Pseudorotaxane-type fluorescent receptor exhibiting unique response to saccharides†

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Pseudorotaxane formed by reacting β-cyclodextrin bearing a phenylboronic acid residue with 1-heptyl-4-(4′-dimethylaminostyryl)pyridinium functioned as a novel fluorescent saccharide receptor having unique responses.

Recent developments in supramolecular chemistry have enabled us to create novel receptors (hosts) for various guest compounds.[1] Whereas excellent receptors have been synthesized with the supramolecular strategy to bind and detect guest compounds in organic solution, the design and construction of receptors that are effective in aqueous solution remain a challenging task.[2] Artificial receptors having rigid structures generally exhibit high affinity to target guest compounds, whereas those having flexible structures are advantageous in signal transduction. Thus, the control of the rigidity–flexibility balance is critical to the design of new receptors possessing excellent binding and signal transduction properties. Rotaxanes[3] may be good scaffolds for the receptors because they are fabricated with several components that confer different properties such as molecular recognition and signal transduction. Rotaxanes and pseudorotaxanes have been studied as molecular machines; however, little attention has been paid to their use as chemical sensors.[4] In this study, we examined a novel chemical sensing system that involves the formation of pseudorotaxane by reacting β-cyclodextrin (β-CD)[5] bearing a phenylboronic acid residue (**1**) with 1-heptyl-4-(4′-dimethylaminostyryl)pyridinium (**C7SP**; Chart 1). The phenylboronic acid residue of **1** is known to be a receptor for saccharides,[6,7] whereas the methyl analogue of **C7SP** is known to show fluorescence depending on the environment.[8] The heptyl and styryl groups make **C7SP** a suitable ditopic guest for β-CD derivatives, thereby leading to the formation of pseudo[3]rotaxane with **1**.[9] The binding of saccharide to **1** is expected to change both stability and conformation, which in turn, should change the fluorescence properties of the pseudorotaxane. The results are noteworthy: the supramolecular pseudorotaxane-type complexes of **C7SP** with **1** respond to D-glucose rather than D-fructose with increased fluorescence.

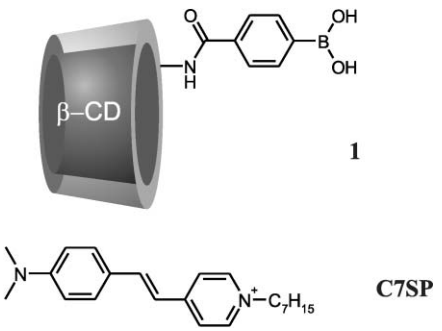

Chart 1

Compound **1** was synthesized by the DCC coupling reaction of amino-β-CD with 4-carboxyphenylboronic acid.‡ The solubility of **1** in water was remarkable. More than 20 mg of **1** dissolves in 0.5 mL of water, resulting in the solubility of >30 mM.

The formation of pseudorotaxane by reacting **1** with **C7SP** was confirmed by fluorescence and ^{1}H NMR measurements. Fig. 1 shows the fluorescence spectra of **C7SP**. **C7SP** alone showed weak fluorescence in water, whereas the presence of **1** enhanced the fluorescence remarkably by more than 100-fold. This notable fluorescence enhancement was due partly to the phenylboronic acid residue, as confirmed by the fact that β-CD alone enhanced the fluorescence of **C7SP** by approximately 10-fold, which is ten times smaller than that induced by **1**. The fluorescence intensity changes induced by **1** were analyzed with the least squares

