Effect of Cyclodextrins on Saccharide Sensing Function of a Fluorescent Phenylboronic Acid in Water

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An inclusion complex consisting of a fluorescent phenylboronic acid (**C1-APB**) and β -cyclodextrin (β -CD) acts as a supramolecular saccharide sensor whose response mechanism is based on photoinduced electron transfer (PET). This study evaluated four kinds of cyclodextrins (α -CD, β -CD, γ -CD, and NH_2 - β -CD) by comparing their pH profiles, and confirmed that β -CD was the best host for **C1-APB** because the **C1-APB**/ β -CD complex exhibited high affinity for saccharides as well as high fluorescent recovery upon saccharide binding. An investigation of the β -CD concentration effect revealed the formation of a 1:1 inclusion complex of **C1-APB** with β -CD. The observed saccharide selectivity of the **C1-APB**/ β -CD complex is in the following order: D-fructose ($4039 \pm 69 \text{ M}^{-1}$) > D-ribose ($1083 \pm 26 \text{ M}^{-1}$) > L-arabinose ($474 \pm 11 \text{ M}^{-1}$) > D-galactose ($318 \pm 3 \text{ M}^{-1}$) > maltotriose ($135 \pm 5 \text{ M}^{-1}$) > D-glucose ($114 \pm 2 \text{ M}^{-1}$) > maltose ($81 \pm 2 \text{ M}^{-1}$). In addition to monomer emission, dimer emission from pyrene dimers was observed in the spectra for the **C1-APB**/ γ -CD complex, which allowed a ratiometric analysis. This study shows that the combination of a simple fluorescent probe, **C1-APB**, with various CDs diversifies the response systems for saccharide recognition.

(Received October 4, 2007; Accepted December 11, 2007; Published February 10, 2008)

Introduction

Sugars are essential biological molecules that play fundamental roles in metabolism and the maintenance of cell structure. They are also related to cell differentiation and immunity.¹ Because of their important properties, researchers need simple methods that can quickly recognize sugars in aqueous solution. Enzyme-based sensors are highly selective, but of limited use for continuous monitoring and *in vivo* analysis because of their poor stability and because they consume their substrate in the recognition process. Although chemosensors based on boronic acid are not as selective, they have attracted much attention because of their greater stability.^{2,3} Phenylboronic acid can readily form stable cyclic esters with the diol moiety of sugars in water,⁴ and various types of the boronic acid chemosensors with response mechanism based on internal charge transfer (ICT),⁵⁻⁸ photoinduced electron transfer (PET),⁹⁻¹¹ and fluorescent resonance energy transfer (FRET)¹² have been developed.

The versatile designs of chemosensors based on supramolecular chemistry are another approach to construct novel sugar sensors.¹³⁻¹⁵ We reported that the complex consisting of the boronic acid fluorophore (**C4-CPB**) and β -cyclodextrin (β -CD) acts as a supramolecular saccharide sensor (Fig. 1).¹⁶ The β -CD solubilizes the water-insoluble **C4-CPB** by forming an inclusion complex. The fluorescence response of this complex is based on the PET mechanism. The inherent fluorescence of **C4-CPB** is quenched by the internal PET from pyrene to the trigonal form of phenylboronic acid, but

saccharide binding converts the boronic acid to the tetrahedral boronate, thereby inhibiting PET quenching and increasing the fluorescence intensity. In a previous study, the spacer effect of the boronic acid probe was examined in detail by using the fluorophores **C1-CPB**, **C4-CPB**, and **C1-APB** (Fig. 1). We found that the **C1-APB**/ β -CD complex was a desirable fluorescent chemosensor for saccharide recognition because of its high affinity to saccharides as well as its relatively high fluorescence recovery upon saccharide binding. Since **C1-APB** is more soluble than **C1-CPB** and **C4-CPB**, because of possessing an amide group, it can be dissolved in water without adding β -CD at the level for the fluorescent measurement. This makes a precise comparison of the CD effect feasible. In the present work, therefore, we focused on **C1-APB**/CD complexes, and investigated how the sensing function of **C1-APB** for saccharide recognition in water was affected by differences in

Fig. 1 Structure of fluorescent probes and CDs.

