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Synthesis of Alkyl Glycosides Catalyzed by β-Glycosidases in a System of Reverse Micelles

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Abstract—A basic possibility of enzymic synthesis of alkyl glycosides in a system of the Aerosol-OT (AOT) reverse micelles was studied. Octyl β-*D*-galactopyranoside and octyl β-*D*-glucopyranoside were synthesized from the corresponding sugars (lactose or glucose) and octyl alcohol under catalysis with glycolytic enzymes, β-galactosidase and β-glucosidase, respectively. The transglycosylation/hydrolysis ratio was shifted toward transglycosylation by using octyl alcohol, one of the substrates, as an organic solvent. The alkyl glycosides were thus obtained in one step from a hydrophilic mono- or disaccharide and a hydrophobic aliphatic alcohol. The direction of the reaction was shown to depend on the pH of aqueous solution solubilized in reverse micelles. The maximum yields were 45% and 40% for octyl galactoside and octyl glucoside, respectively; they markedly exceeded the yields of enzymic syntheses in a two-phase system reported previously.

Key words: alkyl glycosides, β-galactosidase, β-glucosidase, reverse micelle system

INTRODUCTION

Long-chain alkyl glycosides belong to the group of nonionic surfactants with good emulsifying and wetting properties, which also possess antimicrobial activity and are easily biodegradable [1].[2] Some of these compounds are used in the food and pharmaceutical industries [2] and as substrates for producing acyl glycosides (sugar esters of fatty acids) that are applied as practically important surfactants [3]. The traditional chemical synthesis of pure alkyl glycoside anomers proceeds through several stages and requires the protection and subsequent deprotection of the carbohydrate reactive hydroxyl groups, activation of the anomeric carbon atom, and separation of the resulting anomers [4].

Enzymic synthetic methods are advantageous over the chemical ones, as they ensure the regio- and stereoselective one-stage obtaining of the product. The enzymically catalyzed synthesis of an alkyl glycoside can be realized in two routes: direct coupling (inverse hydrolysis) of the corresponding sugar and alkanol (a thermodynamic approach) [5] and transglycosylation (kinetic approach) [6]. Both processes are carried out in two-phase systems, with an aqueous phase containing hydrophilic components, an enzyme (glycosidase) and a substrate (mono- or disaccharide), and a corresponding hydrophobic alcohol being in an organic phase. In such systems, the hydrophobicity of the product is intermediate between those of the two substrates. The product distribution coefficient between the organic and the aqueous phase (α_{app}) depends on the length of the aglycone hydrocarbon chain (n) and its concentration (P); it is equal to $[P]_{ROH}/[P]_{H_2O}$ and has values 5–30 at n = 6–10 [7].

A great inconvenience of two-phase systems evidently consists in reducing the water solubility of an alcohol (and, thus, its availability to the enzyme in the aqueous phase) along with the lengthening of its hydrocarbon chain. This results in the reduced rate of synthesis and an accumulation of the hydrolysis products in the reaction mixture (in the case of transglycosylation). Thus, the synthesis of alkyl glycosides with the alkyl chain lengths more than 12 carbon atoms proceeds in this system in extremely low yields (Table 1). To achieve the yields given in Table 1 within reasonable time periods, elevated temperature should be used. However, under such conditions, the enzyme rapidly denatures, losing its activity within first few hours [7, 8].

Taking into account the disadvantages of the alkyl glycoside synthesis by both chemical and enzymic methods in two-phase systems, we aimed to use for this purpose the system of reverse AOT micelles in octane. In contrast to the conditions discussed above, the system of reverse micelles helps work with both water-soluble and water-insoluble compounds in the pseudohomogeneous medium.

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[2] Abbreviations: AOT, Aerosol-OT, sulfosuccinic acid di(2-ethylhexyl) ester sodium salt; Oc, octyl.

