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Article *in* Langmuir · September 2016

DOI: 10.1021/acs.langmuir.6b02917

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Saccharide Recognition Based on Self-assembly of Amphiphilic Phenylboronic Acid Azoprobes

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ABSTRACT: We designed amphiphilic phenylboronic acid azoprobes (**B-Azo-Cn**) and evaluated their saccharide recognition function in relation to micelle formation changes of the self-assembled **B-Azo-Cn**. First, we evaluated **B-Azo-C8** in 1% methanol-99% water solution under the basic condition. The wavelength of maximum absorption in the UV-Vis spectra of **B-Azo-C8** was shifted and the solution showed color change with the addition of saccharides. The morphology of **B-Azo-C8** was evaluated by dynamic light scattering (DLS) measurements and transmission electron microscopy (TEM) observation. **B-Azo-C8** formed aggregates in the absence of saccharides and the presence of glucose. In the presence of fructose, micelle formed **B-Azo-C8** was dispersed, indicating that **B-Azo-C8** changed its dispersion state by recognizing fructose. The effect of alkyl chain length on saccharide recognition ability was examined as well. **B-Azo-C4** and **B-Azo-C12** did not recognize saccharides in 1% methanol-99% water solution under the basic condition, indicating that an appropriate alkyl chain length was required for recognizing saccharides. The control of hydrophilic-lipophilic balance (HLB) was a key factor for the saccharide recognition.

INTRODUCTION

The design of artificial chemical sensors for biologically important molecules has continued to evolve over the last few decades.[1,2] Saccharides are attractive targets because they play important roles in the metabolic pathways of living organisms. In particular, the detection of monosaccharides, such as glucose, fructose, or galactose, in aqueous solution is necessary in biotechnology, food science, and medicine.[3,4] In nature, lectins recognize various saccharides through the formation of hydrogen bonds with saccharide hydroxyl groups, van der Waals interaction, or hydrophobic interactions.[5,6] However, lectins are easily denatured by environmental factors, such as heat or pH changes, thereby losing their function.[7] In addition, lectins are expensive because of their limited availability. Therefore, the development of artificial saccharide sensors is highly anticipated.[8-10] Phenylboronic acids are known to form reversible covalent bonds with *cis*-1,2- and *cis*-1,3-diol-containing biomolecules,[11,12] such as saccharides and glycoproteins, generating five- and six-membered cyclic boronic esters that are stable in alkaline aqueous solution and dissociate at acidic pH.[13] Because of this unique property, phenylboronic acids have been used in the development of saccharide sensors.[14-19]

In particular, amphiphilic sensors have attracted the interest of researchers because amphiphiles undergo dynamic changes as a result of molecular recognition. Savsunenko *et al.* reported functionalized vesicles based on amphiphilic boronic acids.[20] They developed a functionalized vesicular system that features selective monosaccharide recognition ability. The boronic acid-functionalized vesicles showed significant quenching of dye fluorescence courtesy of an exchange reaction between the dye and the saccharides. It has been reported also that glucose induced aggregation through the formation of gemini-type amphiphiles.[21] Previously, our group developed an amphiphilic molecular recognition system[22,23] consisting of crown ether azo amphiphiles (**15C5-Azo-Cn**) that exhibit K^+-selective spectral responses in water based on selective H-aggregate formation induced by the K^+ binding. In this K^+-selective recognition system, the alkyl spacer length of **15C5-Azo-Cn** was found to strongly affect response efficiency and selectivity. Herein, we have designed

saccharide recognition systems based on the self-assembly of phenylboronic acid azoprobes (**B-Azo-Cn**) and evaluated the effect of the alkyl chain lengths of **B-Azo-Cn**. **B-Azo-Cn** have an amphiphilic structure and a phenylboronic acid recognition site. Phenylboronic acids are known to form hydrophilic anionic esters with the diol moieties of saccharides in water. Thus, the hydrophilic-lipophilic balance (HLB) of **B-Azo-Cn** can be controlled by the saccharide recognition ability, which causes the change in the aggregation mode of **B-Azo-Cn** in water.

