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ARTICLE

Design of phenylboronic acid azoprobe/polyamidoamine dendrimer complexes as a supramolecular sensor for saccharide recognition in water

Yuji Tsuchido,[a] Yuuki Sakai,[a] Keisuke Aimu,[a] Takeshi Hashimoto,[a] Kazunari Akiyoshi,[b,c] and Takashi Hayashita*[a]

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The selective molecular recognition event by physical or chemical signals is a key concept for the design of novel supramolecular sensors. In this study, we designed a novel saccharide recognition system based upon the self-assembly of phenylboronic acid azoprobes (1-BAzo-NPs) on the surface of polyamidoamine (PAMAM) dendrimer in water. The two sulfonic acid moieties in the 1-BAzo-NP enhanced the binding affinity of the azoprobe for PAMAM dendrimer surface. UV-Vis spectral measurements indicated that 1-BAzo-NP showed poor saccharide recognition at pH 7.0, whereas the 1-BAzo-NP/PAMAM complex well recognized the saccharides, particularly glucose. The complexation with glucose yielded aggregates having diameters of 100-200 nm as determined by dynamic light scattering (DLS) measurement and transmission electron microscopy (TEM). The sensitivity to and selectivity for the saccharides were controlled by the density of the assembled phenylboronic acid azoprobes and PAMAM generation. Together, the results revealed that the selective saccharide recognition at pH 7.0 was feasible by boronic acid assembly on PAMAM dendrimer surface.

Introduction

Saccharides play numerous significant roles in living organisms because of their diverse structures.[1,2] In biological systems, saccharides exist on cell and protein surface and mediate intercellular recognition, pathogenesis prevention, and immune responses.[3,4] In nature, lectins, which are saccharide-binding proteins, recognize cell-surface saccharides.[5,6] Lectins bind saccharides via the formation of hydrogen bonds with the saccharide hydroxyl groups, van der Waals forces, or hydrophobic interactions. Expectations are high regarding the application of the sophisticated saccharide recognition ability of lectins in biotechnology, food science, and medicine. However, lectins are expensive because of their limited availability. In addition, lectins are easily denatured by environmental factors, such as thermal or pH change, the addition of ionic detergents, or protease digestion.[7] Therefore, the development of more stable, non-lectin-dependent saccharide sensors is highly awaited.[8,9] Synthetic chemical receptors are robust to environmental changes and can be readily modified to enhance activity and cell permeability. Reporter groups, such as fluorophores or azo moieties, can be easily incorporated into synthetic chemical receptors, resulting in higher sensitivity than protein or biological receptors, including lectins. In this regard, synthetic chemical receptors have attracted much attention for possible analytical and therapeutic applications.[10,11]

Phenylboronic acids are known to form reversible covalent bonds with *cis*-1,2- and *cis*-1,3-diol-containing biomolecules, such as saccharides or glycoproteins, generating five- and six-membered cyclic boronic esters that are stable in alkaline aqueous solution and dissociating at acidic pH.[12] Because of this unique property, phenylboronic acids have been used in the development of saccharide detection and sensing systems as a synthetic mimic of lectins.[13-18] The binding affinity of phenylboronic acids for monosaccharides follows the order of fructose > galactose > mannose > glucose.[19] In general, simple phenylboronic acid sensors have weak affinity and low selectivity for saccharides. To improve binding affinity and selectivity, scientists have endeavored to examine molecular recognition in biological systems as it offers an important clue to solving this problem.

[a] *Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan. Fax: +81-3-3238-3361; Tel: +81-3-3238-3372; E-mail: ta-hayas@sophia.ac.jp*
[b] *Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Kyotodaigaku-Katsura, Nishikyo-ku, Kyoto 615-8510, Japan. Fax: +81-75-383-2590; Tel: +81-75-383-2589; E-mail: akiyoshi@bio.polym.kyoto-u.ac.jp*
[c] *ERATO Bio-nanotransporter Project, Japan Science and Technology Agency (JST), Katsura Int'tech Center, Kyotodaigaku-Katsura, Nishikyo-ku, Kyoto 615-8530, Japan.*

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Biomolecules, such as enzymes or antibodies, can strongly bind their target molecules because of the synergy of multiple binding interactions. In fact, the synergistic effect of simultaneous multiple binding interactions has been employed to enhance binding strength.[20-23]

We have been investigating various types of phenylboronic acid/cyclodextrin (CD) complexes to uncover the selective sensing of monosaccharides.[24-28] Previously, we have reported that the phenylboronic acid azoprobe/γ-CD complex showed high D-glucose selectivity in water by forming a 2:1 inclusion complex of phenylboronic acid azoprobe with γ-CD.[26] We have also revealed that the boronic acid fluorophore/boronic acid-modified-γ-CD complex functioned as an efficient supramolecular sensor for selective glucose recognition in water.[27] Those results indicated that the control of the molecular assembly of phenylboronic acid azoprobes is a key factor to enhancing the selectivity for saccharides.

