Efficient Transport of Saccharides through a Liquid Membrane Mediated by a Cyclodextrin Dimer

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Abstract: A new efficient system for transporting saccharides through a liquid membrane has been constructed. The transport rates of saccharides were accelerated greatly by the cyclodextrin dimer **2**; by contrast, the corresponding cyclodextrin monomer **1** was not effective at mediating saccharide transport. The transport rate of D-ribose through a chloroform liquid membrane was 17 times faster when the cyclodextrin dimer **2** was used as the transporter than when the cyclodextrin monomer **1** was used. Similarly the transport rate of methyl D-galactopyranoside was 16 times faster by **2** than by **1**.

Keywords: carbohydrates · cyclodextrins · host–guest systems · liquid membrane · molecular recognition

Introduction

Recently, much effort has been devoted to the construction of systems for effectively transporting saccharides through a liquid membrane containing of artificial transporters, because such systems are useful not only for separating mixtures of saccharides but also for elucidating the mechanism underlying the transport of saccharides through a biomembrane.[1–4] The separation of a mixture of saccharides is not easy, because most saccharides are isomers that differ only in the configuration of specific hydroxyl groups. An effective method for separating a mixture of saccharides is based on transport of the saccharide through a selective membrane containing transporters that bind only to specific saccharides. The most successful transporters bind to saccharide through the selective formation of hydrogen bonds or covalent bonds. The transport rate of a saccharide through a hydrophobic liquid membrane is quite slow, because saccharides are hydrophilic. However, the transport rate of the saccharide through the liquid membrane would be increased, if the saccharide could be covered by a hydrophobic shell that has functional groups on its inside that interact with the hydroxyl groups of the saccharide. Phenylboronic acid derivatives, reversed micelles, and lipophilic alkaline earth metal complexes have been used previously as saccharide transporters.[1–4] It is reported that a ternary complex of saccharide, water and resorcinarene is effective for the selective transport of saccharide.[5] Cyclodextrins (CDs) are cyclic oligosaccharides composed of six, seven, and eight glucose units joined by α-1,4-linkages named as α-, β-, and γ-CD, respectively.[6–8] Because CD has many hydroxyl groups and, especially, the secondary hydroxyl groups are aligned in the same plane, we thought that a CD dimer would be a powerful transporter for saccharides if the CD dimer could encompass the saccharide, with hydrogen bonds formed between the hydroxyl groups of the saccharide and the hydroxyl groups of CD at the secondary hydroxyl side, and if the primary hydroxyl side of CD were chemically modified with hydrophobic groups. In this paper, we report a new highly efficient system for transporting saccharides through a liquid membrane using a CD dimer.

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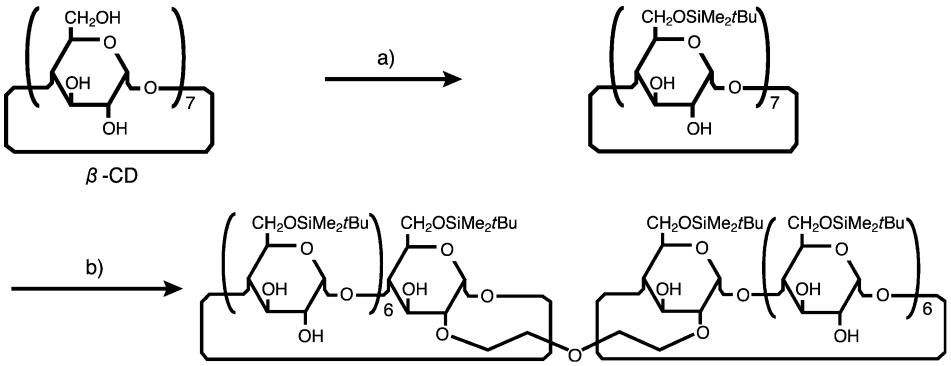
Results and Discussion

A new transporter was prepared as shown in Scheme 1. Saccharides are predicted to be caught effectively by the second hydroxyl groups of the two β-CDs, which are connected at the secondary hydroxyl side. All of the primary hydroxyl groups have to be modified with hydrophobic groups, because β-CD is insoluble in chloroform but soluble in water; this solubility is not suitable for a saccharide transporter. Thus, all of the primary hydroxyl groups of β-CD were first protected with *tert*-butyldimethlylsilyl groups in order to alter the primary hydroxyl side of β-CD to a hydrophobic nature.[9–10] Because heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-CD (**1**) was insoluble in water but was soluble in chloroform, the silylated β-CD **1** was suitable for use as a transporter in a chloroform liquid membrane. Two molecules of silylated β-CD **1** were then linked with bis(2-bromoethyl)

 DOI: 10.1002/chem.200304816



Scheme 1. a) tBuMe$_2$SiCl, pyridine, 4 °C, 18 h, 85.5 %; b) bis(2-bromoethyl) ether, NaH, THF/DMSO, 50 °C, 48 h, 10.9 %.

ether to obtain the CD dimer **2** with a yield of 10 %. After the reaction mixture was purified by column chromatography, the product was identified by elemental analysis, different ^{1}H NMR spectra, and mass spectrometry.