EXPERIMENTAL SECTION

Reagents. Phenol, sodium nitrite, pinacol, sodium hydroxide, hydrochloric acid, potassium carbonate, 1-bromobutane, 1-bromooctane, 1-bromododecane, 1-bromosodium periodate, magnesium sulfate, sodium carbonate, toluene, dichloromethane, hexane, tetrahydrofuran, methanol, fructose, glucose, galactose, and phosphotungstic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)aniline was purchased from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). 1,4-Dioxane and acetone were purchased from Kanto Chemical, Co., Inc. (Tokyo, Japan). Ethanol was purchased from Japan Alcohol Trading, Co., Ltd. (Tokyo, Japan). Dimethyl sulfoxide-d_6 and chloroform-*d* were purchased from Sigma-Aldrich Japan, Co., LLC. (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-3900 UV-Vis spectrophotometer (Hitachi High-Technologies, Co., Tokyo, Japan) equipped with a Peltier thermocontroller and a 10 mm quartz cell.

Synthesis of amphiphilic phenylboronic acid azoprobes. We synthesized the amphiphilic phenylboronic acid azoprobes (**B-Azo-C4, 8, 12**) in accordance with the following scheme (Figure 1). Details of the synthesis are available in our previous article.[24]

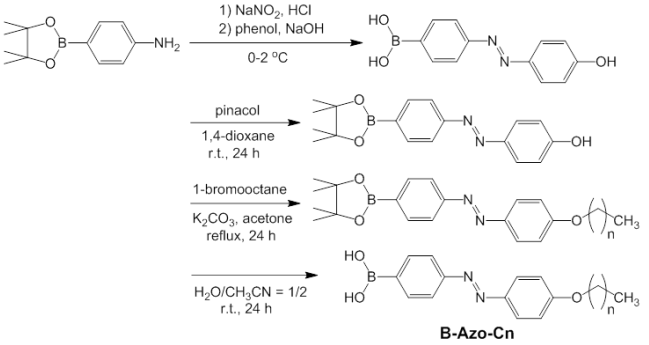

Figure 1. Synthesis of **B-Azo-Cn**.

Evaluation of B-Azo-C8 micelle formation. To evaluate **B-Azo-C8** micelle formation, UV-Vis spectral measurements were performed. **B-Azo-C8** (0.02 mM) and Na_2CO_3 (10 mM, pH 11.0) solutions with different methanol concentrations were prepared. UV-Vis spectra were recorded at 25 °C and methanol concentration was varied from 1% to 80%. Temperature dependence of the **B-Azo-C8** micelle formation was evaluated by preparing **B-Azo-C8** (0.02 mM) and Na_2CO_3 (10 mM, pH 11.0) solution in 1% methanol and changing the temperature from 25 °C to 55 °C.

Saccharide response of B-Azo-C8 micelle. To evaluate saccharide recognition function of **B-Azo-C8** micelle, UV-Vis spectral measurements were performed. **B-Azo-C8** (0.02 mM) and Na_2CO_3 (10 mM, pH 11.0) solutions with different saccharide concentrations were prepared. UV-Vis spectra were recorded at 25 °C and in the 1% methanol concentration. Competitive experiment was carried out for fructose in the presence of glucose. Final concentration of measurement solution was **B-Azo-C8** (0.02 mM), Na_2CO_3 (10 mM, pH 11.0) and glucose (20 mM). UV-Vis spectral measurements were performed by adding fructose to this solution.

Dynamic light scattering (DLS) measurements. Hydrodynamic diameters were determined by DLS measurements. DLS 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 **B-Azo-C8** micelles. **B-Azo-C8** solutions were optically clear suspensions and 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 the solutions.[25] The concentration of **B-Azo-C8** was kept constant at 0.020 mM. The measured autocorrelation function was analyzed by the cumulant method.[26] The hydrodynamic diameter of **B-Azo-C8** was calculated by the Stokes-Einstein equation. The standard deviation is given by three independent measurements.

Negatively stained transmission electron microscopy (TEM). **B-Azo-C8** and **B-Azo-C8** without saccharides or with saccharides (fructose, glucose, galactose) solutions (5 μL) were 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, and the grid was dried in vacuo and washed with a small amount of water.[27] TEM observation was performed at the accelerating voltage of 100 kV by using a HITACHI H-600 and TH-7700 (Hitachi High-Technologies Co., Tokyo, Japan).