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the CD structure. Three of the CDs that we used are widely known (α -CD, β -CD, and γ -CD, respectively), consisting of six, seven and eight glucose units. The diameter of the inner cavity of the CD increases with the number of glucose units, from 5.7 Å for α -CD to 7.8 Å for β -CD and to 9.5 Å for γ -CD.¹⁷ The other CD that we used was NH_2 - β -CD, in which an amino group is substituted for one of the hydroxyls in β -CD (Fig. 1). We compared these CDs, and found that the differences in the cavity size as well as the substituent of the CDs affected the response systems for saccharide recognition.

Experimental

Reagents

2-(4-Phenylboronic acid)-1-pyrenemethamide (**C1-APB**) was synthesized by the following procedure. To a mixture of 4-(1-pyrenyl) methylaminochloride (220 mg, 0.825 mmol), 2-(4-carboxyphenyl)-1,3,2-dioxaborinane (155 mg, 0.825 mmol), triethylamine (75 mg), and 1-hydroxy-7-azabenzotiazole (100 mg, 0.75 mmol) in dry CH_2Cl_2 -DMF (10:1 v/v, 75 ml) was slowly added a solution of 1-(3-dimethylamino) propyl-3-ethylcarbodiimide hydrochloride (140 mg, 0.75 mmol) in dry CH_2Cl_2 -DMF (10:1 v/v, 2 mL) at 0°C. Under dry N_2 , the reaction mixture was stirred at 0°C for 2 h and at room temperature for 2 days. The solvent was evaporated and CH_2Cl_2 (20 mL) was added. The desired product precipitated out and was filtered. Purification by column chromatography on silica gel with 3:1 ethyl acetate:hexane, and subsequently 1:4 methanol:chloroform as eluents gave 60 mg (19.2%) of the product as an off-white solid. The solid was crystallized from ethanol. ^1H -NMR (CDCl_3) δ , 1.01 (t, 3H), 1.6 (q, 2H), 5.2 (s, 2H), 7.8–8.6 (Ar-H, B-OH, 13H), 9.2 (s, 1H). ^{13}C -NMR (CDCl_3) δ , 59, 124, 124.6, 124.8, 125.4, 125.8, 125.9, 127, 127.2, 127.6, 128, 128.2, 128.8, 131.6, 131.8, 133.8, 134.6, 136.2. Positive-ion FAB-MS (glycerol matrix), m/z 436 [$\text{M}+\text{H}+\text{gly}-2\text{H}_2\text{O}$] $^+$.

γ -CD, α -CD, D(-)-fructose, and other saccharides were obtained as special-grade reagents from Wako Pure Chemical Industries, Ltd. The first-reagent grade of β -CD (Wako Pure Chemical Industries, Ltd.) was recrystallized twice from water. 3A-amino-3A-deoxy-(2AS,3AS)- β -cyclodextrin (NH_2 - β -CD) was obtained from Tokyo Chemical Industry Co., 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.

Apparatus

Fluorescence spectra were measured by 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 the excitation wavelength of 328 nm with a 5.0-nm slit width. The scan speed was 60 nm/min. All fluorescence spectra were obtained under an aerated condition. ^1H -NMR spectra were obtained using a Varian INOVA 500 spectrometer (500 MHz, Varian, Inc.). Mass spectrometry was performed on a JEOL JMS-AX505HA spectrometer (JEOL Ltd.). All pH values were determined by a Horiba F-52 pH meter (HORIBA, Ltd.).