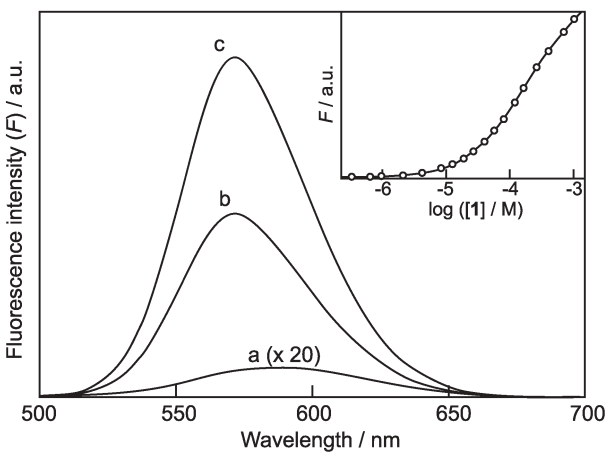

Fig. 1 Fluorescence spectra of **C7SP** (a) alone (30 μM), (b) in the presence of **1** (0.50 mM), and (c) in the presence of **1** (0.50 mM) and D-glc (30 mM) in phosphate buffer (I = 0.07 M, pH 7.2). Inset shows the results of binding analysis considering 1 : 1 and 1 : 2 complexation. The solid line is the best-fit curve with K_1 = 16200 M^{-1} and K_2 = 1760 M^{-1}.

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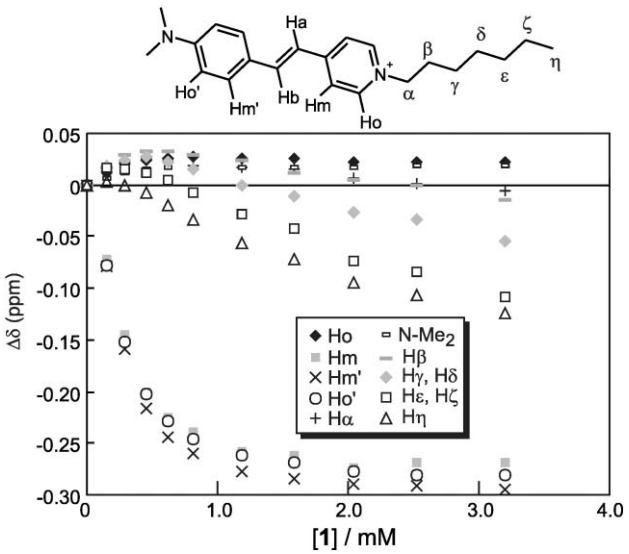

Fig. 2 Chemical shift changes of **C7SP** (0.38 mM) upon the addition of **1** in D_2O buffered by phosphates (I = 0.07 M, pH(D) 7.2). Negative and positive $\Delta\delta$ values correspond to high- and low-field shifts, respectively. Ha and Hb signals were severely broadened to disappear at 0.2 mM **1**.

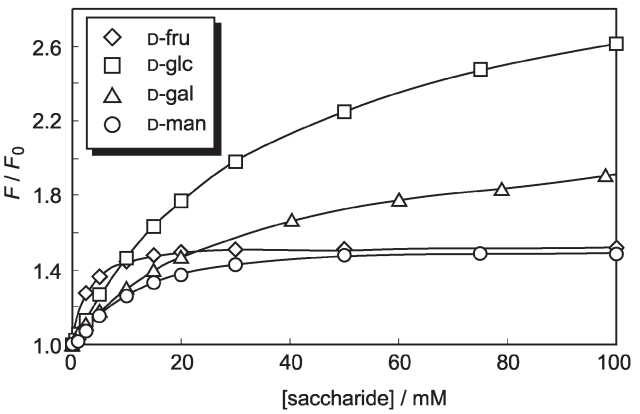

Fig. 3 Fluorescence intensity changes (572 nm) of **C7SP** (30 μM)–**1** (0.1 mM) system induced by D-fru, D-glc, D-gal, and D-man. F/F_0 denotes the fluorescence intensity at a given saccharide concentration (F) normalized to that without saccharide (F_0).

regression method on a 1 : 2 binding model because **C7SP** is a ditopic guest, and the results are shown in the inset of Fig. 1: An excellent fit of the theoretical binding curve to the experimental data was obtained, leading to the association constants for the first and second steps (K_1 and K_2) of 16200 ± 1500 and 1760 ± 190 M^{-1}, respectively. This analysis simultaneously gave the fluorescence intensities of the 1 : 1 and 1 : 2 complexes relative to that of **C7SP** alone of 128 and 241, respectively.†