Circular dichroism (CD) spectroscopy measurements. **B-Azo-C8** (0.02 mM), saccharide (0 or 20 mM) and Na_2CO_3 (10 mM, pH 11.0) solutions with different saccharide concentrations were prepared. Circular dichroism spectra were obtained with a JASCO J-720 circular dichroism spectrophotometer (JASCO Co., Ltd., Tokyo, Japan) equipped with a thermoregulated cell compartment, using a quartz cell with 10 mm light path length. Measurements were carried out under constant gaseous nitrogen flow at 25 °C.

Saccharide response of B-Azo-Cn. To evaluate saccharide recognition function of **B-Azo-Cn**, UV-Vis spectral measurements were performed by the addition of saccharides to the sample solutions. Sample solutions were prepared with

mixing **B-Azo-Cn** (0.02 mM) and Na_2CO_3 (10 mM, pH 11.0) solutions. UV-Vis spectra were recorded at 25 °C and in the 1% methanol concentration.

RESULTS AND DISCUSSION

Evaluation of B-Azo-C8 micelle formation. To investigate the micelle formation characteristics of amphiphilic phenylboronic acid azoprobe **B-Azo-C8**, the organic solvent (methanol)/water ratio of the solution was changed (Figure 2a). In 80% methanol solution, the wavelength of maximum absorption in the UV-Vis spectra of **B-Azo-C8** was 352 nm. As the methanol ratio was decreased, the absorption maximum at 352 nm was decreased and in the 1% and 10% methanol solutions, a new peak maximum appeared at 304 nm. This result indicated that in low methanol ratio solution, **B-Azo-C8** was associated by the hydrophobic interaction of its alkyl chains, forming micelles. The dependence on solution temperature and association state of **B-Azo-C8** was also examined (Figure 2b). For **B-Azo-C8** in 1% methanol solution, the maximum wavelength was 304 nm at 25 °C. Increasing the **B-Azo-C8** solution temperature from 25 °C to 55 °C resulted in a shift of the wavelength of maximum absorption of **B-Azo-C8** from 304 nm to 352 nm. This result indicated that heating the **B-Azo-C8** solution caused **B-Azo-C8** micelle dissociation. To demonstrate **B-Azo-C8** micelle formation, critical micelle concentration (CMC) of **B-Azo-C8** was determined by electrical conductivity measurement. Electrical conductivity was drastically changed when **B-Azo-C8** concentration was changed, and CMC of **B-Azo-C8** was calculated to be 2.1 μM (Figure S1). From this result, we confirmed that **B-Azo-C8** formed micelles in 1% methanol solution.

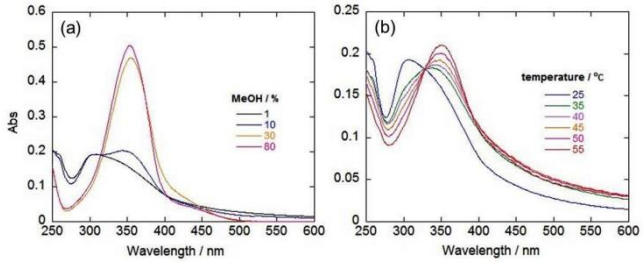

Figure 2. (a) UV-Vis spectra of **B-Azo-C8** measured in solutions containing methanol at various concentrations. (b) UV-Vis spectra of **B-Azo-C8** solutions at various temperatures. [**B-Azo-C8**] = 0.020 mM, [Na_2CO_3] = 10 mM, at pH 11.0.

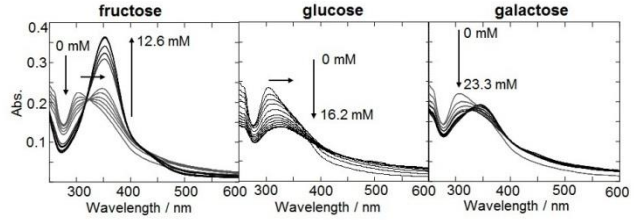

Figure 3. Effects of (a) fructose, (b) glucose, and (c) galactose concentrations on UV-Vis spectra of **B-Azo-C8** in 1% MeOH solution (v/v). [**B-Azo-C8**] = 0.020 mM, [Saccharide] = 0-15 mM, [Na_2CO_3] = 10 mM, pH 11.0, 25 °C.