Results and Discussion

pH dependence of the fluorescence spectra

The fluorescence of the **C1-APB**/CD complexes was

Fig. 2 pH dependence of the fluorescence spectra for **C1-APB** without D-fructose, (a) **C1-APB** alone, (b) **C1-APB**/ γ -CD, (c) **C1-APB**/ β -CD. [**C1-APB**] = 1.0×10^{-6} M in 2% DMSO-98% water (v/v) containing 0.0 or 5.0 mM CD, pH at 25°C adjusted with 0.01 M phosphate buffer, $I = 0.1$ M with NaCl, $\lambda_{\text{ex}} = 328$ nm.

examined under different pH conditions. As shown in Fig. 2a, the fluorescence intensity of **C1-APB** alone increases as the pH increases from 2.2 to 11.0. The presence of 5 mM ($\text{M} = \text{mol dm}^{-3}$) γ -CD (Fig. 2b) or β -CD (Fig. 2c), however, resulted in far more intense fluorescence at any pH. The maximum fluorescence intensity of the **C1-APB**/ NH_2 - β -CD complex was about 50% lower than that of the **C1-APB**/ β -CD complex under basic conditions, and the fluorescence spectra of the **C1-APB**/ α -CD complex were the almost same as those of the **C1-APB** alone (results not shown). As a result, β -CD resulted in the strongest fluorescence response among these four kinds of CDs.

The corresponding fluorescence spectra were also measured in the presence of 30 mM D-fructose. Although similar spectra were obtained for each CD, the pH profiles were clearly different. The maximum fluorescence intensity of the **C1-APB**/ β -CD complex was measured at 377 nm (Fig. 2c), and the I_{377} values are plotted against pH in Fig. 3c. The solid lines of Fig. 3c were obtained from the theoretical calculation,¹⁶ and pK_a values were determined by nonlinear curve-fitting. The pK_a without D-fructose is 8.07, but the apparent pK_a (pK_a^{app}) with D-fructose is 6.01. This apparent pK_a shift ($\Delta\text{pK}_a^{\text{app}}$) enables the **C1-APB**/ β -CD complex to recognize D-fructose under neutral conditions.

The equilibria in Scheme 1 show the proton-dissociation and saccharide-binding equilibria in water. The PET-based fluorescence change without D-fructose occurs upon conversion from the trigonal form under acidic conditions to the tetrahedral form under basic conditions. The presence of D-fructose shifts the apparent pK_a lower, which suggests that the tetrahedral form is produced at a lower pH because boronic acid and saccharides form anionic esters.

The I_{377} vs. pH profiles of **C1-APB** alone (Fig. 3a), the **C1-APB**/ α -CD complex (Fig. 3d) and the **C1-APB**/ NH_2 - β -CD complex (Fig. 3e) reveal two pK_a 's. The lower pK_a (pK_{a1}) is close to that of the **C1-APB**/ β -CD complex, suggesting that the pK_{a1} is induced by the proton dissociation of boronic acid in the same way as in Scheme 1. The higher pK_a (pK_{a2}) seems to be caused by proton dissociation of the amide group, which should have a higher pK_a than that of the boronic acid. The **C1-APB**/ β -CD complex and the **C1-APB**/ γ -CD complex display only simple one- pK_a pH profiles (Figs. 3b and 3c) indicating that the pK_a of the amide group increased in the hydrophobic cavity of CD, and the pK_{a2} was not observed under pH 11 (Table 1).

The formation of an inclusion complex of **C1-APB** with β -CD or γ -CD increases the fluorescence intensity above that of **C1-APB** alone at any pH. In general, the fluorescence intensity increases in a hydrophobic environment, like that in the CD

Table 1 Binding constants for D-fructose calculated from pK_a s with D-fructose and pK_a^{app} 's with D-fructose

CD	Without D-fructose		With 30 mM D-fructose		ΔpK_a^{app}	K_{LS}/M^{-1}
	pK_{a1}	pK_{a2}	pK_{a1}^{app}	pK_{a2}^{app}		
None	8.09 ± 0.03	10.15 ± 0.01	6.59 ± 0.04	9.12 ± 0.05	1.50 ± 0.07	1000
α -CD	8.18 ± 0.02^a	10.11 ± 0.02^a	6.51 ± 0.05^a	9.35 ± 0.05^a	1.67 ± 0.07	1500 ^b
γ -CD	7.85 ± 0.01^a	—	5.82 ± 0.01^a	—	2.03 ± 0.01	3600 ^b
β -CD	8.07 ± 0.01^a	—	6.01 ± 0.01^a	—	2.06 ± 0.02	3800 ^b
NH_2 - β -CD	8.11 ± 0.01^a	10.70 ± 0.01^a	6.01 ± 0.02^a	8.94 ± 0.03^a	2.11 ± 0.03	4200 ^b

a. Conditional apparent acidity constants in the presence of 5.0 mM CDs. b. Conditional binding constants in the presence of 5.0 mM CDs.