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Table 1. The results of the synthesis of *n*-alkyl-β-*D*-glucosides from glucose and the corresponding alcohol ($C_nH_{2n+1}OH$) in a two-phase system catalyzed by β-glucosidase*

n-Alcohol	Alkyl	Reaction time, days	Alcohol–water, vol/vol	Yield, %**
6	Hexyl	4	16.7	20
7	Heptyl	5	16.7	13
8	Octyl	3	10	6
		4	16.7	9
		6	30	14
		10	50	2
9	Nonyl	7	16.7	5
10	Decyl	7	16.7	4
12	Dodecyl	7	16.7	<1

* The reaction was carried out at 60°C at vigorous stirring (adapted from [6, 7]).

** From the starting glucose.

Reverse micelles in organic medium are the associates of surfactant molecules, whose internal and external layers are formed by the polar heads and the hydrocarbon tails of these molecules, respectively. The ability to solubilize (to include into micelles) water and other polar substances is the most important property of reverse micelles, with the solubilizing capacity strongly depending on the nature of the micelle-forming surfactant (see, e.g., [9]). Enzymes solubilized in organic solvents by reverse micelles of surfactants can retain their catalytic activity [10–13].

The possibility of the exact dosage of water content and the purposeful variation of the basic physicochemical factors is one of the main advantages of the micellar systems.

We herein studied the possibility of enzymic synthesis of surfactants (alkyl glycosides) by the example of octyl glucosides and octyl galactosides in the system of reverse AOT micelles, with one of the substrates, aliphatic alcohol *n*-octanol, being the organic solvent. We also investigated the conditions favoring the formation of alkyl glycosides and developed a procedure for the analytical isolation of resulting products.

RESULTS AND DISCUSSION

Synthesis of Octyl Galactopyranoside from Lactose Catalyzed by β-*Galactosidase*

One can see from the results of the reaction of lactose and octanol as substrates catalyzed by β-galactosidase (Fig. 1) that octyl galactoside accumulates in the system along with the disaccharide hydrolysis products (glucose and galactose). The reaction evidently proceeds according to the following scheme:

$$\text{Gal}\beta 1\text{-4Glc-OH} \xrightarrow[\text{OcOH}]{\text{H}_2\text{O}} \begin{array}{l}\text{Gal-OH + Glc-OH (hydrolysis)}\\ \text{Gal-Oc + Glc-OH (transglycosylation).}\end{array}$$

The absence of a maximum on the galactose accumulation curve suggests that either the galactose moiety of lactose is a donor in the transglycosylation reaction or the rate of octyl galactoside formation exceeds the rate of the lactose hydrolysis. Note that the yield of octyl galactoside in the system of reverse AOT micelles in octane at pH 4.5 at 30°C (20%) markedly exceeds the results of similar syntheses reported previously [6, 7]. The raise of temperature up to 60°C results in an equal increase in the rates of both reactions, hydrolysis and transglycosylation, so that this does not affect the yield of the transglycosylation product. Thus, on the one hand, the synthesis can be substantially accelerated by raising the temperature if necessary, but, on the other hand, performing the synthesis at room temperature obviously simplifies the process without loss of the reaction productivity.

The equilibrium between the enzymic hydrolysis and transglycosylation is markedly shifted upon changing pH of the solution from 4.5 to 7.5 (Fig. 2). The maximum yield of octyl galactoside (45%) is observed at pH 5.5; however, the degree of hydrolysis is also rather high at this pH (62%). The accumulation of the transglycosylation product was somewhat less (40%) at pH 6.5, but, in this case, only 50% of the substrate was hydrolyzed, that is, almost entire substrate was subjected to transglycosylation. Under these conditions (pH 6.5), we succeeded in achieving an advantageous ratio between the transglycosylating and hydrolytic activities of β-galactosidase. After 5 days, 80% of

