Preparation of Saccharide Exchange Membrane Modified by Phenylboronic Acid Azoprobe/Polyamidoamine (PAMAM) Dendrimer

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Abstract
Phenylboronic acid azoprobe (B-Azo-Cb) for saccharide recognition was synthesized. B-Azo-Cb/polyamidoamine (PAMAM) complex was synthesized by the condensation reaction of terminal carboxylic group of B-Azo-Cb and surface amine of PAMAM dendrimer. Thin porous cellulose acetate membrane embedded with B-Azo-Cb/PAMAM dendrimer complex was prepared from cellulose acetate, formamide, acetone, and B-Azo-Cb/PAMAM dendrimer complex by casting on the glass plate at various temperatures. The saccharide transportability was evaluated by measuring the amount of saccharide in receiving solution. The amount of saccharide transport of porous cellulose acetate membrane embedded with B-Azo-Cb/PAMAM dendrimer complex was higher at low temperature-treated membrane, and low pH of receiving solution. In contrast, the saccharide transportabilities through non-treated cellulose acetate membrane were the same at any pH of receiving solution. Saccharide transportability was in the order of galactose > glucose > fructose (B-Azo-Cb/PAMAM G4 modified membrane), and glucose > galactose > fructose (B-Azo-Cb/PAMAM G5 modified membrane). These orders were the same of the saccharide response in solution. These results indicated that the saccharide transport of B-Azo-Cb/PAMAM-embedded membrane is successfully mediated by the phenylboronic acid.

Keywords: Saccharide transport, Phenylboronic acid, Membrane, PAMAM dendrimer

1. Introduction
Saccharides are essential biological molecules as regards nutrition, metabolism, and cell structure, so many researchers are devoted to develop selective and sensitive method for in vivo saccharide recognition.1-7) Although enzyme-based sensors are highly selective and efficient, there are some problems from the aspect of durability, continuous monitoring, and in vivo analysis.8) In contrast, chemical sensors based on phenylboronic acid have attracted much attention because of their stability and the binding behavior that form stable cyclic esters with diol moieties of saccharides in aqueous solution. Previously, we have reported the first success in the design of a supramolecular phenylboronic acid azoprobe/γ-CyD complex sensor.9) The phenylboronic acid azoprobe/γ-CyD complex sensor was found to show high glucose selectivity in water by forming a 2:1 inclusion complex of phenylboronic azoprobe with γ-CyD. Herein, we designed phenylboronic acid azoprobe (PBA)-modified polyamidoamine (PAMAM) dendrimer as a saccharide recognition sensor. PAMAM dendrimer is a highly branched, monodisperse, and nanospherical macromolecule (Fig. 1(a)). PAMAM dendrimer is easy to modify various functional molecules to its terminal amines. We prepared PBA/PAMAM dendrimer sensor and embedded into the porous cellulose acetate membrane. The saccharide transportability of the prepared membrane was also evaluated.

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2. Material and Methods

2.1 Apparatus

\(^1\)H NMR spectra were measured with a Lambda GX-500 (JEOL Ltd., Tokyo, Japan) at 300 K. Elemental analysis was measured with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer, Inc., MA, USA). All pH values were recorded with a Horiba F-52 pH meter (HORIBA, Ltd., Kyoto, Japan). UV-Vis absorption spectra were measured by using a Hitachi U-3000 UV-Vis spectrophotometer (Hitachi High-Technologies Co., Tokyo, Japan) equipped with a Peltier thermocontroller with a 10 mm quartz cuvette at 25 °C.

2.2 Materials

2.2.1 Reagents

B-Azo-Cb was synthesized as Fig. 1 (b). B-Azo and Bp-Azo were synthesized by the reported procedure. Ethyl bromoacetate, potassium carbonate, dichloromethane, methanol, n-hexane, tetrahydrofuran, cellulose acetate, formamide, sodium hydroxide, hydrochloric acid, fructose, glucose, and galactose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetone was purchased from Kanto Chemical, Co., Inc. (Tokyo, Japan). DMT-MM was purchased from Kokusan Chemical, Co., Ltd. (Tokyo, Japan). PAMAM dendrimer, ethylenediamine core, generation 4.0 solution, and PAMAM dendrimer, ethylenediamine core, generation 5.0 solution were purchased from Sigma-Aldrich Japan, Co., LLC. (Tokyo, Japan). Methanol-\(d_4\) was purchased from Merck Japan (Tokyo, Japan). All other organic solvents and reagents were commercially available with guaranteed grades and used without further purification. Water was doubly distilled and deionized by a Milli-Q water system (WG222, Yamato Scientific Co., Ltd., Tokyo, Japan and Autopure WR-600G, Merck Millipore, MA, USA) before use.