The transport experiments were performed in a U-tube glass cell across a chloroform liquid membrane from an aqueous source phase containing a saccharide to an aqueous receiving phase (Figure 1). Three kinds of liquid membranes

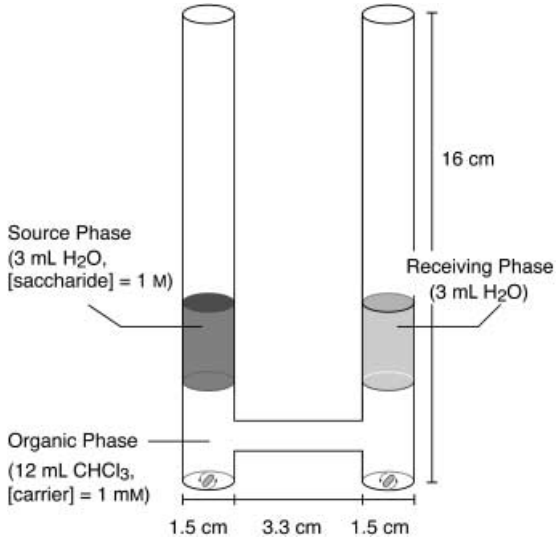


Figure 1. U-type glass cell used for the transport experiments.

were examined for each saccharide: the chloroform liquid membrane in the presence of ether **1** or **2**, or in their absence. Three kinds of methyl pyranosides (methyl α-D-glucopyranoside, methyl α-D-galactopyranoside, and methyl α-D-mannopyranoside), two kinds of pentoses (D-ribose, and D-2-deoxyribose), and two kinds of disaccharides (D-maltose, and D-lactose) were used as substrates of the transport experiments (Figure 2).

At regular intervals, a small aliquot of the aqueous receiving phase was sampled, suitably diluted, and assayed by HPLC. The amount of saccharide transported to the receiving phase increased linearly with time in the all cases (Figure 3). The transport rate was determined by the slope of the time course of the amount of the transported saccharide (Table 1). The transport rates of methyl α-D-glucopyranoside and methyl α-D-mannopyranoside were accelerated more than fourfold by the addition of the β-CD dimer **2**, but was accelerated less than twofold by the addition of the β-CD monomer **1**. The transport rate of D-ribose across the liquid membrane in the presence of the β-CD dimer **2** was 2.5 times faster than that in its absence, by contrast, the transport rate of D-ribose across the liquid membrane in the presence of the

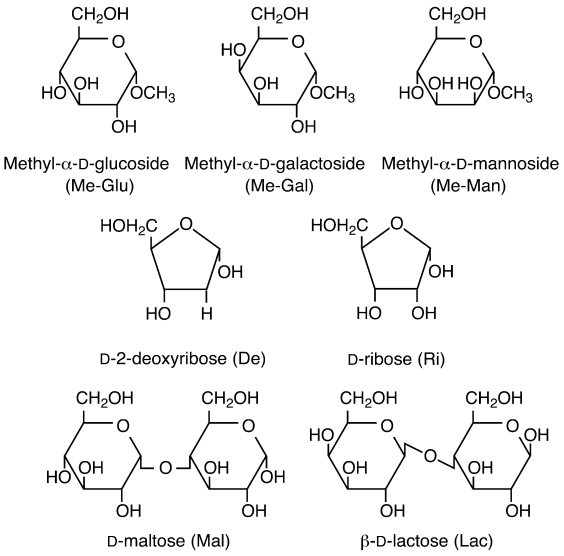


Figure 2. Substrates used in the transport experiments.

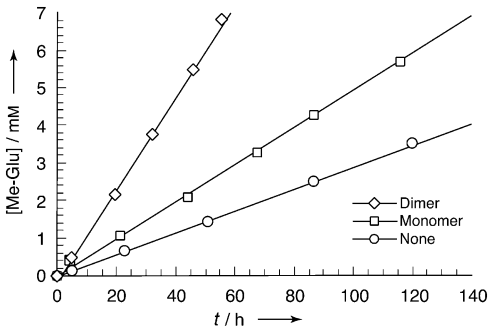


Figure 3. Time course of the concentration of methyl α-D-glucopyranoside in the receiving phase. Source phase (3 mL H_2O, [Me-Glu] = 1.0 M); organic phase (12 mL $CHCl_3$, [**1**] = [**2**] = 1.0 mM); receiving phase (3 mL H_2O), 25 °C.