Dendrimers are a useful scaffold to create multivalent binding sites.[29-32] Dendrimers are highly branched, nanospherical, and monodisperse macromolecules having three-dimensional structures. Polyamidoamine (PAMAM) dendrimer is easy to modify functional molecules such as PEG, RGD peptides, or antibodies to terminal amines.[31,32] Because of this feature, PAMAM dendrimer is well used in biosensor applications.[33-36]

Herein, we designed a phenylboronic acid azoprobe/PAMAM dendrimer for use as a supramolecular complex sensor for saccharide recognition. First, we designed a phenylboronic acid azoprobe with two sulfonic acid moieties in the azoprobe to attain water solubility and to enhance electrostatic interaction with the terminal amine of PAMAM dendrimer (Figure 1).

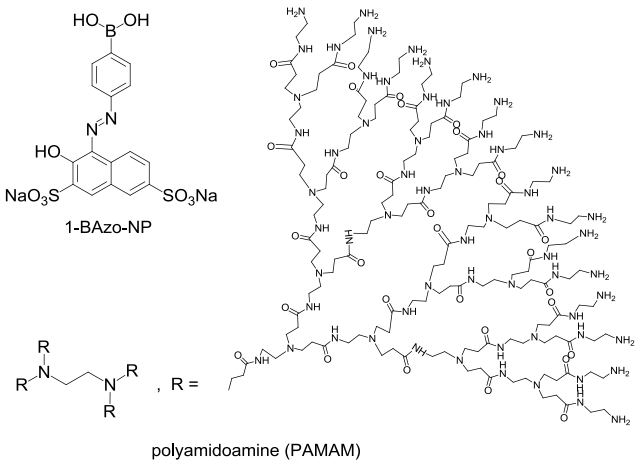

Figure 1. Chemical structure of 1-BAzo-NP and PAMAM dendrimer.

Then, we measured the UV-Vis spectra of the phenylboronic acid azoprobe and the phenylboronic acid azoprobe/PAMAM dendrimer complex to determine their saccharide recognition ability. In addition, the morphology of the phenylboronic acid azoprobe/PAMAM dendrimer complex was examined by dynamic light scattering (DLS) measurements and transmission electron microscopy (TEM). We report herein selective saccharide recognition based on the aggregate formation of the supramolecular boronic acid azoprobe/PAMAM dendrimer complex.

Experimental

Reagents

1-Naphthol-4-sulfonic acid sodium salt, sodium nitrite, sodium hydroxide, hydrochloric acid, phosphoric acid, fructose, glucose, galactose, and phosphotungstic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Disodium 3-hydroxy-2,7-naphthalenedisulfonate, (4,4,5,5-Tetramethyl-1,3,2-dioxabororan-2-yl) aniline, and *N*-(2-aminoethyl)acetamide were purchased from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). Acetone was purchased from Kanto Chemical, Co., Inc. (Tokyo, Japan). Ethanol was purchased from Japan Alcohol Trading, Co., Ltd. (Tokyo, Japan). PAMAM dendrimer, ethylenediamine core, generation 3.0 solution, 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). Polyallylamine (M_w = 5,000) was purchased from Nittobo Medical Co., Ltd. (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.

Apparatus

^{1}H NMR spectra were measured with a Lambda GX-500 (JEOL Ltd., Tokyo, Japan) at 300 K. Elemental analysis was performed with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer, Inc., MA, USA). Mass spectrometry was performed on a JMS-T100LC (JEOL, Ltd., Tokyo, Japan). All pH values were recorded with a Horiba F-52 pH meter (HORIBA, Ltd., Kyoto, Japan). UV-Vis absorption spectra were measured with a Hitachi U-3000 UV-Vis spectrophotometer (Hitachi High-Technologies, Co., Tokyo, Japan) equipped with a Peltier thermocontroller with a 10-mm quartz cell at 25 °C.