Scheme 1 Proton-dissociation and saccharide-binding equilibria of **C1-APB** in aqueous solution.

Fig. 3 I_{377} versus pH profiles for **C1-APB**/CD with and without D-fructose. (a) **C1-APB** alone, (b) **C1-APB**/ γ -CD, (c) **C1-APB**/ β -CD, (d) **C1-APB**/ α -CD, (e) **C1-APB**/ NH_2 - β -CD. [**C1-APB**] = 1.0×10^{-6} M in 2% DMSO–98% water (v/v) containing 0.0 or 5.0 mM CD, pH at 25°C adjusted with 0.01 M phosphate buffer, $I = 0.1$ M with NaCl, $\lambda_{ex} = 328$ nm. (1) [fructose] = 0 mM, (2) [fructose] = 30 mM.

cavity. In addition to this, the formation of the inclusion complex of **C1-APB** with the suitable size of CDs restricts the molecular motion of **C1-APB** and inhibits the radiationless transition process. These phenomena efficiently increase the fluorescence quantum yield.

β -CD is large enough to enclose the pyrene moiety of **C1-APB** and produces the greatest fluorescence intensity whereas α -CD is too small to enclose **C1-APB** and γ -CD is too large to fit a single **C1-APB** molecule. On the other hand, γ -CD can incorporate two pyrene moieties, as reported in earlier studies.^{18,19} In the fluorescence spectra of the **C1-APB**/ γ -CD complex, a slight level of dimer emission is evident at longer wavelengths (Fig. 2b). The formation of pyrene dimers decreases the monomer emission, and hence the maximum fluorescence intensity (I_{377}) of the **C1-APB**/ γ -CD complex is less than that of the **C1-APB**/ β -CD complex. The I_{377} vs. pH profiles of the **C1-APB**/ NH_2 - β -CD complex (Fig. 3e) are quite different from those of the **C1-APB**/ β -CD complex (Fig. 3c). The amino group ($-NH_2$) on the β -CD rim appears to inhibit the formation of an inclusion complex with the pyrene moiety of **C1-APB**. It should be noted, however, that the fluorescence intensity of the **C1-APB**/ NH_2 - β -CD complex is higher than that of **C1-APB** alone.

The pK_a^{app} shift of the pH profiles with and without D-fructose can be used for determining the conditional binding constants of the **C1-APB**/CD complexes for D-fructose. It is noted that the equilibria shown in Scheme 1 should include the inclusion complex formation of **C1-APB** with CDs. However, the equilibrium analysis including all the system is too complicated to evaluate the binding constants of **C1-APB**/CD complexes for saccharides. Thus, for simplicity, we used a conditional equilibrium of **C1-APB** with saccharide at the fixed CD concentration. As we reported earlier,¹⁶ the conditional binding constant (K_{LS}) at the fixed CD condition is expressed as

$$\Delta pK_a^{app} = \log(1 + K_{LS}[S]), \quad (1)$$

where [S] is the saccharide concentration. The results are summarized in Table 1. The larger ΔpK_a^{app} represents the higher

Scheme 2 Equilibrium of 1:1 inclusion complex of **C1-APB** with β -CD.

