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Glucose Recognition by a Supramolecular Complex of Boronic Acid Fluorophore with Boronic Acid-Modified Cyclodextrin in Water

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A boronic acid fluorophore (**C1-APB**)/boronic acid-modified γ-cyclodextrin (3-PB-γ-CyD) complex as a supramolecular sensor has been designed for selective glucose recognition in water. The fluorescent response behavior of the **C1-APB**/3-PB-γ-CyD complex under various pH conditions revealed that a **C1-APB**/3-PB-γ-CyD complex solution containing glucose showed a large increase in the fluorescence intensity under alkaline pH conditions. In contrast, only small increases in the fluorescence intensity were noted for fructose and without sugar solutions. The observed response selectivity for the **C1-APB**/3-PB-γ-CyD complex was on the order of glucose >> galactose, mannose > fructose. The evidence on a large value of the inclusion constant ($K_{L\cdot CyD} = 6.5 \times 10^3$ M^{-1}), a marked broadening of the ^{1}H NMR spectra, and an enhancement of induced circular dichloism (ICD) intensity for the **C1-APB**/3-PB-γ-CyD complex by glucose binding supported the multi-point interaction of the **C1-APB**/3-PB-γ-CyD complex with glucose. These results demonstrated that the **C1-APB**/3-PB-γ-CyD complex functioned as an efficient supramolecular sensor for selective glucose recognition in water.

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Introduction

Sugars are essential biological molecules regarding nutrition, metabolism, and cell structure, and play elemental roles in controlling an individual's birth, differentiation, and immunity.[1-3] Because of their specific properties, simple methods are to be developed for sugar recognition in water. Enzyme-based sensors are highly selective, but their stabilities are not sufficient. Therefore, there are some problems from the aspect of durability, continuous monitoring, and *in vivo* analysis.[4] Chemosensors based on boronic acid have attracted much attention because of their greater stability.[5,6] Most mono-phenylboronic acids exhibit fructose selectivity. But due to the importance of glucose in diabetes management, much attention has been paid to the design of glucose-selective sensors.[7-9]

The versatile designs of chemosensors based on supramolecular chemistry are another approach to construct novel sugar sensors.[10,11] We have been examining the sugar-recognition function of supramolecular complexes based on the combination of boronic acid fluorophore (**C1-APB**) and various cyclodextrins (native CyD and aminated CyD).[12] The CyDs consist of cyclic glucose units, and their inner cavity diameter increases with the number of glucose units, from 5.7 Å for α-CyD to 7.8 Å for β-CyD and to 9.5 Å for γ-CyD. The advantages of CyD complex sensors are: (1) an improvement in the water solubility of the hydrophobic probe **C1-APB**, (2) an enhancement of the fluorescence emission of **C1-APB** in water, and (3) the capability of multi-point recognition by using CyD derivatives. Though almost all CyD complexes displayed no glucose selectivity, only the 3-NH_2-γ-CyD complex was found to exhibit high glucose recognition ability, due to a multi-point interaction (Fig. 1).[12] Further, a supramolecular sensor of γ-CyD having a functional group at the secondary hydroxyl side with **C1-APB** is expected to realize multipoint recognition for glucose. It is known that glucose can bind with two phenylboronic acids because it has two *cis-diol* moieties.[13-15] Therefore, in this study, we examined the sugar recognition function of the supramolecular

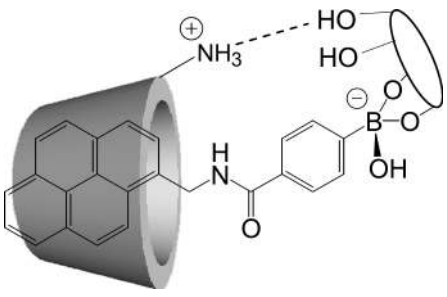

Fig. 1 Multi-point recognition of the **C1-APB**/3-NH_2-γ-CyD complex.