Synthesis of 1-BAzo-NP

A solution of sodium nitrite (165 mg, 2.39 mmol) in water (4.0 mL) was added to a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) aniline (501 mg, 2.29 mmol) in distilled water (8.50 mL) and 12 M HCl (0.75 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. Then, disodium 3-hydroxy-2,7-naphthalenedisulfonate (851 mg, 2.44 mmol) dissolved in aqueous NaOH (5.50 mL) was added drop-by-drop to the reaction mixture. After stirring for 45 min, pH was adjusted to 6 and the reaction mixture turned red. The diazotization product was obtained by filtration and washed with acetone and a small amount of water. Then, it was recrystallized twice from acetone with a small amount of water and dried *in vacuo* to give a red solid (248 mg, 21.8%). ^{1}H NMR (Figure S1(a) in supporting information, 500 MHz, D_2O): δ (ppm) 7.02 (d, J = 8.5 Hz, 4H), 7.24 (d, J = 9.0 Hz, 4H), 7.54 (s, 2H), 7.76 (d, J = 10.0 Hz, 2H), 7.82 (s, 2H), 7.96 (d, J = 8.5 Hz, 2H); ^{13}C NMR (Figure S1(b), 125 MHz, D_2O): 173.6, 142.7, 141.7, 140.4, 137.1, 136.4, 135.0, 129.2, 127.9, 127.5, 124.7, 122.4, 116.6. Negative ESI-MS (*m*/*z*): Calcd. for $C_{16}H_{11}BN_2O_9S_2Na$ ([M-Na]$^-$): 501.2. Found: 501.0. Anal. Calcd. for $C_{16}H_{11}BN_2O_9S_2Na_2\cdot4H_2O$ (%): C, 33.82; H,

3.37; N, 4.93. Found: C, 34.09; H, 3.38; N, 5.14.

pH profile of 1-BAzo-NP

To evaluate the saccharide recognition ability of 1-BAzo-NP, UV-Vis spectral measurements were performed. Solutions containing 1-BAzo-NP (0.02 mM), NaCl (100 mM), and H_3PO_4 (1 mM) were prepared and UV-Vis spectra were measured at 25 °C at various pH values in the absence or presence of saccharide (30 mM) with the addition of $NaOH_{aq}$.

Saccharide recognition by 1-BAzo-NP/PAMAM-G4 complex

To evaluate the saccharide recognition ability of 1-BAzo-NP and the 1-BAzo-NP/PAMAM complex, UV-Vis spectral measurements were performed. Solutions containing 1-BAzo-NP (0.02 mM), PAMAM-G4 (0 or 0.62 μM, amine unit base: 0.04 mM), and H_3PO_4 (1 mM, pH 7.0, adjusted with NaOH) were prepared and UV-Vis spectra were measured at 25 °C while fructose, glucose, and galactose concentrations were varied from 0 to 1 mM.

Saccharide recognition by 1-BAzo-NP/PAMAM-G4 complex in the presence of NaCl

To evaluate the effect of sodium chloride on the saccharide recognition ability of the 1-BAzo-NP/PAMAM complex, UV-Vis spectral measurements were performed in the presence of sodium chloride. Solutions containing 1-BAzo-NP (0.02 mM), PAMAM-G4 (0.62 μM, amine unit base: 0.04 mM), NaCl (100 mM), and H_3PO_4 (1 mM, pH 7.0, adjusted with NaOH) were prepared and UV-Vis spectra were measured at 25 °C while fructose, glucose, and galactose concentrations were varied from 0 to 1 mM.

Dynamic light scattering (DLS) and ζ-potential measurements

Hydrodynamic diameters were determined by DLS measurements. DLS and ζ-potential measurements were carried out at 25 °C using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom) at the wavelength of 633 nm and the detection angle of 173° to determine the size of the 1-BAzo-NP/PAMAM-G4 complex. Two optically clear suspensions, 1-BAzo-NP solution and PAMAM dendrimer solution, were filtered through a poly(vinylidene fluoride) (PVDF) membrane filter (MILLEX GV, Millipore Co., Ltd., MA, USA; pore size, 0.45 μm) prior to the preparation of measurement solutions to remove dust in water.[37] The concentration of the 1-BAzo-NP/PAMAM-G4 complex was kept constant at 1.25 μM (amine unit base: 0.08 mM). The measured autocorrelation function was analyzed by the cumulant method.[38] The hydrodynamic diameter of the 1-BAzo-NP/PAMAM-G4 complex was calculated by the Stokes-Einstein equation. The standard deviation of three independent measurements is given in parenthesis. The ζ-potential measurements were performed using a capillary ζ-potential cell in the automatic mode.

Negatively stained transmission electron microscopy (TEM)

The 1-BAzo-NP/PAMAM-G4 complex with fructose or glucose solution (5 μL) was applied onto a 200 mesh copper grid deposited with carbon and dried in vacuo. Ten microliters of an aqueous solution of phosphotungstic acid (approximately 2.5 wt%) was applied onto the grid, dried in vacuo, and washed with a small amount of water.[37] TEM observation was performed at the accelerating voltage of 100 kV by using a HITACHI H-600 (Hitachi High-Technologies Co., Tokyo, Japan).

Results and Discussion

Saccharide recognition by 1-BAzo-NP/PAMAM-G4 complex

To examine the saccharide recognition ability of 1-BAzo-NP, pH titration experiments were carried out. The pH profiles of 1-BAzo-NP in the absence or presence of fructose are shown in Figure 2 (for UV-Vis spectra, see Figure S2).