β-CD monomer **1** was almost the same as in its absence. Because D-deoxyribose is more hydrophobic than the other saccharides and its transport rate in the absence of the transporter is fast, the difference in transport rates among the three kinds of membrane condition was small. Nevertheless, the transport rate of D-deoxyribose was faster in the presence of **2** than in the presence of **1**.

Table 1. Transport rates of saccharides through the liquid membrane mediated by **1** or **2**.

Substrate	Transport rate [10^{-2} mM h^{-1}]		
	non	**1**	**2**
Me-Glu	2.9	5.0	12.0
Me-Gal	5.8	6.3	13.7
Me-Man	3.7	5.5	14.9
Ri	3.4	3.7	8.4
De	33.0	36.0	39.0
Mal	0	0	0
Lac	0	0	0

The transport rate in the absence of transporter was subtracted from the transport rate in the presence of the transporter to determine the transport rate of the complex of the saccharide with the transporter (Figure 4). The transport rate for the complex of methyl α-D-galactopyranoside with

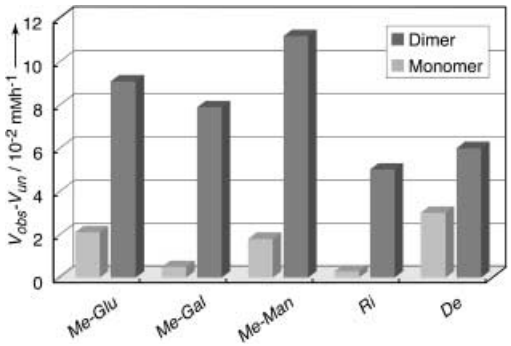


Figure 4. Transport rates of complexes of monosaccharides with cyclodextrin derivatives through the liquid membrane.

the β-CD dimer **2** was 16 times larger than that with the β-CD monomer **1**. The transport rate for the complex of D-ribose with the β-CD dimer **2** was 17 times larger than that with the β-CD monomer **1**. The transport rates of the three types of methyl pyranoside in complex with the β-CD dimer **2** were almost same. The transport rates of the complex of ribose with the β-CD dimer **2** are similar to that of deoxyribose with the β-CD dimer **2**. These results can lead to an estimated mechanism that the saccharides are completely covered by the β-CD dimer **2** and that the hydrophobicities of these complexes are almost same, although the structure of the complex of the saccharide with the β-CD dimer **2** is not identified.

Because we could not detect a complex of the β-CD dimer and the saccharide in chloroform by a ^{1}H NMR spectroscopy, the concentration of this complex in chloroform is not high. However, the complex is quite effective for transporting saccharides, even at this low concentration. A successful transporter has not only to trap a saccharide effectively at the interface between the aqueous source phase and the chloroform liquid membrane, but also to release the saccharide rapidly at the interface between the aqueous receiving phase and the chloroform liquid membrane. Therefore, a good transporter should have a moderate binding affinity for a saccharide rather than a high binding affinity.

Unfortunately, the β-CD dimer **2** was not effective for transporting disaccharides. Because disaccharides are themselves insoluble in chloroform and it is impossible to transport disaccharides through the chloroform membrane in the absence of an effective transporter, it is necessary to wrap the disaccharide completely by the transporter for passage through the chloroform layer. If the binding constant of a transporter for a disaccharide is not large, the disaccharide will be released in the chloroform layer during the transport and will subsequently precipitate in this layer. The binding affinity of the β-CD dimer **2** is sufficient for transporting a monosaccharide but not a disaccharide. It will be therefore necessary to introduce effective functional groups to the β-CD dimer **2** in order to improve the binding ability for disaccharides.

Although the β-CD dimer **2** is not effective for transporting disaccharides, our experimental results indicate that it functions quite effectively in the liquid membrane as the transporter for the monosaccharides. Whereas the β-CD dimer **2** can almost completely encompass the monosaccharides, the β-CD monomer **1** is not enough to cover up the monosaccharides. Although in theory two β-CD monomers could make a complex with a saccharide in which the saccharide is covered in a similar manner to that achieved by the β-CD dimer, the concentration of this complex in the chloroform layer would be quite small. Therefore, the β-CD dimer is more effective than the β-CD monomer for the transporting the saccharide through the liquid membrane. If functional groups that could interact selectively with the hydroxyl groups of the saccharide were introduced into the secondary hydroxyl side of β-CD, then a more efficient transporter might be constructed.

Conclusion

A new and effective system for transporting saccharides through a liquid membrane has been constructed using a β-CD dimer as a transporter. This system is more successful for transporting saccharides through a liquid membrane than is a system using a β-CD monomer as a transporter.