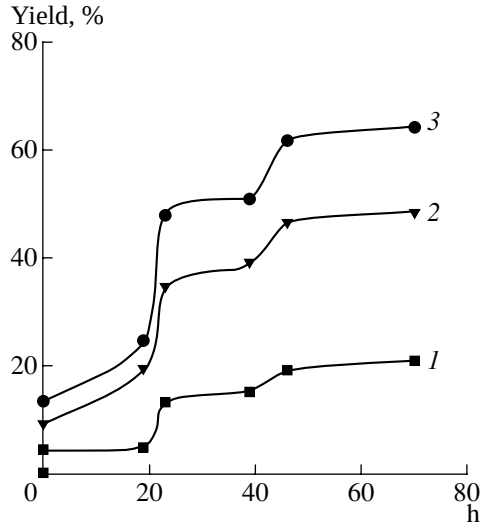

Fig. 1. Accumulation of (*1*) octyl galactoside, (*2*) galactose, and (*3*) glucose upon the synthesis of octyl galactoside from lactose and octanol catalyzed by β-galactosidase (30°C, pH 4.5). The yields are calculated from the starting lactose using the TLC data; $[Lac\text{-}OH]_0$ = 3 mM; $[E]_0$ = 2 μg/ml.

galactose released after the lactose hydrolysis was in the form of octyl galactoside.

Thus, we have achieved an advantageous ratio between the transglycosylating and hydrolytic activities of β-galactosidase by the example of octyl galactoside using the system of reverse micelles. Variation of substrates under these conditions can result in the formation of a variety of other alkyl glycosides. In the same way, we have obtained heptyl galactoside and nonyl galactoside in 43 and 37% yields, respectively (data not shown).

Synthesis of Octyl Glucoside Using β-Glucosidase

To reveal other possibilities of synthesis of long-chain alkyl glycosides in the system of reverse micelles, we used another glycosylating enzyme, β-glucosidase from sweet almond (*Amygdalus communis var. dulcis*). Unlike β-galactosidase, which displayed no transglycosylating activity toward the monosaccharide (galactoside) (data not shown) under our experimental conditions, β-glucosidase turned out to be suitable for the coupling (direct glycosylation). Thus, we have reached a substantial yield (20%) of octyl glucoside starting from glucose and octanol. In addition to the target product, the reaction mixture contained a compound (Fig. 3, curve *3*) that turned out to be alkyl glucobioside as followed from its TLC mobility. It accumulated in the reaction mixture in quantities exceeding the yield of the target product (by 10–15%) within up to 120 h of the reaction. Then its content began to fall, and after 200 h it completely disappeared. It is likely that glucobiose is synthesized at the early stages of the process and is immediately (probably, without leaving the enzyme active site) transferred as a

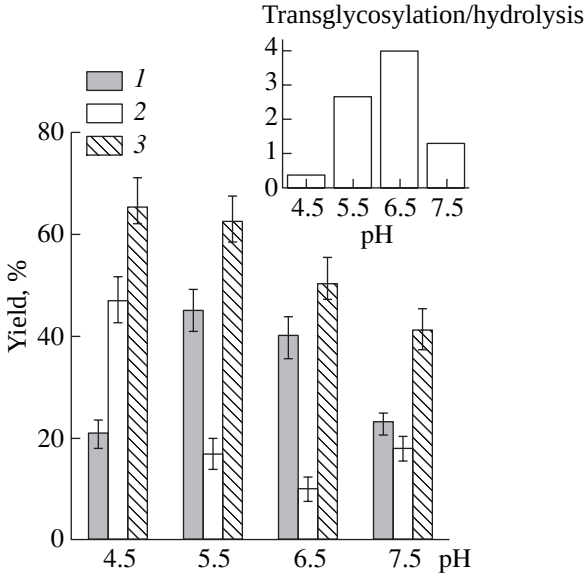

Fig. 2. The yield (from the starting lactose) of (*1*) octyl galactoside, (*2*) galactose, and (*3*) glucose in the synthesis of octyl galactoside from lactose and octanol catalyzed by β-galactosidase at various pH values. The products were analyzed after 5 days. $[Lac\text{-}OH]_0$ = 4.5 mM; $[E]_0$ = 2 μg/ml; 20°C. Standard deviations are given as a result of three independent measurements.