The absorption maximum at 494 nm decreased abruptly above pH 10, which indicated that the hydroxyl group of the naphthol ring was dissociated. At pH 6 to 8, the absorption maximum at 494 nm was almost the same regardless of the presence or absence of fructose. This result indicated that fructose was hardly recognized at neutral pH. Then, the UV-Vis spectra of 1-BAzo-NP and 1-BAzo-NP/PAMAM-G4 complex solutions were measured at pH 7.0 while varying the concentrations of fructose, glucose, and galactose. The ratio of 1-BAzo-NP/PAMAM-G4 complex solutions was fixed at 1:2 (amino unit base), because 1-BAzo-NP was densely packed on PAMAM-G4. No UV-Vis spectral changes were observed when fructose, glucose, and galactose (0 to 1 mM) were added to 1-BAzo-NP solution. In contrast, apparent UV-Vis spectral changes were noted when saccharides were added to 1-BAzo-NP/PAMAM-G4 complex solution. The UV-Vis spectral changes upon addition of glucose for 1-BAzo-NP and 1-BAzo-NP/PAMAM-G4 complex solutions were depicted in Figure S3 (supporting information). As we

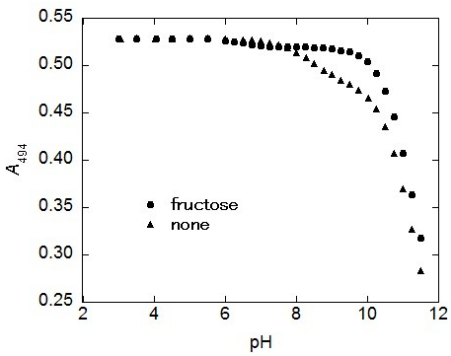

Figure 2. pH profiles of 1-BAzo-NP. [1-BAzo-NP] = 0.02 mM, [fructose] = 30 mM (closed circle) or 0 mM (closed triangle), [H_3PO_4] = 1.0 mM.

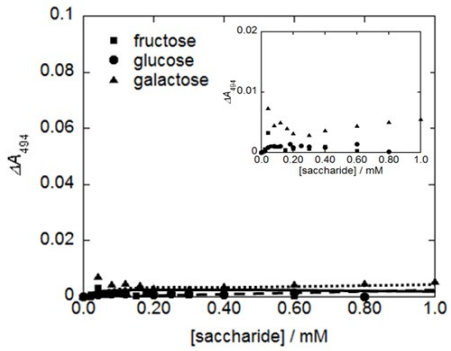

Figure 3. Saccharide recognition by 1-BAzo-NP. [1-BAzo-NP] = 0.02 mM, [saccharide] = 0-1 mM, [H_3PO_4] = 1.0 mM, pH 7.0. (Closed square = fructose, closed circle = glucose, closed triangle = galactose).

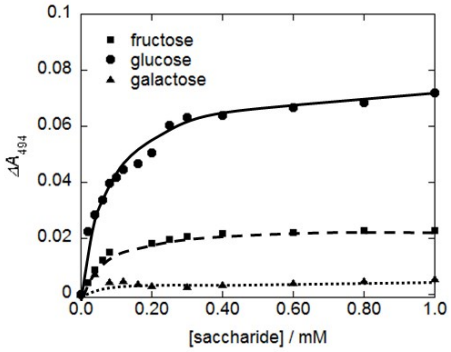

Figure 4. Saccharide recognition by 1-BAzo-NP/PAMAM-G4 complex in the absence of NaCl. [1-BAzo-NP] = 0.02 mM, [PAMAM-G4] = 0.62 μM (amine unit base: 0.04 mM), [saccharide] = 0-1 mM, [H_3PO_4] = 1.0 mM, pH 7.0. (Closed square = fructose, closed circle = glucose, closed triangle = galactose).

discussed later, the decrease in absorbance intensity was attributable to aggregate formation of 1-BAzo-NP/PAMAM-G4 complex upon saccharide binding. The absorption maximum at 494 nm is plotted against the saccharide concentration in Figures 3 and 4.

Usually, boronic acid forms a bond with saccharide under the alkaline condition.[12] 1-BAzo-NP did not form a bond with saccharide at neutral pH (Figure 3). However, the 1-BAzo-NP/PAMAM-G4 complex showed saccharide recognition ability at pH 7.0, and the absorbance changes at 494 nm were in the order of glucose > fructose > galactose (Figure 4). The saccharide recognition by the 1-BAzo-NP/PAMAM-G4 complex at pH 7.0 was achieved by decreasing the apparent pK_a. The pK_a decrease was a result of the increase of the electron-withdrawing ability of the boronic acid azoprobe induced by interactions between the terminal amino groups of PAMAM-G4 and the sulfonate groups of the naphthalene ring of 1-BAzo-NP. The results indicated that 1-BAzo-NPs assembled on the surface of PAMAM-G4. To clarify the saccharide recognition resulting from the assembly of 1-BAzo-NPs on the PAMAM-G4 surface through the electrostatic interaction, saccharide recognition was evaluated in the presence of a large excess (100 mM) of sodium chloride (Figure 5).