2.2.2 Synthesis of Bp-Azo-Es

Bp-Azo (0.68 g, 2.46 mmol) was dissolved in acetone (50 mL) and potassium carbonate (2.70 g, 19.5 mmol) added to the solution. Ethyl bromoacetate (2.81 g, 16.5 mmol) dissolved in acetone (50 mL) was slowly added to the mixture at room temperature and refluxed for 24 h. The reaction mixture was cooled at room temperature and filtered out potassium carbonate. The filtrate solution was evaporated to obtain orange oily products. The product was purified by column chromatography (silica gel/ dichloromethane : methanol = 9:1). The product was recrystallized by n-hexane and dichloromethane to obtain orange solid. The structure of the product was confirmed by \(^1\)H NMR.

\(^1\)H NMR Spectra of Bp-Azo-Es (300 MHz, methanol-\(d_4\)): \(\delta\) (ppm): 1.36 (s, 12H, H\(_a\)), 4.18-4.28 (m, 5H, H\(_g\), H\(_h\)), 4.87 (s, 2H, H\(_f\)), 7.82-7.85 (d, 2H, H\(_e\), \(J_{ee} = 9.02\)), 7.90-7.93 (m, 6H, H\(_b\), H\(_c\), H\(_d\)).

2.2.3 Synthesis of B-Azo-Cb

Bp-Azo-Es was dissolved in 50 mL of tetrahydrofuran (THF) and 40 mL of 10% sodium hydroxide was added. The reaction mixture was refluxed for 24 h and cooled to room temperature. THF was evaporated from the reaction mixture and 6 M hydrochloric acid was added. The pH of reaction mixture was adjusted to pH 2 and obtained yellow precipitate. The precipitate was filtered and washed by 1 M hydrochloric acid (30 mL), water (40 mL×2). Obtained product was red solid (253 mg, 0.843 mmol). The structure of the product was confirmed by \(^1\)H NMR and elemental analysis.

\(^1\)H NMR Spectra of B-Azo-Cb (300 MHz, methanol-
4.79 (s, 2H, H_h), 7.05-7.08 (d, 2H, H_e, J_{ed} = 9.02), 7.90-7.93 (d, 2H, H_d, J_{de} = 9.02), 8.16 (s, 2H, Ha). Anal. Calcd. for C_{14}H_{13}BN_{2}O_{5}.2.2.4 Synthesis of B-Azo-Cb/PAMAM G4 dendrimer complex

B-Azo-Cb (707 mg, 2.47 mmol) was dissolved in methanol (70 mL). PAMAM G4 dendrimer (1.33 g, 0.0386 mmol) was added to the reaction mixture and stirred for 30 min. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM, 1.06 g, 3.84 mmol) was added to the reaction mixture and stirred at room temperature for five days. The precipitate was obtained and filtered. The filtrate was dissolved in sodium hydroxide solution and hydrochloric acid was added to the solution to form the precipitate. The precipitate was filtered to obtain the purified product (373 mg, 51.9%). The product was analyzed by 1H NMR (300 MHz, DMSO-d_6). The peak of secondary amine of PAMAM dendrimer was observed at 8.2 ppm, and the peak derived from B-Azo-Cb was observed at 4.7 ppm.

2.2.4 Synthesis of B-Azo-Cb/PAMAM G5 dendrimer complex

B-Azo-Cb (707 mg, 2.47 mmol) was dissolved in methanol (70 mL). PAMAM G5 dendrimer (1.33 g, 0.0386 mmol) was added to the reaction mixture and stirred for 30 min. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM, 1.06 g, 3.84 mmol) was added to the reaction mixture and stirred at room temperature for five days. The precipitate was obtained and filtered. The filtrate was dissolved in sodium hydroxide solution and hydrochloric acid was added to the solution to form the precipitate. The precipitate was filtered to obtain the purified product (272 mg, 50.1%). The peak of secondary amine of PAMAM dendrimer was observed at 8.2 ppm, and the peak derived from B-Azo-Cb was observed at 4.7 ppm.

2.3 Preparation of membrane

Cellulose acetate (3.0 g), formamide (5.0 g), and acetone (10.0 g) were mixed and B-Azo-Cb/PAMAM dendrimer complex (0.180 g) was added to the solution and shaken at room temperature for 24 h. The membranes were prepared by casting method with a doctor blade.11) This reaction mixture (ca. 2.0 mL) was casted on the glass plate (10×10 cm²) and sliced by doctor blade (thickness: 200 μm). The casted glass plate was put in cold water for 1 h. The obtained membrane was put in hot water (70, 80, 90 °C) for 10 min followed by immersing in cold water. Then, the membrane was cut circle (diameter: 9 mm) to obtain thin porous cellulose acetate membrane. The membrane thickness was ca. 150 μm.