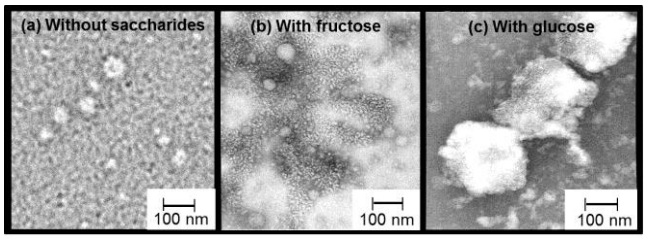

Figure 4. Negatively stained TEM images of **B-Azo-C8** without or with saccharides. [**B-Azo-C8**] = 0.020 mM, [Saccharide] = (a) 0 mM or (b), (c) 20 mM, [Na_2CO_3] = 10 mM, pH 11.0.

Saccharide response of B-Azo-C8. We evaluated the saccharide recognition function of **B-Azo-C8**. In 1% methanol solution, the maximum wavelength in the UV-Vis spectra of **B-Azo-C8** was 304 nm (Fig. 3a). When fructose was added to this solution, the maximum wavelength of **B-Azo-C8** was shifted from 304 nm to 352 nm with isosbestic points. The maximum wavelength of 352 nm was the same as that of **B-Azo-C8** without saccharides in the methanol-rich solution. This result indicated that equilibrium was established between the micelle and the monomer. The morphology changes of micelle to monomer caused a large spectral change (ca. 50 nm) upon fructose binding due to the changes in stacking mode of azobenzene moieties. Thus, fructose could be easily detected by naked eye as color changes from colorless to yellow (Figure S2).

Similarly, glucose and galactose were added to the 1% methanol solution of **B-Azo-C8**. When the amount of glucose added to **B-Azo-C8** solution was increased, the absorption maximum at 304 nm was decreased and the wavelength of maximum absorption was shifted to 323 nm (Figure 3b). Also, the optical density at 600 nm (Figure 3b) was increased by the addition of glucose. These results indicated that **B-Azo-C8** formed aggregates with the addition of glucose. When the amount of galactose added to **B-Azo-C8** solution was increased, the absorption maximum at 304 nm was decreased and the wavelength of maximum absorption was slowly shifted to 352 nm (Figure 3c).

To evaluate the saccharide selectivity of **B-Azo-C8**, the absorbance changes at 352 nm, which were estimated to exist as

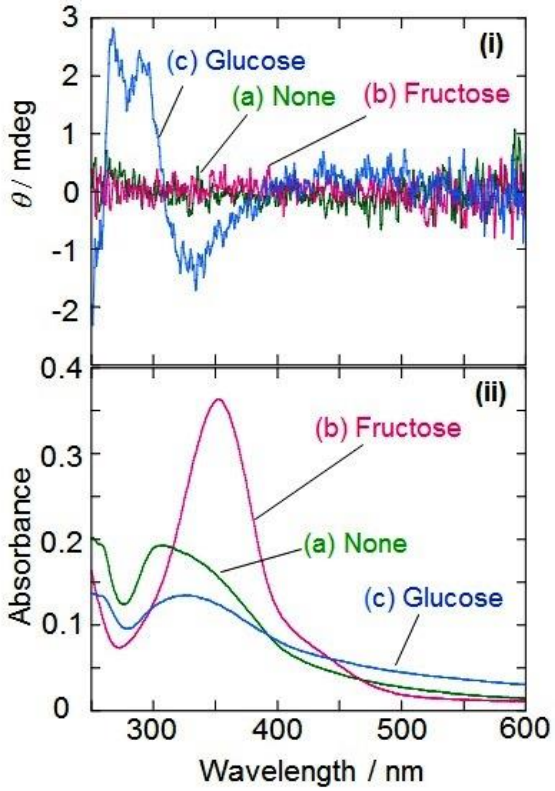

Figure 5. CD spectra and UV-Vis spectra of **B-Azo-C8**. [**B-Azo-C8**] = 0.020 mM, [Saccharide] = 20 mM, [Na_2CO_3] = 10 mM, pH 11.0.