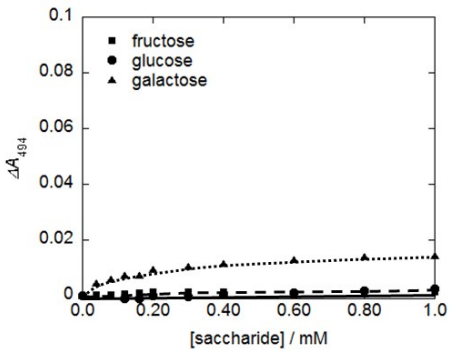

Figure 5. Saccharide recognition by 1-BAzo-NP/PAMAM-G4 in the presence of NaCl. [1-BAzo-NP] = 0.02 mM, [PAMAM-G4] = 0.62 μM (amine unit base: 0.04 mM), [saccharide] = 0-1 mM, [NaCl] = 100 mM, [H_3PO_4] = 1.0 mM, pH 7.0. (Closed square = fructose, closed circle = glucose, closed triangle = galactose).

The increase of the ionic strength in bulk solution should diminish the electrostatic interaction between 1-BAzo-NP and PAMAM-G4. As expected, UV-Vis spectral changes due to saccharide recognition were not observed in the presence of 100 mM NaCl, which was mostly the same behaviour of 1-BAzo-NP only. It was evident that the high concentration of NaCl affected the electrostatic interaction of anionic sulfonate probe with positively charged amines on the surface of dendrimer. We have also examined the saccharide recognition ability by using polyallylamine (M_w = 5,000), which is a linear polymer containing primary amines (Figure S4a). When polyallylamine was mixed with an equivalent amount of 1-BAzo-NP, absorbance at 494 nm was similarly decreased by the addition of saccharides (Figure 6a). However for 1-BAzo-NP in the presence of monomeric *N*-(2-aminoethyl)acetamide (Figure S4b), the absorbance at 494 nm was little changed by the addition of saccharides (Figure 6b). These results indicated that the saccharide recognition was achieved by self-assembly of 1-BAzo-NPs based on the interaction between sulfonic acids of 1-BAzo-NP and amino groups in polyallylamine. However, due to random assembly of 1-BAzo-NPs in polyallylamine, no specific selectivity was noted for saccharides (Figure 6a). These results demonstrated that the 1-BAzo-NP/PAMAM-G4 complex exhibited the saccharide recognition function through the electrostatically induced assembly of 1-BAzo-NPs on the surface of PAMAM-G4 in water.

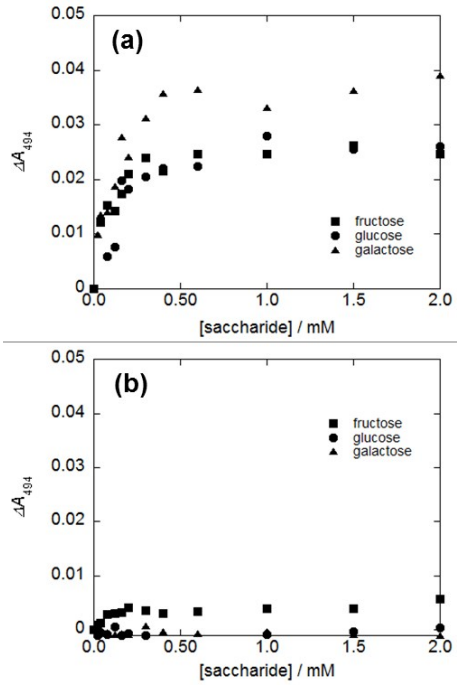

Figure 6. Changes of the absorption at 494 nm of 1-BAzo-NP with (a) polyallylamine and (b) *N*-(2-aminoethyl)acetamide by the addition of various saccharides (fructose, glucose, galactose). [1-BAzo-NP] = 0.02 mM, [saccharide] = 0-2 mM, [H_3PO_4] = 1.0 mM, pH 7.0, and (a) [polyallylamine] = 0.45 μM (amino unit base: 0.04 mM), (b) [*N*-(2-aminoethyl)acetamide] = 0.04 mM.

Diameter and morphology of 1-BAzo-NP/PAMAM-G4 complex

The morphology of the 1-BAzo-NP/PAMAM-G4 complex in the presence of glucose or fructose was examined by transmission electron microscopy (TEM) (Figure 7).