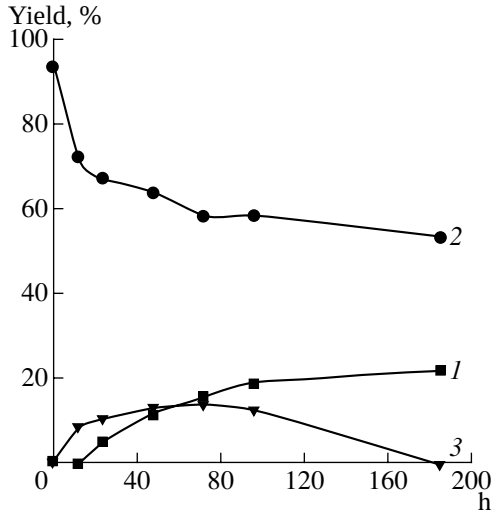

Fig. 3. Accumulation of (*1*) octyl glucoside, (*2*) glucose, and (*3*) an unidentified product (see the Results and Discussion section) upon the synthesis of octyl glucoside from glucose and octanol catalyzed by β-glucosidase. The yields were calculated from the starting glucose using the TLC data; $[Glc\text{-}OH]_0$ = 4.5 mM; $[E]_0$ = 15 μg/ml; pH 4.5; 20°C.

glucobioside residue onto the aliphatic alcohol molecule to give octyl glucobioside.

The hypothetical scheme of the glucobioside formation is as follows:

$$2\text{Glc-OH} \xrightarrow{\text{E}} \text{Glc-Glc-OH}$$
$$\xrightarrow{\text{OcOH}} \text{Glc-Glc-Oc} \xrightarrow{\text{H}_2\text{O, E}} \text{Glc-Oc} + \text{Glc-OH}.$$

This scheme is confirmed by the fact that glucobiose itself was not found in observable quantities at any stage of the process.

As in the case of the β-galactosidase transglycosylation, the maximum yield of the transglycosylation product (40%) is observed at pH 5.5, whereas at pH 6.5 and 7.5 the yield is higher than that in the optimum for hydrolysis (pH 4.5) (data not shown). The main results of the alkyl glycoside synthesis in the system of reverse micelles are given in Table 2.

Separation of Alkyl Glycosides from Other Components of Reaction Mixtures by Capillary Electrophoresis

An important practical problem was the separation of alkyl glycosides not only from hydrophilic mono- and disaccharides but also from the micelle-forming surfactant, AOT, which was present in the amount several orders of magnitude greater than our synthetic surfactant. This was solved by capillary electrophoresis, which allows a wide and flexible variation of the separation parameters on the basis of molecular characteristics of compounds (size, charge, chirality, hydrophobicity, etc.) and is highly selective and sensitive.

The components of the reaction mixture were transferred into the borate buffer by extraction with water (buffer). This helped considerably reduce the lap between the alkyl glycoside and AOT concentrations: the distribution coefficient of octyl glucoside between octane and water is about 15, whereas the AOT solubility in water is about 10^{-3} M and practically limitless in octane. The compounds were separated due to the formation of negatively charged borate complexes, which had different molecular masses and overall charges for the substances analyzed. Their intensive UV absorption allowed the process monitoring with a sensitive UV detector at 192 nm. The electrophoregrams of the separation of AOT and octyl glycoside are given in Fig. 4.

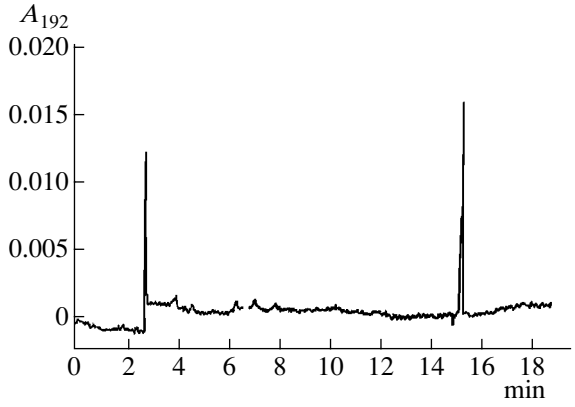

Fig. 4. Electrocapillogram with the peaks of octyl glucoside (right peak) and AOT (left peak) separated by capillary electrophoresis.