Experimental Section

General: Mass spectra were obtained on a Shimadzu MALDI-III (TOF-MS). ^{1}H NMR spectra were recorded on a Varian VXR-500S FT NMR spectrometer. Chloroform ($CHCl_3$, $\delta = 7.26$) was used as an internal standard, and tetramethylsilane (TMS, $\delta = 0$) was used as an external standard. Elemental analyses were performed by the Analytical Division in Research Laboratory of Resources Utilization of Tokyo Institute of Technology. β-Cyclodextrin was a kind gift from Nihon Shokuhin Kako Ltd. Other reagents were purchased from Tokyo Kasei. $CDCl_3$ with an isotopic purity of 99.95%, was purchased from Merck.

Heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-CD (1): A solution of *tert*-butyldimethylchlorosilane (14.5 g) in pyridine (150 mL) was added to a solution of β-CD (9 g) in pyridine (100 mL) for 3.5 h at 0 °C under nitrogen. The reaction mixture was stirred for 18 h at room temperature under nitrogen. After the solvent was removed, the residue was dissolved in chloroform, washed with 1N hydrochloric acid aqueous solution, saturated aqueous solution of sodium hydrogen carbonate, and saturated brine, and dried over magnesium sulfate. The crude product was purified by column chromatog-

raphy on silica gel (chloroform/ethyl acetate 7:0.1) and by recrystallization from methanol/chloroform (95:5) to yield 13.1 g, 85.5%. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.03$ (s, 21H; Si-CH_3), 0.04 (s, 21H; Si-CH_3), 0.87 (s, 63H; C-$(CH_3)_3$), 3.56 (t, $J = 9.2$ Hz, 7H; H-4), 3.61 (m, 7H; H-5), 3.64 (dd, $J = 3.3$, 9.2 Hz, 7H; H-2), 3.71 (br d, $J = 10.0$ Hz, 7H; H-6), 3.90 (dd, $J = 3.1$, 11.4 Hz, 7H; H-6′), 4.04 (t, $J = 9.2$ Hz, 7H; H-3), 4.89 (d, $J = 3.4$ Hz, 7H; H-1); MALDI-TOFMS: *m*/*z*: calcd for $C_{84}H_{168}O_{35}Si_7Na$: 1956; found: 1956 [$M^+$+Na]; elemental analysis calcd (%) for $C_{84}H_{168}O_{35}Si_7 \cdot H_2O \cdot \frac{1}{3}CHCl_3$: C 50.83, H 8.60, Cl 1.78; found: C 50.79, H 8.39, Cl 1.89.

***β*-CD dimer (2)**: NaH (18 mg) was added to a solution of **1** (1.0 g) in a mixed solvent of THF (30 mL) and DMSO (3 mL) and stirred at room temperature. A solution of bis(2-bromoethyl) ether (57 mg) in THF (20 mL) was added to the reaction mixture for 10 h at 50 °C. After the solvents were removed, the residue was dissolved in chloroform, washed with saturated aqueous solution of ammonium chloride and dried over magnesium sulfate. The crude product was purified by column chromatography on silica gel (chloroform/methanol/water 5:1:0.1). The fractions containing the desired product were concentrated under reduced pressure to give a white powder (221.9 mg, 10.9%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.03$ (br s, 42H; Si-CH_3), 0.87 (s, 63H; C-$(CH_3)_3$), 3.22 (br d, 2H; H-2), 4.82–5.02 (m, 14H, H-1); MALDI-TOFMS: *m*/*z*: calcd for $C_{172}H_{342}O_{71}Si_{14}K$: 3965; found: 3965 [$M^+$+K]; elemental analysis calcd (%) for $C_{172}H_{342}O_{71}Si_{14} \cdot 3H_2O \cdot \frac{1}{3}CHCl_3$: C 51.32, H 8.70, Cl 0.88; found: C 51.35, H 8.68, Cl 0.90.

Transport experiments: Transport experiments were performed in a U-tube glass cell across a chloroform liquid membrane from an aqueous source phase (3 mL) containing a saccharide (1M) to an aqueous receiving phase (3 mL) (Figure 1). Three kinds of liquid membranes were examined for each saccharide: chloroform liquid membrane (12 mL) in the presence of either **1** or **2** (1 mM), or in their absence at 25 °C. At regular intervals, a small aliquot of the aqueous receiving phase was sampled, suitably diluted, and assayed by HPLC using Tosoh TSKgel Amide-80. Saccharides were detected by a refractive index meter.

Acknowledgements

We are grateful to Nihon Shokuhin Kako Co., Ltd. for a generous supply of cyclodextrins. This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology and by the Grant of the 21st Century COE Program of Ministry of Education, Culture, Sports, Science and Technology.

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Received: February 6, 2003 [F4816]