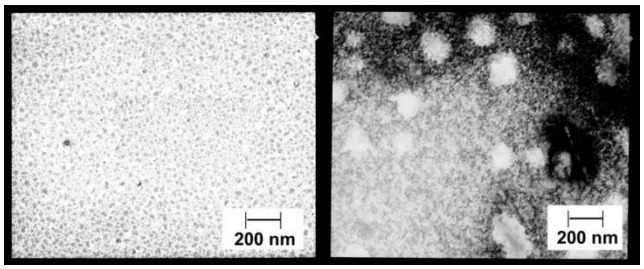

Figure 7. Negatively stained TEM images of 1-BAzo-NP/PAMAM-G4 complex in the presence of fructose (left) or glucose (right). [1-BAzo-NP] = 0.02 mM, [PAMAM-G4] = 0.41 μM (amino unit base: 0.026 mM), [saccharide] = 30 mM, [H_3PO_4] = 1.0 mM, pH 7.0.

Samples on TEM grids were negatively stained with phosphotungstic acid. In the presence of glucose (30 mM), aggregate formation was clearly observed and the average diameter of the aggregates was estimated to be ca. 150 nm. In contrast, the aggregate formation was not observed in the presence of fructose (30 mM). It is known that boronic acid usually forms a 1:1 complex with fructose and a 2:1 complex with glucose by forming reversible covalent bonds with *cis*-1,2- and *cis*-1,3-diols.[13] In the presence of fructose, the 1-BAzo-NP/PAMAM-G4 complex did not form aggregates because of the 1:1 ester formation of 1-BAzo-NP on the PAMAM-G4 surface and fructose. Whereas in the presence of glucose, 1-BAzo-NP on the PAMAM-G4 surface formed 2:1 binding with glucose. The cross-linked 1-BAzo-NP/PAMAM-G4 complex with glucose due to the formation of 1,2- and 1,3-diols resulted in the aggregate formation. The results demonstrated that the 1-BAzo-NP/PAMAM-G4 complex selectively recognized glucose.

The hydrodynamic diameters (D_{hy}) of the 1-BAzo-NP/PAMAM-G4 complex were also determined by DLS measurements (Table 1). D_{hy} of PAMAM-G4 in phosphoric acid buffer was 4.7 ± 0.4 nm and that of the 1-BAzo-NP/PAMAM-G4 complex was 5.2 ± 0.3 nm. The assembly of 1-BAzo-NP with PAMAM-G4 increased D_{hy}. The ζ-potentials of PAMAM-G4 and the 1-BAzo-NP/PAMAM-G4 complex were +7.8 ± 2.4 mV and +1.4 ± 1.2 mV, respectively. The decrease in ζ-potential indicated that the sulfonic acid groups in 1-BAzo-NP assembled with the cationic form of the amino groups on PAMAM-G4 surface by electrostatic interaction, and the particle surface was covered with 1-BAzo-NP. In the presence of glucose (30 mM), D_{hy} and ζ-potential of the 1-BAzo-NP/PAMAM-G4 complex were 158 ± 19 nm and −1.4 ± 1.2 mV, respectively. This slightly negative electric charge was due to the formation of aggregates composed of the 1-BAzo-NP/PAMAM-G4 complex and glucose. In the presence of fructose, however, D_{hy} was 4.4 ± 0.8 nm. This result corresponded well with TEM observation, indicating that the 1-BAzo-NP/PAMAM-G4 complex and fructose did not form aggregates. In this condition, the ζ-potential was +21.4 ± 0.4 mV. The result indicated that the 1-BAzo-NP/PAMAM-G4 complex was decomposed by the 1:1 ester formation with fructose.

Table 1. Characteristics of 1-BAzo-NP/PAMAM-G4 complex

Sample [a]	D_{hy} / nm [b]	ζ-potential / mV [b]
PAMAM-G4	4.7 ± 0.4	+ 7.8 ± 2.4
1-BAzo-NP/PAMAM-G4	5.2 ± 0.3	+ 1.4 ± 1.2
1-BAzo-NP/PAMAM-G4 with glucose	158 ± 19	−1.4 ± 1.2
1-BAzo-NP/PAMAM-G4 with fructose	4.4± 0.8	+ 21.4 ± 0.4

[a] Measurements were performed under the following conditions: [1-BAzo-NP] = 0.02 mM, [PAMAM-G4] = 1.25 μM (amino unit base: 0.08 mM), [saccharide] = 0 or 30 mM, [H_3PO_4] = 1.0 mM, pH 7.0. [b] The standard deviation is given by three independent measurements.

