows. The two-phase system of aqueous buffer solution (10 mL, 0.01 M phosphate, pH 5.1) of the lactose monohydrate (36 mg, 10 mM) and 2-propyl ether solution (10 mL) of a lipid-coated enzyme (1–2 mg, 0.1 mg of protein) and an alcohol substrate (1.4–8.5 mg, 1.0 mM) was vigorously stirred at 30 °C. Within the prescribed time interval, the reduction of alcohol acceptors was followed by liquid chromatography: TSK-gel ODS-80Ts (4.6 mm i.d. × 25 cm) in a TOSOH CCPD-system liquid chromatography equipped with a UV (at 254 nm) and RI detector (elution, 1:1 acetonitrile–water; flow rate, 1 mL/min). 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 in the aqueous phase was followed with an enzymatic detection using D-galactose dehydrogenase [D-galactose: NAD^+ 1-oxido-reductase, EC 1.1.1.48] [36].

The transgalactosylation products (β-D-Gal-OR) in Table 3 were isolated from the reaction mixture by evaporating the organic phase and purified by a large-scale separation on a preparative HPLC column (TSK-gel ODS-80Ts, 21.5 mm i.d. × 30 cm). The chemical structures of the isolated products were confirmed by elemental analyses (C and H) and by ^1H and ^{13}C NMR spectroscopy. The elemental analyses for all products were consistent with the calculated values within 0.2%. Spectral data of ^1H (CD_3OD, 300 MHz) and ^{13}C NMR (CD_3OD, 75 MHz) of the products were as follows.

Butyl β-D-galactopyranoside: ^1H NMR (CD_3OD, 300 MHz): d 0.8 (3 H, t), 1.2 (4 H, m), 3.4 (2 H, t), 3.5–4.2 (5 H, m), 4.7 (1 H, d, *J* 7.0 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 12.1 (C-4′), 23.0 (C-3′), 32.6 (C-2′), 62.9 (C-6), 70.1 (C-1′), 71.3 (C-4), 72.0 (C-2), 75.4 (C-3), 78.5 (C-5), 104.3 (C-1, β-bond).

sec-Butyl β-D-galactopyranoside: ^1H NMR (CD_3OD, 300 MHz): d 0.9 (3 H, t), 1.2 (2 H, m), 1.5 (3 H, m), 3.2 (1 H, m), 3.5–4.2 (5 H, m), 4.6 (1 H, d, *J* 7.1 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 10.4 (C-3′), 21.8 (C-1″), 33.6 (C-2′), 62.8 (C-6), 71.4 (C-4), 72.0 (C-2), 75.3 (C-3), 76.9 (C-1′), 78.1 (C-5), 104.1 (C-1, β-bond).

tert-Butyl β-D-galactopyranoside: ^1H NMR (CD_3OD, 300 MHz): δ 1.3 (9 H, s), 3.4–4.1 (5 H, m), 4.7 (1 H, d, *J* 7.0 Hz). ^{13}C NMR (CD_3OD, 75 MHz): d 37.1 (C-1′), 62.9 (C-6), 66.4 (C-2′), 71.1 (C-4), 72.9 (C-2), 75.4 (C-3), 78.4 (C-5), 104.1 (C-1, β-bond).

5-Phenylpentyl β-D-galactopyranoside: ^1H NMR (CD_3OD, 300 MHz): d 1.2 (4 H, m), 1.6 (2-H, t), 2.1 (2 H, t), 3.3 (2 H, t), 3.4–4.1 (5 H, m), 4.7 (1 H, d, *J* 7.0 Hz), 7.2–7.5 (5 H, m). ^{13}C NMR (CD_3OD, 75 MHz): d 27.4 (C-3′), 31.5 (C-2), 33.3 (C-5′), 48.8 (C-4′), 63.1 (C-6), 65.6 (C-1′), 71.2 (C-4), 72.0 (C-2), 75.5 (C-3), 78.5 (C-5), 104.6 (C-1, β-bond), 128.2 (Ph), 128.5 (Ph), 129.6 (Ph).