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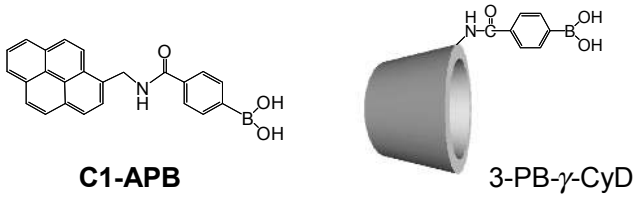
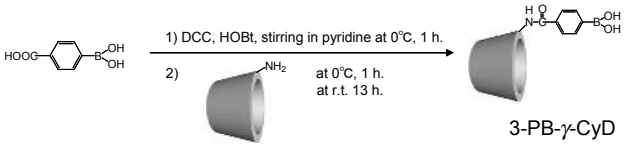


Fig. 2 Structure of fluorophore and the CyD derivative.



Scheme 1 Synthesis of 3-PB-γ-CyD.

complex based on **C1-APB** and γ-CyD modified with phenylboronic acid at the secondary hydroxyl side (3-PB-γ-CyD) (Fig. 2). The results revealed that the **C1-APB**/3-PB-γ-CyD complex functioned as an efficient supramolecular sensor for selective glucose recognition in water.

Experimental

Apparatus

Fluorescence spectra were measured with a Hitachi F-4500 fluorescence spectrometer (Hitachi, Ltd.) equipped with a 1.0-cm quartz cell. The fluorescence emission from 350 to 550 nm was monitored at an excitation wavelength of 328 nm with a 5.0-nm slit width. The scan speed was 60 nm min^{-1}. All fluorescence spectra were recorded under an aerated condition. UV-Vis spectra were measured by a Hitachi U-3000 UV-Vis spectrophotometer (Hitachi, Ltd.). ^{1}H NMR spectra were obtained with a JEOL GX-300 (Lambda). For 2D-ROESY analysis, JEOL GX-500 (Lambda) was used. The circular dichloism (CD) spectra were recorded with a JASCO J-820 spectrophotometer (JASCO Co.) having a 1.0-cm quartz cell. All pH values were recorded with a Horiba F-52 pH meter (HORIBA, Ltd.).

Reagents and chemicals

C1-APB, having the function of a photo-induced electron transfer (PET), was synthesized as previously reported.[16]

3-PB-γ-CyD was synthesized by the following procedure (Scheme 1).[17,18] 4-Carboxyphenylboronic acid (278 mg, 1.68 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC; 345 mg, 1.67 mmol), and 1-hydroxybenzotriazole monohydrate (252 mg, 1.65 mmol) were dissolved in dried pyridine (20 mM), and stirred for 1 h in an ice bath. 3A-Amino-3A-deoxy-(2AS,3AS)-γ-CyD (922 mg, 0.690 mmol) was added to the solution at once, which was stirred for 1 h in an ice bath and for 13 h at room temperature. Precipitates formed were removed by filtration, and the filtrate was concentrated to approximately 5 mL. The concentrated mixture was poured into acetone (200 mL) with vigorous stirring. White precipitates formed were collected, washed with acetone, followed by ether, and dried *in vacuo* (60°C). This material was dissolved with water (20 mL) and passed through a reverse-phase column (Chromatorex DM-1020T, 2 × 10 cm, pre-equilibrated with 10% MeOH). Step gradient elution was carried out with 10 and 20% MeOH. The fractions of 20% MeOH were collected and concentrated. Glassy solids obtained were again dissolved in water to be lyophilized to afford white fluffy powders (163 mg, 16.4%). ^{1}H NMR (D_2O): δ, 3.3 – 3.9 (m, other H of γ-CyD), 4.9 – 5.1 (m, 8H), 7.5 – 7.8 (m, 4H). Found; C, 43.0; H, 6.2; N, 1.0%. $C_{55}H_{86}BNO_{42}\cdot 5H_2O$ requires C, 43.1; H, 6.3; N, 0.9%.

Glucose, fructose, galactose and mannose were obtained as special-grade reagents (D-form) from Wako Pure Chemical Industries, Ltd. Water was doubly distilled and deionized by a Milli-Q water system (Millipore) before use. Other chemicals were of analytical reagent grade, and were used as received.

Measurements

For pH titration, the pH of a 2% DMSO–98% water (v/v) solution containing 1.0×10^{-6} M (M = mol dm^{-3}) **C1-APB**, 0.20 mM 3-PB-γ-CyD, 0.010 M phosphoric acid, and 0.10 M NaCl was controlled by titrating with 0.50 M NaOH at 25°C. The fluorescence spectra were recorded at each pH in both the absence and presence of 30.0 mM sugars. An excitation wavelength of 328.0 nm was selected as an isosbestic point of **C1-APB** in its UV-Vis spectra. A similar procedure was carried out for the sugar titration using a 1.0 M sugar solution.