Effect of PAMAM generation on saccharide recognition

To evaluate the relation between 1-BAzo-NP density on the surface of PAMAM dendrimer and the response selectivity of saccharides, we examined UV-Vis measurements by changing the concentration of PAMAM by using various generations of PAMAM while keeping 1-BAzo-NP concentration constant (Figure 8). As a reference of turbidity for the aggregate formation of 1-BAzo-NP/PAMAM complex, the absorbance at 640 nm for detection of light scattering was recorded as a function of PAMAM concentration in the presence of various saccharides at pH 7.0 (for the absorbance changes at 493 nm, see Figure S5). Saccharide recognition was increased by changing the concentration of PAMAM from 0 to 1 eq, and this tendency was observed regardless of PAMAM generation. This indicates that the number of PAMAM molecules forming complexes with closely packed 1-BAzo-NPs was increased. However, saccharide recognition was decreased by the addition of excess PAMAM. It is evident that saccharide recognition ability was altered by the density of 1-BAzo-NP molecules assembling on PAMAM dendrimer surface, and 1-BAzo-NP/PAMAM complex showed

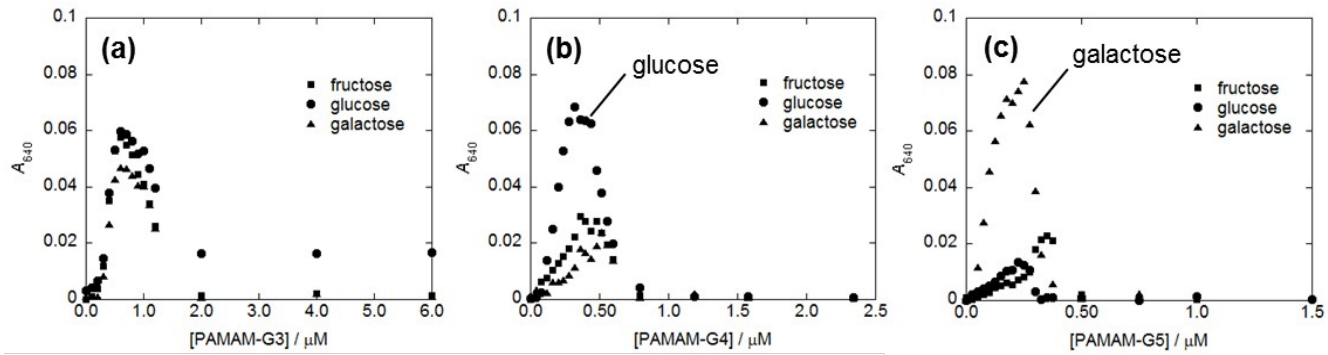

Figure 8. Saccharide recognition by 1-BAzo-NP and various generations of PAMAM dendrimer ((a) PAMAM-G3, (b) PAMAM-G4, (c) PAMAM-G5). [1-BAzo-NP] = 0.02 mM, [saccharide] = 30 mM, [H_3PO_4] = 1.0 mM, pH 7.0. (Closed square = fructose, closed circle = glucose, closed triangle = galactose).

the best saccharide recognition ability when 1-BAzo-NP molecules were densely assembled on the surface of PAMAM dendrimer in the presence of the excess of amount of saccharides. In addition, the effect of PAMAM generation on the saccharide recognition ability was evaluated. PAMAM-G3 did not show any saccharide recognition ability, whereas PAMAM-G4 recognized glucose and PAMAM-G5, galactose. The results indicated that the density of boronic acids is an important factor for saccharide recognition. The difference in saccharide recognition ability was due to the difference in the density of boronic acids assembling on the PAMAM dendrimer surface because the number of amino groups on the PAMAM dendrimer surface varied with PAMAM generation.

Conclusions

We have explored the potential of the electrostatically assembled phenylboronic acid azoprobe/PAMAM dendrimer complexes as a supramolecular sensor for saccharide recognition in water. The phenylboronic acid azoprobe/PAMAM dendrimer complex responded to saccharides and exhibited selective aggregation particularly with glucose at neutral pH. The saccharide recognition ability was improved by decreasing the ionic strength of bulk solution. This result revealed that saccharide recognition at pH 7.0 was promoted by the assembly of boronic acid molecules on the surface of PAMAM dendrimer via electrostatic interaction. Saccharide recognition at physiological pH region (pH 7.0) is much superior as an actual saccharide sensing system. Both sensitivity to and selectivity for saccharides were dependent on the density of the assembled phenylboronic acid azoprobes and the PAMAM generation. The results indicated that controlling the density of molecular recognition sites is vital to the separation and recognition of saccharides in water.