Fig. 4 Effect of the β -CD concentration on the fluorescence spectra and the fluorescence intensity at 377 nm (inset figure) for **C1-APB** without saccharide at pH 9.2. [**C1-APB**] = 1×10^{-6} M in 2% DMSO–98% water (v/v), pH at 25°C adjusted to 9.2 with 0.01 M sodium hydrogencarbonate, $I = 0.1$ M with NaCl, $\lambda_{\text{ex}} = 328$ nm.

binding ability. The conditional binding constant of the **C1-APB**/ β -CD complex is 3.8 times higher than that of **C1-APB** alone, suggesting that there is an interaction between a hydroxyl group of β -CD and the boronic acid ester, and this hydrogen bond makes the boronic acid ester formation more stable. The **C1-APB**/ γ -CD complex showed the lowest $\text{p}K_{\text{a}}$ in the absence of D-fructose (7.85). Although the reason is unclear at present, the hydrogen bonding of the boronic acid with the hydroxyl group of γ -CD, or the formation of **C1-APB** dimers may affect the proton dissociation. The **C1-APB**/ α -CD complex, in contrast, showed the highest $\text{p}K_{\text{a}}$ in the absence of D-fructose (8.18). We suspect that this is because the boronic acid moiety of **C1-APB** interacted with the α -CD cavity. It is reasonable that the $\text{p}K_{\text{a}}$ of boronic acid exhibited a slight increase in the narrowest CD cavity because of the hydrophobic effect.²⁰ The conditional binding constant of the **C1-APB**/ NH_2 - β -CD complex is comparable to that of the **C1-APB**/ β -CD complex, although the fluorescence intensity is lower than that of the **C1-APB**/ β -CD complex. Since the amino group on the β -CD rim is protonated under acidic and neutral conditions,²¹ the electrostatic interaction stabilizing the anionic boronate ester should be taken into consideration.

Overall, of these four CDs, β -CD is the best host for **C1-APB**. The **C1-APB**/ β -CD complex showed the largest fluorescence intensity change as well as a large $\text{p}K_{\text{a}}^{\text{app}}$ shift in the presence of D-fructose, evidence of a high conditional binding constant with D-fructose.

Effect of β -CD concentration

Because of the small fluorescence intensity change for **C1-APB** under acidic conditions, the effect of β -CD on the fluorescence intensity of **C1-APB** without D-fructose was investigated at pH 9.2, at which the boronate anion (L^-) is the

main species, so as to elucidate an inclusion ability (Scheme 2). The fluorescence intensity increased drastically as the β -CD concentration increased (Fig. 4). Based on the 1:1 inclusion complex formation for **C1-APB** (L^-) and CD, the relationship between fluorescence intensity (I) and the CD concentration [CD] can be expressed by the following equations:

$$\text{L}^- + \text{CD} \rightleftharpoons \text{L}^-\text{-CD}, \quad (2)$$

$$K_{\text{LCD}} = \frac{[\text{L}^-\text{-CD}]}{[\text{L}^-][\text{CD}]}, \quad (3)$$

and

$$I = \frac{\beta[\text{L}]_t(\phi_{\text{L}} + \phi_{\text{LCD}}K_{\text{LCD}}[\text{CD}])}{1 + K_{\text{LCD}}[\text{CD}]}, \quad (4)$$

where ϕ is the fluorescence quantum yield for the corresponding chemical species, β is a constant proportional to the intensity of the excitation light and the molar extinction coefficient of **C1-APB**, and $[\text{L}]_t$ is the total concentration of **C1-APB**. The fluorescence intensity changes at 377 nm are shown in the inset of Fig. 4 as a function of CD concentration. The observed results were well fitted with Eq. (4) (solid line), and the binding constant (K_{LCD}) of **C1-APB** with β -CD was calculated from the theoretical curve to be $820 \pm 60 \text{ M}^{-1}$. The binding constant was also calculated using the Benesi-Hildebrand method,²² and both results were in good agreement. This strongly supports 1:1 inclusion complex formation of **C1-APB** with β -CD.