Thus, we have demonstrated the basic possibility of synthesis of alkyl glycosides in the system of reverse micelles by the example of enzymic synthesis of octyl glycosides. We have shown that the synthesis of long-chain alkyl glycosides can be performed in yields significantly exceeding those previously reported for the enzymic methods in two-phase systems. The direction of the process depends on pH of the aqueous solution captured in micelles. Unlike the chemical synthetic methods, the reaction proceeds in one stage and under mild conditions. The results of this study open up new opportunities for enzymic synthesis of surfactants in the systems of reverse micelles.

EXPERIMENTAL

β-Galactosidase (β-*D*-galactoside–galactohydrolase, EC 3.2.1.123) from *Penicillium canescencs* was isolated, purified, and kindly given by Prof. A.P. Sinitsyn's laboratory (Department of Chemical Enzymology, Faculty of Chemistry, Moscow State University). β-Glucosidase (EC 3.2.1.21) from sweet almond of

Table 2. TLC mobility (R_f) and yields (after 5 days) of the products of alkyl glycoside synthesis

Product	Yield, %	R_f	TLC developing system
Synthesis of octyl galactoside from lactose and octanol, pH 5.5			
Octyl galactoside	45	0.85	5 : 4 : 1 isopropanol–ethyl acetate–water
Galactose	17	0.58	
Glucose	62	0.69	
Synthesis of octyl glucoside from glucose and octanol, pH 5.5			
Octyl glucoside	40	0.80	80 : 35 : 5 chloroform–methanol–water

chromatographic purify (Sigma, United States) was desalted and lyophilized. Sodium citrate was from Chemapol (Czech Republic). Aerosol-OT, glucose, lactose, galactose, and octyl β-*D*-glucopyranoside were purchased from Sigma (United States). Octane, octanol, α-naphthol, isopropanol, ethyl acetate, chloroform, and methanol were from Reakhim (Russia).

Synthesis of octyl glycosides in the system of reverse micelles. A citrate buffer (0–60 μl) with the required pH and a substrate solution (100–155 μl, 0.174 M lactose, 0.83 M galactose, or 1.11 M glucose) were added to a mixture of *n*-octane (2 ml) containing 0.3 M AOT and *n*-octanol (4 ml) in a screw-cap tube. The mixture was vigorously shaken for 20–30 min, and a solution of the corresponding enzyme (12 μl, 1–10 mg/ml of β-galactosidase or 1–5 mg/ml of β-glucosidase) was added. The tube with the reaction mixture was closed and continuously shaken. Aliquots (20 μl) were taken when necessary and analyzed by TLC on the Sorbfil precoated plates (AO Sorbpolimer, Krasnodar, Russia) using an 80 : 35 : 5 chloroform–methanol–water or a 5 : 4 : 1 isopropanol–ethyl acetate–water developing system. The plates were treated with an α-naphthol solution (0.159 g of α-naphthol was dissolved in 51 ml of ethanol and 4 ml of water and 6.5 ml of 18 M sulfuric acid were added) and heated at 110°C for 6 min. The spots were detected by absorption at 545 nm on a Shimadzu CS-9000 scanning spectrophotometer. Octyl-β-*D*-glucopyranoside was used as a standard for identification of the spots of alkyl glycosides.

Capillary electrophoresis. The compounds under study were separated in the form of negatively charged borate complexes by capillary electrophoresis. A borate buffer solution (50 mM, pH 9.1, 5 ml) used as an electrolyte was added to the reaction mixture (3.5 ml) in a screw-cap tube, the mixture was shaken for 15 min, and the aqueous phase of the resulting two-phase system was analyzed. This procedure was also used to transfer into the borate buffer the solutions of pure lactose, glucose, galactose, and octyl glucoside used for the identification of the reaction products from the reverse micelles. The separation was performed on a Bio-Rad Biofocus 3000 Capillary Electrophoresis System with uncoated fused silica capillaries (24 cm × 25 μm) at 6 kV and 25°C. Detection at 192 nm was performed using a highly sensitive UV detector built into the system.

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