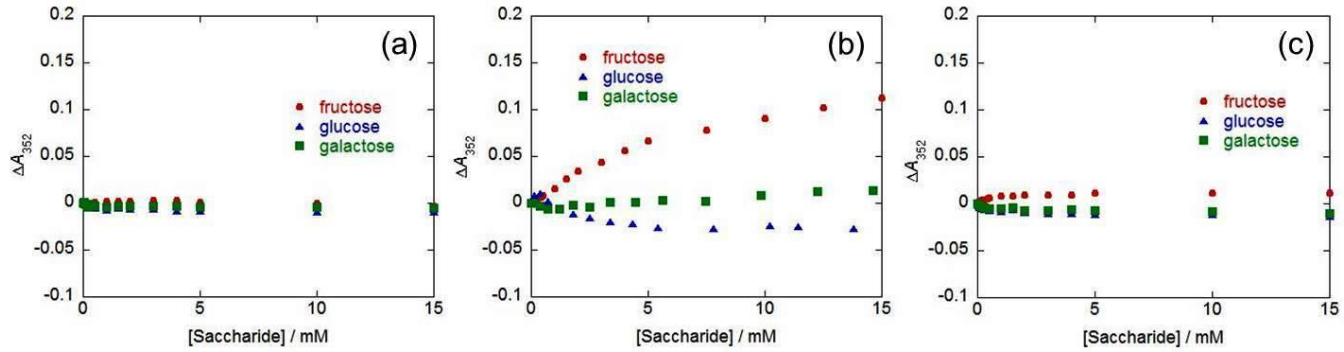

Figure 6. Effect of saccharide concentration on absorbance at 352 nm of **B-Azo-Cn** (n = (a) 4, (b) 8, (c) 12) in 1% MeOH solution (v/v). [**B-Azo-Cn**] = 0.020 mM, [Saccharide] = 0-15 mM, [Na_2CO_3] = 10 mM, pH 11.0, 25 °C.

the monomer, due to the addition of various saccharides were plotted (Figure 6b). The absorbance at 352 nm was increased as the amount of fructose added was increased, indicating that micelle formed **B-Azo-C8** bound to fructose and was stably dispersed. In contrast, the absorbance at 352 nm was not changed by the addition of glucose or galactose, demonstrating that the number of **B-Azo-C8** monomers did not increase. The association state of **B-Azo-C8** was changed by the saccharide species. Phenylboronic acid is known to form anionic ester with *cis*-diol moiety of 1,2-diols for fructose, 1,2- and 4,6-diols for glucose and 1,2- and 3,4-diols for galactose.[11] Phenylboronic acids could form 1 : 1 complex with fructose and 2 : 1 complex with glucose and galactose. Thus the glucose is considered to behave as glue between B-Azo-C8 micelles to form the micelle aggregation.

The competitive experiment was carried out for fructose in the presence of 20 mM glucose. The aggregation of **B-Azo-C8** micelles by glucose binding was dispersed by the addition of fructose, and the maximum wavelength of UV-Vis spectra was shifted from ca. 320 nm to 352 nm, which were the similar UV-vis spectral changes by the addition of fructose to **B-Azo-C8** micelle (Figure S3). This result indicated that the glucose bindings were replaced with the fructose bindings because of the stronger binding ability of fructose for **B-Azo-C8**. Also, we examined the time-dependent response of the micelle by the addition of fructose. As shown in Figure S4, the **B-Azo-C8** micelle dissociation took place within 10 min after the addition of fructose. By the addition of fructose in the **B-Azo-C8** micelle aggregate solution containing 20 mM glucose. The micelle dissociation equilibrium was achieved

within 30 min. These results revealed that **B-Azo-C8** micelle was easily dissociated by the addition of fructose.