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Notes and references

1 R. A. Laine, *Glycobiology*, 1994, **4**, 759-767.
2 B. G. Davis, *Chem. Rev.*, 2002, **102**, 579-601.
3 A. Helenius and M. Aebi, *Science*, 2001, **291**, 2364-2369.
4 T. Angata and A. Varki, *Chem. Rev.*, 2002, **102**, 439-469.
5 K. Ohtsubo and J. D. Marth, *Cell*, 2006, **126**, 855-867.
6 A. Varki, *Nature*, 2007, **446**, 1023-1029.
7 H. Wei and E. Wang, *Anal. Chem.*, 2011, **83**, 2829-2833.
8 A. P. Davis, *Org. Biomol. Chem.*, 2009, **7**, 3629-3638.
9 S. Jin, Y. F. Cheng, S. Reid, M. Li and B. Wang, *Med. Res. Rev.*, 2010, **30**, 171-257.
10 G. F. Whyte, R. Vilar and R. Woscholski, *J. Chem. Biol.*, 2013, **6**, 161-174.
11 Y. Egawa, T. Seki, S. Takahashi and J. Anzai, *Mat. Sci. Eng. C*, 2011, **31**, 1257-1264.
12 T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem. Int. Ed.*, 1996, **35**, 1910-1922.
13 D. K. Scrafton, J. E. Taylor, M. F. Mahon, J. S. Fossey and T. D. James, *J. Org. Chem.*, 2008, **73**, 2871-2874.
14 S. A. Elfeky, F. D'Hooge, L. Poncel, W. Chen, S. P. Perera, J. M. H. van den Elsen, T. D. James, A. T. A. Jenkins, P. J. Cameron and J. S. Fossey, *New J. Chem.*, 2009, **33**, 1466-1469.
15 F. D'Hooge, S. A. Elfeky, S. E. Flower, S. I. Pascu, A. T. A. Jenkins, J. M. H. V. D. Elsen, T. D. James and J. S. Fossey, *RSC Adv.*, 2012, **2**, 3274-3280.
16 M. Sanjoh, Y. Miyahara, K. Kataoka and A. Matsumoto, *Anal. Sci.*, 2014, **30**, 111-117.
17 M. Ikeda, K. Fukuda, T. Tanida, T. Yoshii and I. Hamachi, *Chem. Commun.*, 2012, **48**, 2716-2718.
18 M. Lee, T.-I. Kim, K.-H. Kim, J.-H. Kim, M.-S. Choi, H.-J. Choi and K. Koh, *Anal. Biochem.*, 2002, **310**, 163-170.
19 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769-774.
20 M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 1998, **37**, 2754-2794.
21 G. M. Pavan, A. Danani, S. Pricl and D. K. Smith, *J. Am. Chem. Soc.*, 2009, **131**, 9686-9694.
22 H. Wang, Z. Bie, C. Lü and Z. Liu, *Chem. Sci.*, 2013, **4**, 4298-4303.
23 X. Wu, Z. Li, X. X. Chen, J. S. Fossey, T. D. James and Y. B. Jiang, *Chem. Soc. Rev.*, 2013, **42**, 8032-8048.
24 A.-J. Tong, A. Yamauchi, T. Hayashita, Z. Zhang, B. D. Smith and N. Teramae, *Anal. Chem.*, 2001, **73**, 1530-1536.
25 R. Ozawa, T. Hashimoto, A. Yamauchi, I. Suzuki, B. D. Smith and T. Hayashita, *Anal. Sci.*, 2008, **24**, 207-212.
26 C. Shimpuku, R. Ozawa, A. Sasaki, F. Sato, T. Hashimoto, A. Yamauchi, I. Suzuki and T. Hayashita, *Chem. Commun.*, 2009, 1709-1711.
27 M. Kumai, S. Kozuka, M. Samizo, T. Hashimoto, I. Suzuki and T. Hayashita, *Anal. Sci.*, 2012, **28**, 121-126.
28 T. Hashimoto, M. Yamazaki, H. Ishii, T. Yamada, and T. Hayashita, *Chem. Lett.*, 2014, **43** (2), 228-230.
29 A. Endo, H. Minesaka, T. Hashimoto and T. Hayashita, *Anal. Methods*, 2012, **4**, 2657-2660.
30 Y. Tsuchido, K. Aimu, Y. Toda, T. Hashimoto, and T. Hayashita, *J. Ion Exchange*, 2014, **25**(4), 146-150.
31 A. E. Beezer, A. S. H. King, I. K. Martin, J. C. Mitchel, L. J. Twyman and C. F. Wain, *Tetrahedron*, 2003, **59**, 3873-3880.
32 S. E. Stiriba, H. Frey and R. Haag, *Angew. Chem., Int. Ed.*, 2002, **41**, 1329-1334.
33 M. Akin, M. Yuksel, C. Geyik, D. Odaci, A. Bluma, T. Höpfner, S. Beutel, T. Scheper and S. Timur, *Biotechnol. Prog.*, 2009, **26**, 896-906.
34 D. Kim, M. Rahman, A. Do, M. H. C. Ban and Y. B. Shim, *Biosens. Bioelectron.*, 2010, **25**, 1781-1788.
35 R. P. Singh, *Analyst*, 2011, **136**, 1216-1221.
36 J. Luo, M. Dong, F. Lin, M. Liu, H. Tang, H. Li, Y. Zhang and S. Yaoa, *Analyst*, 2011, **136**, 4500-4506.
37 Y. Sasaki, Y. Tsuchido, S. Sawada and K. Akiyoshi, *Polym. Chem.*, 2011, **2**, 1267-1270.
38 D. E. Koppel, *J. Chem. Phys.*, 1972, **57**, 4814-4820.