To determine the apparent inclusion constant of **C1-APB** with 3-PB-γ-CyD, five different concentrations of 3-PB-γ-CyD sample solutions were separately prepared, and their fluorescence spectra were measured in both the absence and presence of 30.0 mM glucose or fructose at pH 11.3.

^{1}H NMR spectra of the **C1-APB**/3-PB-γ-CyD complex were recorded with solutions of 20% DMSO-d_6–80% D_2O (v/v) containing 0.5 mM **C1-APB**, 1.0 mM 3-PB-γ-CyD, pD at 25°C adjusted to 11.3 with 2.5 mM Na_2CO_3 buffer in both the absence and presence of 30 mM glucose or fructose.

CD spectra were measured with solutions of 2% DMSO–98% water (v/v) solution containing 1.0×10^{-5} M **C1-APB**, 0.20 mM 3-PB-γ-CyD, 0.10 M NaCl, and pH at 25°C adjusted to 11.3 with 0.01 M Na_2CO_3 buffer in both the absence and presence of 30 mM glucose or fructose.

Results and Discussion

*Effect of pH upon the fluorescence response of the **C1-APB**/3-PB-γ-CyD complex*

The fluorescence response of **C1-APB** is based on the PET mechanism. The inherent fluorescence of **C1-APB** is quenched by the internal PET from pyrene to the trigonal form of phenylboronic acid, but the presence of sugar under alkaline conditions converts boronic acid to the tetrahedral boronate, thereby inhibiting PET quenching and increasing the fluorescence intensity of the pyrene monomer.[18–20] In addition, the formation of an inclusion complex of **C1-APB** with a suitable conformation of CyD derivatives restricts the molecular motion of **C1-APB**, and inhibits the radiationless transition process. Fluorescence quenching in water is also prevented inside the CyD cavity. These phenomena efficiently increase the fluorescence quantum yield of **C1-APB**.

The fluorescence behaviors of the **C1-APB**/3-PB-γ-CyD complex in 2% DMSO–98% water (v/v) were examined under different pH conditions. The pK_a of 3-PB-γ-CyD in 2% DMSO–98% water (v/v) has been determined as 7.96 ± 0.02 based on the UV-Vis spectral analysis as a function of the solution pH. Figure 3a shows typical fluorescence spectra of the **C1-APB**/3-PB-γ-CyD complex. The fluorescence intensity