2.4 Evaluation of saccharide transport

The saccharide transport was evaluated by phenol-sulfuric acid colorimetric method. Phenol solution (0.1 mL) was added to sample solution (0.1 mL). Sulfuric acid (0.5 mL) was added to this solution. After 1 h, the absorption maximum of 490 nm was measured.

3. Results and Discussion

3.1 Preparation of membrane

The porous cellulose acetate membrane is composed from active layer and sponge layer. The pore size of active layer is known to be able to control by treated temperature (high temperature: small pore, low temperature: large pore). Figure 2 shows the sugar (glucose) transportability of cellulose acetate membrane prepared by various temperatures (70, 80, 90 °C). Sugar transportability was in the order of 70 > 80 > 90 °C. This result showed that sugar transportability was controlled by temperature to form the pore size of active layer.

![Fig. 2. Effect of treated temperature upon permeability of sugar (glucose). Feed: 18 mM glucose, pH 10 adjusted with 10 mM NaHCO₃. Receiving: pH 10 adjusted with 10 mM NaHCO₃.](image)

3.2 pH effect for glucose transport

B-Azo-Cb/PAMAM dendrimer complex (1 wt%, PAMAM G4 or G5) modified and non-modified porous membranes were prepared. Saccharide (glucose) transportability under pH gradient between feed (pH 10) and receiving solutions (pH 3, 5, 7, 10) were evaluated (Fig. 3). By using non-modified porous membrane, concentration of glucose in receiving solution did not change at any pH. This result showed that glucose was transported by physical diffusion through non-modified porous membrane. In contrast, by using B-Azo-Cb/PAMAM dendrimer complex modified membrane, glucose concentration in receiving solution was increased according to pH of receiving solution. Proton transport from receiving solution to feed solution was observed through the membrane under the condition that the feed solution was pH 10 and the receiving solution kept low pH. In the feed solution side of the
membrane, the phenylboronic acid binding sites were easy to form anionic esters with saccharides, and in the receiving solution side of the membrane, the anionic esters were easy to dissociate. Thus the phenylboronic acid-mediated transport of saccharides through the membrane embedded with B-Azo-Cb/PAMAM dendrimer complex took place under proper pH gradient through the membrane (Fig. 4). Saccharide formed anionic ester with boronic acid and the saccharide-binding boronic acid transferred next to the boronic acid in membrane. This indicated that the ability of saccharide transport was conducted by the phenylboronic acid binding sites inside the membrane.
3.3 Evaluation of saccharide separation ability

Various saccharide solutions (glucose, galactose, fructose) were added to feed solution. The concentration changes of saccharide in receiving solution as a function of time were evaluated by phenol-sulfuric acid colorimetric method (Fig. 5). The saccharide concentrations in receiving solution were in the order of galactose > glucose > fructose (B-Azo-Cb/PAMAM G4 modified membrane) and glucose > galactose > fructose (B-Azo-Cb/PAMAM G5 modified membrane). UV-Vis spectral changes of B-Azo-Cb/PAMAM complexes in solution based on the saccharide recognition was shown in Fig. 6. B-Azo-Cb/PAMAM G4 showed strong response to galactose, and B-Azo-Cb/PAMAM G5 exhibited glucose selectivity. These results revealed that the ability of saccharide transport was in consistent with the saccharide response of B-Azo-Cb/PAMAM complexes in solution. Thus, the selective saccharide transport was achieved by not only the diffusion of saccharide in solution but also saccharide binding with B-Azo-Cb/PAMAM complex embedded in the porous cellulose acetate membrane.

4. Conclusions

The porous cellulose acetate membrane embedded with B-Azo-Cb/PAMAM complex was prepared. The saccharide transportability through the porous cellulose acetate membrane bearing B-Azo-Cb/PAMAM complex was higher for the low temperature-treated membrane, and for the larger pH gradient between the feed and receiving solutions. Saccharide transportability was in the order of galactose > glucose > fructose (B-Azo-Cb/PAMAM G4 modified membrane), and glucose > galactose > fructose (B-Azo-Cb/PAMAM G5 modified membrane). These orders were the same of the saccharide recognition selectivity of B-Azo-Cb/PAMAM complex in solution. Thus, these results indicated that the selective saccharide transport was realized through B-Azo-Cb/PAMAM-modified membrane based on the phenylboronic acid-mediated transfer mechanism.

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6. References