Octyl β-D-galactopyranoside: ^1H NMR (CD_3OD, 300 MHz): δ 0.9 (3 H, t), 1.3 (10 H, m), 1.6 (2 H, t), 3.2 (2 H, t), 3.6–4.1 (5 H, m), 4.7 (1 H, d, *J* 7.1 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 12.6 (C-8′), 21.6 (C-7′), 26.3, 28.8, 29.0, 29.3, 30.1 (C-2′–C-6′), 60.9 (C-6), 69.1 (C-1′), 71.3 (C-4), 72.0 (C-2), 75.4 (C-3), 78.5 (C-5), 104.1 (C-1, β-bond).

Decyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 0.9 (3 H, t), 1.3 (14 H, m), 1.6 (2 H, t), 3.3 (2 H, t), 3.6–4.1 (5 H, m), 4.6 (1 H, d, *J* 7.1 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 10.9 (C-10′), 24.1 (C-9′), 25.9, 26.4, 29.1, 30.0, 31.2 (C-2′–C-8′), 63.1 (C-6), 68.4 (C-1′), 70.9 (C-4), 73.1 (C-2), 74.6 (C-3), 79.0 (C-5), 104.1 (C-1, β-bond).

Dodecyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 0.9 (3 H, t), 1.3 (18 H, m), 1.6 (2 H, t), 3.2 (2 H, t), 3.5–4.1 (5 H, m), 4.6 (1 H, d, *J* 7.1 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 11.3 (C-12′), 23.7 (C-9′), 26.3, 27.2, 27.8, 28.6, 30.1, 31.4 (C-2′–C-10′), 62.1 (C-6), 67.4 (C-1′), 70.6 (C-4), 74.0 (C-2), 75.9 (C-3), 79.1 (C-5), 103.8 (C-1, β-bond).

1,2,3,4-Tetra-*O*-acetyl-D-glucopyranosyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 2.0–2.2 (12 H, m), 3.5–4.1 (7 H, m), 4.6 (1 H, d, *J* 7.1 Hz) 5.0–5.4 (3 H, m), 5.7 (1 H, d). ^{13}C NMR (CD_3OD, 75 MHz): δ 103.0 (C-1, β-bond).

2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 2.0–2.2 (12 H, m), 3.5–4.1 (5 H, m), 4.1–4.3 (3 H, m), 4.6 (1 H, d, *J* 7.1 Hz), 4.9–5.2 (3 H, m), 5.3 (1 H, t). ^{13}C NMR (CD_3OD, 75 MHz): δ 102.4 (C-1, β-bond).

Methacryloyloxyethyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 1.95 (3 H, s), 3.70 (2 H, t), 3.75 (2 H, t), 3.8–4.1 (6 H, m), 4.3 (1 H, d, *J* 7.1 Hz), 5.75 (1 H, d), 6.2 (1 H, d). ^{13}C NMR (CD_3OD, 75 MHz): δ 18.5 (Me), 55.2 (C-2′), 60.3 (C-1′), 60.9 (C-6), 72.6 (C-4), 73.1 (C-2), 75.4 (C-3), 78.9 (C-5), 103.7 (C-1, β-bond), 124.7 (*C*=C–Me), 137.2 (C=*C*–Me), 167.9 (C=O).

p-Nitrophenyl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 3.6–4.1 (5 H, m), 4.9 (1 H, d, *J* 6.5 Hz), 7.2, 7.5, 8.2 (4 H, d). ^{13}C NMR (CD_3OD, 75 MHz): δ 63.6 (C-6), 71.3 (C-4), 73.3 (C-2), 75.4 (C-3), 78.6 (C-5), 103.0 (C-1, β-bond), 119.4 (C-2′, C6′), 129.0 (C-3′, C5′), 114.2 (C-4′), 164.8 (C-1′).

Cholesteryl β-D-galactopyranoside: ^{1}H NMR (CD_3OD, 300 MHz): δ 0.9–2.4 (44 H, t), 3.3 (1 H, m), 3.6–4.2 (5 H, m), 4.6 (1 H, d, *J* 6.9 Hz), 5.4 (1 H, m). ^{13}C NMR (CD_3OD, 75 MHz): δ 105.8 (C-1, β-bond).

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