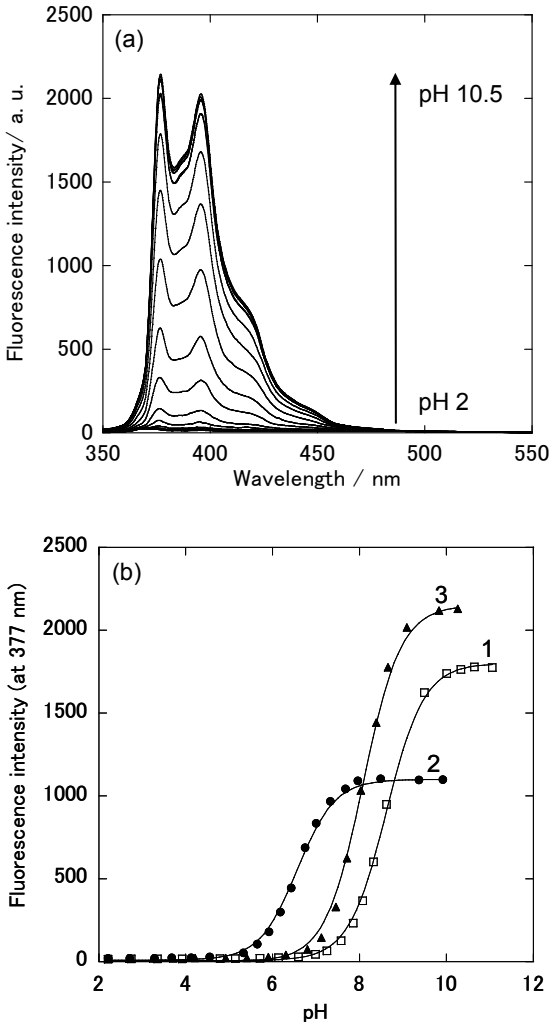


Fig. 3 Fluorescence spectra of the **C1-APB**/3-PB-γ-CyD complex with 30 mM glucose (a), and pH profile of the **C1-APB**/3-PB-γ-CyD complex with and without sugar (b). [**C1-APB**] = 1.0×10^{-6} M in 2% DMSO-98% water (v/v), [3-PB-γ-CyD] = 0.2 mM. (1) □, Without sugar, (2) ●, 30 mM fructose, and (3) ▲, 30 mM glucose; pH at 25°C adjusted with 0.01 M phosphate buffer; I = 0.1 M with NaCl; λ_{ex} = 328 nm.

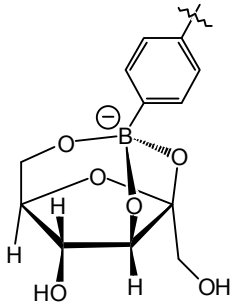


Fig. 4 Fructose recognition by mono-phenylboronic acid.

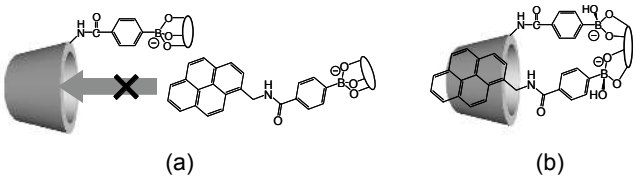


Fig. 5 Sugar recognition by the **C1-APB**/3-PB-γ-CyD. (a) Fructose recognition, (b) glucose recognition.

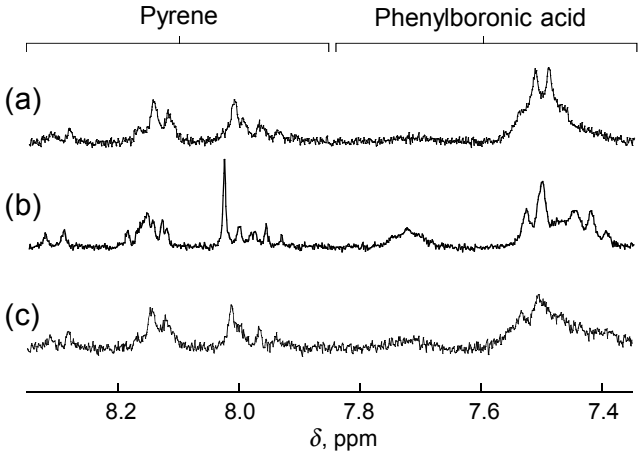


Fig. 6 ^{1}H NMR spectra of the **C1-APB**/3-PB-γ-CyD complex. [**C1-APB**] = 0.5 mM in 20% DMSO-d_6-80% D_2O (v/v), [3-PB-γ-CyD] = 1 mM, pD at 25°C adjusted to 11.3 with 2.5 mM Na_2CO_3 buffer. (a) Without sugar, (b) 30 mM fructose, (c) 30 mM glucose.

increased with an increase of the solution pHs. The changes in the fluorescence intensity at 377 nm were plotted against pH (Fig. 3b). In the presence of 30 mM fructose (plot 2 in Fig. 3b), the fluorescence intensity under the alkaline pH condition was much smaller than that of without a sugar solution (plot 1). On the other hand, in the case of a glucose solution, as shown in Fig. 3b (plot 3), the fluorescence intensity under the alkaline pH condition was much larger than that of without a sugar solution. Under the alkaline conditions, the phenylboronic acid of **C1-APB** was completely converted to the tetrahedral boronate in the presence or absence of sugar. Therefore, the differences in the fluorescence intensity may be due to the difference in the inclusion ability of 3-PB-γ-CyD for **C1-APB** under different sugar binding modes.

The mono-phenylboronic acids are known to bind fructose strongly and selectively by three-point recognition (Fig. 4).[21] In the case of a fructose solution, the results of fluorescence intensity imply that fructose binds each phenylboronic acid of **C1-APB** and 3-PB-γ-CyD (Fig. 5a). The binding of fructose to phenylboronic acid of 3-PB-γ-CyD may disturb the inclusion complex formation of **C1-APB** for 3-PB-γ-CyD. However, in the case of a glucose solution, the results of the fluorescence intensity suggest that glucose is efficiently bound with two phenylboronic acids, **C1-APB** and 3-PB-γ-CyD, as expected in Fig. 5b. The multi-point interaction may strengthen the inclusion of **C1-APB** with 3-PB-γ-CyD.