The formation of the 1 : 2 complex, and hence pseudo[3]rotaxane, was confirmed from ^{1}H NMR titration results. Fig. 2 shows changes in the chemical shifts of **C7SP** upon the addition of **1**. The aromatic proton signals (Hm, Ho′, Hm′) were monotonically shifted to the high-field region and reached constant levels at [**1**] > 2 mM. In contrast, the alkyl proton signals were transiently shifted to the low-field region followed by a high-field shift as the concentration of **1** was increased. These shift patterns do not occur in a simple 1 : 1 binding model. Thus, we conclude that **C7SP** and **1** form a 1 : 2 complex together with a 1 : 1 complex. The observed high-field shifts are due to the ring current effect of the phenylboronic acid residue of **1**, indicating that both the aromatic and alkyl groups interact with the phenylboronic acid residue in the 1 : 2 complex. The marked fluorescence enhancement induced by **1** may be due to the restriction of the molecular motion of **C7SP** by the π–π interaction between the aromatic groups of **C7SP** and **1**.†

Since **1** is a phenylboronic acid derivative, it is expected to bind saccharides. The saccharide binding ability of **1** was determined from the apparent pK_a values obtained in the presence of saccharides. The pK_a value of **1** alone is 7.63 ± 0.01, as determined from the pH-dependent UV-vis absorption spectral variations. This pK_a value was decreased when saccharides (30 mM) were added, because the formation of cyclic boronate ester promoted the acid dissociation of water bound to the boronic acid. The magnitudes of pK_a decrease were 1.91, 0.49, 0.87, and 0.70 for D-fructose (D-fru), D-glucose (D-glc), D-galactose (D-gal), and D-mannose (D-man), respectively. Using the known equation[7] (ΔpK_a = log (K_s [**S**] + 1)) where [**S**] denotes saccharide concentration, the binding constants of **1** with saccharides (K_s) were determined to be 2700 ± 140 (D-fru), 69.1 ± 6.6 (D-glc), 213 ± 36 (D-gal), and 134 ± 38 M^{-1} (D-man). The saccharide selectivity of **1** follows the order D-fru ≫ D-gal > D-man > D-glc, and agrees with the saccharide selectivity of phenylboronic acid derivatives. The high solubility in water and the pK_a value of **1**, together with this saccharide binding ability, demonstrate that **1**, in combination with **C7SP**, is a promising fluorescent receptor for saccharides in neutral aqueous solution.

The ability of the **C7SP**–**1** complex to act as a fluorescent saccharide receptor was noteworthy, as revealed by the further enhancement of **C7SP** fluorescence by the addition of saccharides. As seen in curve c of Fig. 1, the fluorescence of **C7SP** in the presence of **1** was further intensified in the presence of D-glc. This indicates that the **C7SP**–**1** complex shows favorable fluorescence enhancement upon the addition of saccharides. The fluorescence responses of the **C7SP**–**1** system to saccharides are displayed in Fig. 3. The data were measured at 30 μM **C7SP** and 0.1 mM **1**. Interestingly, although D-fru shows the highest binding ability for **1**, the fluorescence intensity changes induced by D-fru are limited. D-Fru induced steep fluorescence enhancement at low concentrations (<5 mM), but was unable to intensify the fluorescence further at high concentrations. By contrast, D-glc gradually enhanced the fluorescence of **C7SP** as its concentration was increased, and the fluorescence of **C7SP** did not reach a plateau even at the D-glc concentration of 100 mM. A similar gradual increase in fluorescence intensity was observed for D-gal, although the magnitude of the increase was less pronounced. D-Man was the least effective in inducing a fluorescence response. Above the saccharide concentration of 10 mM, the highest fluorescence response was obtained when D-glc was used, which showed the lowest binding ability for **1**, followed by D-gal.