Selectivity of **C1-APB**/ β -CD for saccharides

The precise values of the conditional binding constants (K_{LS}) in the presence of 5 mM β -CD can be obtained from the saccharide titration analysis by using the $\text{p}K_{\text{a}}$ value in the absence of saccharide.¹⁶ Thereby, the effect of the concentration of seven kinds of saccharides was examined at pH 7.1 (Fig. 5). The fluorescence intensity of the **C1-APB**/ β -CD complex increases upon the addition of saccharides. The binding constant at a given pH is readily determined by nonlinear curve-fitting analysis of the following equation giving fluorescence intensity (I) as a function of saccharide concentration ($[\text{S}]$):

$$I = \frac{\beta[\text{L}]_t \left(\phi_{\text{HL}} + \phi_{\text{L}} \frac{K_{\text{a}}}{[\text{H}^+]} (1 + K_{\text{LS}}[\text{S}]) \right)}{1 + \frac{K_{\text{a}}}{[\text{H}^+]} (1 + K_{\text{LS}}[\text{S}])}. \quad (5)$$

As shown in Fig. 5, the observed fluorescence changes against the saccharide concentration are well fitted by Eq. (5) (solid lines). In the order whose conditional K_{LS} value is high among monosaccharides, D-fructose ($4039 \pm 69 \text{ M}^{-1}$) > D-ribose ($1083 \pm 26 \text{ M}^{-1}$) > L-arabinose ($474 \pm 11 \text{ M}^{-1}$) > D-galactose ($318 \pm 3 \text{ M}^{-1}$) > D-glucose ($114 \pm 2 \text{ M}^{-1}$). These results follow the well-known selectivity order of phenylboronic acid towards saccharides.^{2,4} The conditional binding constants for maltose and maltotriose were also calculated as 81 ± 2 and $135 \pm 5 \text{ M}^{-1}$,

Scheme 3 Equilibria of 2:2 and 1:1 inclusion complex of **C1-APB** with γ -CD.

Fig. 5 Effect of the saccharide concentration on the fluorescent response of **C1-APB**/ β -CD at pH 7.1. $[\text{C1-APB}] = 1.0 \times 10^{-6}$ M in 2% DMSO-98% water (v/v) containing 5.0 mM β -CD, pH at 25°C adjusted to 7.1 with 0.01 M phosphate buffer, $I = 0.1$ M with NaCl, $\lambda_{\text{ex}} = 328$ nm. (1) D-Fructose, (2) D-ribose, (3) L-arabinose, (4) D-galactose, (5) maltotriose, (6) D-glucose, (7) maltose.

Fig. 6 Spectra of **C1-APB** with 15 mM γ -CD without saccharide at (1) pH 7.1 and (2) pH 10.6. Inset: effect of the γ -CD concentration on I_{465}/I_{377} at pH 7.1 ($\blacktriangledown$) and pH 10.6 ($\blacksquare$). $[\text{C1-APB}] = 1.0 \times 10^{-6}$ M in 2% DMSO-98% water (v/v), pH at 25°C adjusted to 7.1 with 0.01 M phosphate buffer and to 10.6 with 0.01 M sodium carbonate, $I = 0.1$ M with NaCl, $\lambda_{\text{ex}} = 328$ nm.

respectively. The conditional binding constants of the **C1-APB**/ β -CD complex for D-fructose were obtained in two ways: from $\Delta pK_{\text{a}}^{\text{app}}$ (3800 M^{-1} in Table 1) and from the titration curve of D-fructose (4039 M^{-1}). The difference between these results is within the experimental error.

Ratiometric response

The fluorescence spectra of the **C1-APB**/ γ -CD complex display a slight dimer emission at longer wavelengths (Fig. 2b), but those of **C1-APB** alone and of the other **C1-APB**/CD complexes do not. It has been reported that pyrenes form a 2:2 inclusion complex with γ -CD as a barrel-type complex (a head-to-head complex with secondary hydroxyl sides facing each other) and show dimer emission.²³ A similar 2:2 inclusion complex of **C1-APB** with γ -CD is expected (Scheme 3). The possibility of a 2:1 inclusion complex formation should not be excluded, though. If the formation of boronate ester anions in a 2:2 (or a 2:1) inclusion complex causes the electrostatic repulsion and forms a 1:1 inclusion complex, the ratio of dimer (I_{465})/monomer emission (I_{377}) would be used for the ratiometric sensing of saccharides.²⁴ The effect of the γ -CD concentration on the fluorescence response of the **C1-APB**/ γ -CD complex was therefore examined under basic and neutral conditions (Inset in Fig. 6). As expected, strong dimer emission was found to appear for the **C1-APB**/ γ -CD complex when the γ -CD concentration was increased, and the highest dimer/monomer ratio was obtained with 15 mM γ -CD at neutral pH. The spectra of the **C1-APB**/ γ -CD inclusion complex formed with 15 mM γ -CD under neutral and basic conditions are shown in Fig. 6. It is evident that the high dimer emission was seen only under the neutral condition. The monomer emission, in contrast, is much higher than dimer emission at any concentrations of γ -CD at a