To clarify the complex structure of **C1-APB**/3-PB-γ-CyD, we measured the ^{1}H NMR spectra in 20% DMSO-d_6-80% D_2O (v/v) at 300 MHz. The ^{1}H NMR spectra at 7.8 – 8.3 ppm (Fig. 6) is ascribed to the resonance corresponding to the pyrene protons of **C1-APB**. In the case of a fructose solution, sharper signals were noted, as compared with the signals without sugar and the glucose solution. This result supported that all parts of the fructose/**C1-APB** complex were not included inside the cavity of 3-PB-γ-CyD. Thus, the free fructose/**C1-APB** complex exhibited sharp proton signals in the solution. The ^{1}H NMR

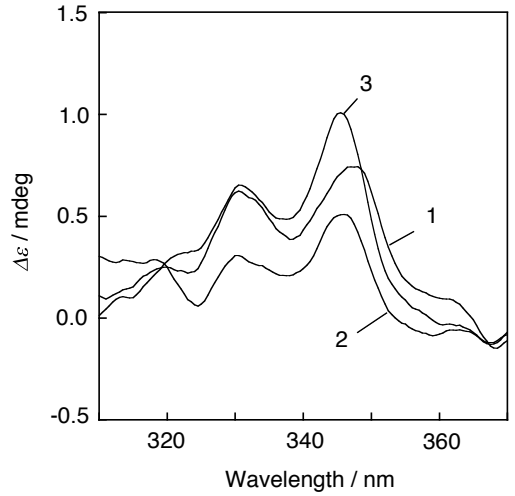


Fig. 7 ICD spectra of the **C1-APB**/3-PB-γ-CyD complex. [**C1-APB**] = 1.0×10^{-5} M in 2% DMSO–98% water (v/v), [3-PB-γ-CyD] = 0.2 mM. (1) Without sugar, (2) 30 mM fructose, and (3) 30 mM glucose; pH 11.3 at 25°C adjusted with 0.01 M Na_2CO_3 buffer; I = 0.1 M with NaCl.

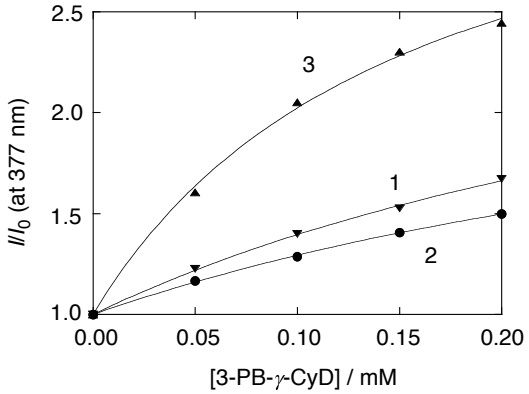


Fig. 8 Effect of the 3-PB-γ-CyD concentration on the fluorescence intensity at 376.4 nm for **C1-APB**. [**C1-APB**] = 1.0×10^{-6} M in 2% DMSO–98% water (v/v). (1) ▼, Without sugar, (2) ●, 30 mM fructose, and (3) ▲, 30 mM glucose; pH 11.3 at 25°C adjusted with 0.01 M Na_2CO_3 buffer; I = 0.1 M with NaCl; λ_{ex} = 328 nm.

spectra at 7.35 – 7.8 ppm (Fig. 6) depict the resonance signals corresponding to the phenylboronic acid protons of both **C1-APB** and 3-PB-γ-CyD. In the case of the glucose solution, broader peaks were observed as compared with the signals of without sugar and the fructose solution. This result suggested that in the presence of glucose the **C1-APB** was efficiently included inside the cavity of 3-PB-γ-CyD, and the molecular motion of the phenylboronic acids was restricted to result in signal broadening. In a similar way, we measured the 2D-ROESY spectra of the **C1-APB**/3-PB-γ-CyD complex in the presence of 30 mM glucose or fructose. In the presence of fructose, no significant cross peak based on the nuclear overhauser effect (NOE) between pyrene protons with CyD protons was noted (Fig. S2a, Supporting Information).[22] On the other hand, a NOE peak between the pyrene proton and the CyD proton appeared in the presence of glucose (Fig. S2b, Supporting Information), indicating that the inclusion complex formation between **C1-APB** and 3-PB-γ-CyD was enhanced by glucose binding.