The morphology of **B-Azo-C8** was evaluated on the basis of DLS measurements (Table S1) and TEM observation. DLS measurements revealed that the diameter of **B-Azo-C8** in the absence of saccharides was 126±43 nm. In the presence of glucose, the diameter of **B-Azo-C8** was increased to 329±9 nm. In the presence of fructose, the diameter of **B-Azo-C8** could not be determined because of low scattering. Then, we observed the morphology of **B-Azo-C8** with fructose and glucose by TEM (Figure 4). Aggregates measuring ca. 250~300 nm in diameter were observed in the presence of glucose, but not in the presence of fructose. These results were the same as those of DLS measurements. We also measured circular dichroism (CD) spectra in the presence or absence of saccharides. CD response was noted only in the presence of glucose (Figure 5). This result indicated that each **B-Azo-C8** probe was closely packed via 2:1 binding with glucose.

Evaluation of saccharide response to B-Azo-Cn having different alkyl chain lengths. We evaluated saccharide recognition function by changing the alkyl chain lengths of **B-Azo-Cn** (n = 4, 12). In 1% methanol solution, the maximum wavelength in the UV-Vis spectra of **B-Azo-C4** was 352 nm (Figure S5). The maximum wavelength of **B-Azo-C4** did not change even when methanol concentration was increased (Figure S6). In addition, the electrical conductivity of **B-Azo-C4** was decreased with increasing concentration of **B-Azo-C4**, and CMC of **B-Azo-C4** was not determined (Figure S1). This result demonstrated that **B-Azo-C4** did not form micelles and existed as a monomer. That the UV-Vis spectra were not affected by the type of saccharide added (Figure 6a) was an indication that the hydrophobicity of **B-Azo-C4** was increased by binding with saccharides and **B-Azo-C4** was dispersed.

Similarly, in the 1% methanol solution, the maximum wavelength in the UV-Vis spectra of **B-Azo-C12** was 304 nm and CMC of **B-Azo-C12** was 2.3 μM (Figures S1 and S7). When methanol concentration exceeded 50%, the maximum wavelength of **B-Azo-C12** was shifted from 304 nm to 352 nm (Figure S8). This suggested that the micelle was very stable in aqueous solution because the hydrophilicity of **B-Azo-C12** was much higher than that of **B-Azo-C4** or **B-Azo-C8**. The UV-Vis spectra of **B-Azo-C12** did not change when saccharides were added to 1% methanol solution of **B-Azo-C12** (Figure 6c). The UV-Vis spectra of **B-Azo-C12** were slightly shifted when fructose was added to 30% methanol solution of **B-Azo-C12** (Figure S9), indicating that **B-Azo-C12** micelles were slightly dissociated. Saccharide binding to **B-Azo-C12** increased the hydrophobicity of **B-Azo-C12**; nevertheless, **B-Azo-C12** still formed micelles stably because each **B-Azo-C12** molecule exhibited much higher interaction than **B-Azo-C8**.

Together, the results demonstrated that saccharide response was altered by the alkyl chain lengths of **B-Azo-Cn** and an appropriate alkyl chain length of **B-Azo-Cn** was important for this saccharide recognition system based on micelle formation and dissociation.

CONCLUSION

We have explored the potential of the amphiphilic phenylboronic acid azoprobes (**B-Azo-Cn**) as saccharide recognition sensors. The saccharide recognition function was derived from the micelle formation changes of self-assembled **B-Azo-Cn**. The wavelength of maximum absorption of **B-Azo-C8** in 1% methanol-99% water solution was shifted and solution color change with the addition of fructose was observed. **B-Azo-C8** formed aggregates with glucose and the diameters of the aggregates were approximately 250 nm based on DLS measurement and TEM observation. This result showed that **B-Azo-C8** changed its association state by recognizing saccharides. The effect of alkyl chain length on the saccharide recognition was examined as well. The fact that **B-Azo-C4** and **B-Azo-C12** did not respond to saccharides demonstrated that an appropriate alkyl chain length was required for recognizing saccharides. These results signify that the control of HLB was a key factor for the saccharide recognition based on micelle formation and dissociation.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was financially supported by JSPS KAKENHI Grant Numbers JP26248038 and JP15K05548. We are grateful to Associate Professor Sada-atsu Mukai and Assistant Professor Akihiko Watanabe for technical assistance in TEM measurements.

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Table of Contents