Fig. 7 Spectral changes of **C1-APB**/ γ -CD upon the addition of D-fructose. Inset: changes in I_{465}/I_{377} of **C1-APB**/ γ -CD as a function of saccharide concentration at pH 7.1. D-Fructose ($\bullet$), D-galactose ($\blacklozenge$), and D-glucose ($\blacktriangle$). $[\text{C1-APB}] = 1.0 \times 10^{-6}$ M in 2% DMSO-98% water (v/v) containing 15.0 mM γ -CD, pH at 25°C adjusted to 7.1 by 0.01 M phosphate buffer, $I = 0.1$ M with NaCl, $\lambda_{\text{ex}} = 328$ nm.

basic pH, indicating inhibition of the 2:2 (or 2:1) complex formation due to electrostatic repulsion of the two boronate anions.

Accordingly, the **C1-APB**/ γ -CD complex formed with 15 mM γ -CD was used at neutral pH for the ratiometric sensing of saccharides. Figure 7 shows the ratiometric fluorescence spectral changes upon the addition of D-fructose. The corresponding changes upon the addition of D-glucose and

D-galactose were also measured, and the I_{465}/I_{377} results are shown in the inset of Fig. 7. The selectivity for D-fructose is evident. Although the results of selectivity were the same as those of the **C1-APB**/ β -CD complex, the ratiometric analysis is more useful than analysis by single monomer emission, because it compensates for any minor changes of the sample concentration and fluctuations in the lamp irradiation intensity. At present, however, the response mechanism includes the transfer of the **C1-APB**/ γ -CD complex from a 2:2 complex or a 2:1 complex (dimer) to a 1:1 complex (monomer), and PET mechanism, making a quantitative analysis difficult. To overcome this problem, the development of a non-PET type of pyrene probe is actively underway in our laboratory.

Conclusions

In this study, the inclusion complexes of a fluorescent phenylboronic acid (**C1-APB**) and four kinds of cyclodextrins (CDs) were compared by examining their pH profiles. Of these four CDs, β -CD was found to be the best host for **C1-APB**. The fluorescence quantum yield was greatly enhanced by the formation of a **C1-APB**/ β -CD inclusion complex. The pH profiles of the **C1-APB**/ β -CD complex exhibited increased fluorescence upon the addition of D-fructose under neutral conditions. The **C1-APB**/ β -CD complex also exhibited increased fluorescence when various other saccharides were added, and the conditional binding constant was highest for D-fructose. The results are consistent with the known binding selectivity of phenylboronic acid. An examination of the β -CD concentration effect revealed the formation of a 1:1 inclusion complex of **C1-APB** and β -CD. The ratiometric approach was also evaluated for the **C1-APB**/ γ -CD complex. High changes in the dimer emission response were obtained for the **C1-APB**/ γ -CD complex formed with 15 mM γ -CD. Ratiometric fluorescence spectral changes were obtained upon the addition of saccharides, and changes in the intensity ratio of dimer and monomer emission revealed the selectivity for D-fructose. The selectivity results were the same as those of the **C1-APB**/ β -CD complex. This work demonstrates that different response systems for saccharide recognition are available by combining a simple probe (**C1-APB**) with various CDs.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Japan Society of Promotion of Science (18350043 and 19550130) and the Toray Scientific Foundation.

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