The circular dichloism analysis is an additional approach to confirm the complex structure. The achiral chromophores are known to exhibit an induced circular dichloism (ICD) by forming an inclusion complex with CyDs based on the chiral nature of CyD cavities.[23] As shown in Fig. 7, positive cotton effects based on ICD were noted at the UV-Vis absorption region of pyrene, indicating the formation of an inclusion complex between **C1-APB** and 3-PB-γ-CyD. However, the ICD intensities were apparently reduced in the presence of fructose (line 2). This indicates that fructose binding disturbs inclusion complex formation, as expected in Fig. 5a. In fact, a similar destabilization of the inclusion complex formation between phenylboronic acid modified β-CyD and styrylpyridinium dyes by fructose binding has been reported.[18] However, in the presence of glucose, the ICD intensities were efficiently enhanced (line 3), demonstrating that glucose binding promoted inclusion complex formation, possibly as a result of a multi-point interaction (Fig. 5b).

Table 1 Apparent inclusion constant (1:1) of **C1-APB** with 3-PB-γ-CyD

Condition	Inclusion constant $K_{L\cdot CyD}$/M^{-1}
Without sugar	$(2.4 \pm 0.4) \times 10^3$
30 mM fructose	$(2.1 \pm 0.3) \times 10^3$
30 mM glucose	$(6.5 \pm 0.9) \times 10^3$

*Inclusion ability of the **C1-APB**/3-PB-γ-CyD complex*

To examine the inclusion ability of the **C1-APB**/3-PB-γ-CyD complex, we measured the fluorescence behaviors under different concentrations of 3-PB-γ-CyD at pH 11.3 (Fig. 8). The fluorescence intensity of the glucose solution was much larger than that of other solutions. On the basis of 1:1 inclusion complex formation for **C1-APB** (L) and 3-PB-γ-CyD, the relationship between the fluorescence intensity (I) and the 3-PB-γ-CyD concentration [CyD] can be expressed by the following equations:[16]

$$\mathrm{L} + \mathrm{CyD} \rightleftharpoons \mathrm{L{\cdot}CyD}, \tag{1}$$

$$K = \frac{[\mathrm{L{\cdot}CyD}]}{[\mathrm{L}][\mathrm{CyD}]}, \tag{2}$$

and

$$\frac{I}{I_0} = \frac{\phi_{\mathrm{L}} + \phi_{\mathrm{L{\cdot}CyD}} K_{\mathrm{L{\cdot}CyD}}[\mathrm{CyD}]}{\phi_{\mathrm{L}}(1 + K_{\mathrm{L{\cdot}CyD}}[\mathrm{CyD}])}, \tag{3}$$

where ϕ is the fluorescence quantum yield for the corresponding chemical species, I_0 the initial fluorescence intensity ($I_0 = \beta\phi_{\mathrm{L}}[\mathrm{L}]_t$), β a constant proportional to the intensity of the excitation light and the molar extinction coefficient of **C1-APB**, and $[\mathrm{L}]_t$ the total concentration of **C1-APB**. It should be noted that the concentration of 3-PB-γ-CyD added to the solution (0.05 – 0.20 mM) was large enough to that of **C1-APB** (1.0×10^{-6} M). The observed results were well fitted with Eq. (3) (solid line), and the apparent inclusion constant ($K_{\mathrm{L{\cdot}CyD}}$) of **C1-APB** with 3-PB-γ-CyD was determined from a theoretical curve. The results are summarized in Table 1. The $K_{\mathrm{L{\cdot}CyD}}$ value of 6.5×10^3 M^{-1} observed for the glucose solution was larger

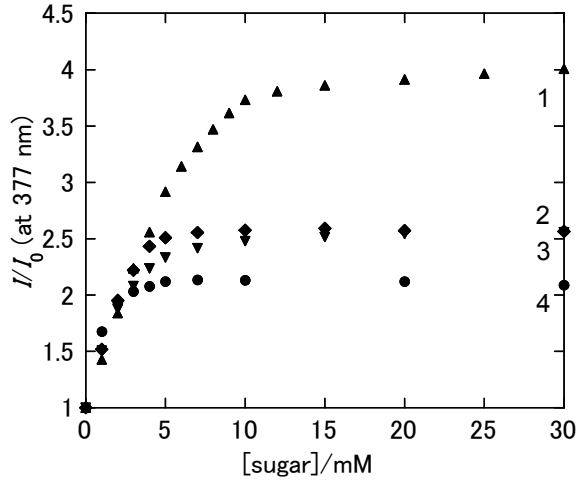


Fig. 9 Effect of the sugar concentration on the fluorescent response of **C1-APB**/3-PB-γ-CyD. [**C1-APB**] = 1.0×10^{-6} M in 2% DMSO–98% water (v/v), [3-PB-γ-CyD] = 0.2 mM. (1) ▲, Glucose, (2) ◆, galactose, (3) ▼, mannose, and (4) ●, fructose; pH 8.5 at 25°C adjusted with 0.01 M phosphate buffer; I = 0.1 M with NaCl; λ_{ex} = 328 nm.

than that for the solutions without sugar (2.4×10^3 M^{-1}) and with fructose (2.1×10^3 M^{-1}), demonstrating that **C1-APB** was efficiently included inside the 3-PB-γ-CyD cavity based on the multi-point interaction.

*Glucose recognition selectivity for the **C1-APB**/3-PB-γ-CyD complex*

To examine glucose recognition selectivity for the **C1-APB**/3-PB-γ-CyD complex, we measured the fluorescence behaviors under different concentrations of sugar species (glucose, fructose, galactose, mannose, Fig. 9). Because of the small fluorescence intensity change for the **C1-APB**/3-PB-γ-CyD complex under acidic conditions, the effect of the sugar concentrations on the fluorescence intensity of the complex was investigated at pH 8.5. As shown in Fig. 9, the glucose-added solution showed a large increase in fluorescence intensity. In contrast, only small increases in the fluorescence intensity were noted for fructose-, galactose-, mannose-added solutions. These results showed that galactose and mannose bound each phenylboronic acid of the **C1-APB** and 3-PB-γ-CyD, like fructose, resulting in a high glucose recognition selectivity for **C1-APB**/3-PB-γ-CyD complex (at pH 9.0, see Fig. S3).

To examine the interference of competing materials (fructose, galactose, mannose) to glucose recognition by the **C1-APB**/3-PB-γ-CyD complex, we briefly measured the fluorescent responses under similar sugar concentrations to those in blood serums (4.61 mM glucose, 0.0483 mM fructose, 0.0389 mM galactose, and 0.0638 mM mannose). The 2% DMSO–98% water (v/v) solutions (pH 8.5 adjusted with 0.01 M phosphate buffer, I = 0.1 M with NaCl) of 1.0×10^{-6} M **C1-APB** and 0.20 mM 3-PB-γ-CyD containing sugars were prepared as sample solutions. The results revealed that sample solutions containing the competing sugars exhibited a fluorescence intensity of 96.3 ± 0.4% (average of three individual experiments) for that of only glucose-added (4.61 mM) solution. This result suggests that competing sugars give only a small interference under physiological conditions at pH 8.5.

Conclusions

A supramolecular complex based on boronic acid fluorophore (**C1-APB**) and γ-CyD bearing boronic acid binding site at the secondary hydroxyl side (3-PB-γ-CyD) has been designed, and its sugar sensing functions were examined. The pH-dependent fluorescence changes of the **C1-APB**/3-PB-γ-CyD complex revealed selective increases in the fluorescence intensity for glucose under alkaline pH conditions, compared with those for fructose and in the absence of sugars. Evidence of a large value of the inclusion constant ($K_{L\cdot CyD} = 6.5 \times 10^3$ M^{-1}), a marked broadening of the ^{1}H NMR spectra, and an enhancement of the ICD intensity for the **C1-APB**/3-PB-γ-CyD complex by glucose binding supported the multi-point interaction of the **C1-APB**/3-PB-γ-CyD complex with glucose. As a result, selective glucose recognition by the **C1-APB**/3-PB-γ-CyD complex over other sugars, such as fructose, galactose, and mannose, was achieved under physiological conditions at pH 8.5. The combination of various types of boronic acid probes with 3-PB-γ-CyD is expected to realize additional sophisticated sugar sensors based on efficient multi-point interactions. Some of these approaches are actively under way in our laboratory.

Acknowledgements

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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