One possible explanation for the fluorescence enhancement induced by saccharides is the negative charge generated on the phenylboronic acid residue of **1**. We found that the complexation of **C7SP** with **1** was dependent on pH; both K_1 and K_2 values were larger at pH 9.6 (K_1 = 23100 M^{-1} and K_2 = 2320 M^{-1}) than at pH 5.6 (K_1 = 5100 M^{-1} and K_2 = 350 M^{-1}). The pK_a value of **1** (7.63) indicates that **1** bears a negative charge at pH 9.6 and this negative charge stabilizes the **C7SP**–**1** complex. In addition, the

Table 1 Conditional binding constants (K_{1app}, K_{2app}) and fluorescence enhancement factors (R_1, R_2) of **1** with **C7SP** in the presence of 30 mM saccharide[a]

Saccharide	K_{1app}/M^{-1}	R_1[b]	K_{2app}/M^{-1}	R_2[b]
none (K_1 and K_2)	16200	128	1760	241
D-fru	10100	276	981	355
D-glc	22700	248	2750	477
D-gal	4720	421	265	489
D-man	18800	213	2030	382

[a] Errors were estimated to be ±15% except for D-man, the errors of which were estimated to be ±30%. [b] R_1 and R_2 are defined as $F_{\mathbf{C7SP}-\mathbf{1}}/F_{\mathbf{C7SP}}$ and $F_{\mathbf{C7SP}-2\cdot\mathbf{1}}/F_{\mathbf{C7SP}}$, where F denotes the fluorescence intensity at 572 nm.

fluorescence enhancement was more pronounced at pH 9.6 than at pH 5.6 or 7.2.† The apparent pK_a values became smaller when the phenylboronic acid residue of **1** formed a cyclic boronate ester with the saccharides. This pK_a shift indicates that **1** bears a negative charge in the presence of saccharides. Therefore, the presence of the saccharides is partly related to the fluorescence enhancement.

However, the above discussion fails to explain why D-glc, which shows the lowest binding ability for **1**, induced the largest fluorescence enhancement. To clarify this point, the equilibrium (binding) constants must be determined in solutions containing **C7SP**, **1**, and saccharides. Unfortunately, since the **C7SP**–**1**–saccharide system shows complicated equilibrium states, it is difficult to determine the binding constants for each equilibrium state. Alternatively, we determined the conditional binding constants (K_{1app} and K_{2app}) of **1** with **C7SP** in the presence of 30 mM saccharide by measuring **1**-induced fluorescence variations. The K_{1app} and K_{2app} values listed in Table 1 indicate that 1 : 1 complexation, together with 1 : 2 complexation, was promoted by D-glc. In other words, **1** becomes a better host for **C7SP** when it forms a cyclic boronate ester with D-glc. Moreover, the fluorescence of **C7SP** was intensified when **1** formed cyclic boronate esters with the saccharides, as proven by the large R_1 and R_2 values in the presence of the saccharides. Since the negative charge on **1** results in the fluorescence enhancement, the obtained large R_1 and R_2 values in the presence of saccharides are reasonable. Among the saccharides investigated, D-glc has the most favorable properties (increased K_{1app} and K_{2app} values as well as large R_1 and R_2 values) for the enhancement of the fluorescence of **C7SP** when it was bound by **1**. Taking the fluorescence intensity changes (Fig. 3) and the binding and fluorescence parameters (Table 1) into consideration, the second binding step in the complexation of **C7SP** with **1** (resulting in K_{2app} and R_2) may be more important for the large fluorescence enhancement induced by high saccharide concentrations. It is noted that some phenylboronic acid derivatives bind D-glc at the ratio of 2 : 1 (phenylboronic acid residue : saccharide). The possibility that the two molecules of **1** in the **C7SP**–**1** complex cooperatively bind D-glc should not be excluded.[6]

In early studies on saccharide sensors based on phenylboronic acid derivatives, photo-induced electron transfer (PET) was utilized as the signaling mechanism. Recent studies have used more sophisticated signaling mechanisms such as twisted-intramolecular charge transfer (TICT) emission.[6,10] Since the fluorescence signaling mechanism of the **C7SP**–**1** complex is completely different from those of other fluorescent saccharide receptors, our results demonstrate the utility of pseudorotaxanes in the construction of supramolecular saccharide sensors. To further develop supramolecular sacchride sensors based on pseudorotaxanes, we will continue in investigate the detailed mechanisms of the fluorescence enhancements in our laboratory.

In conclusion, we demonstrated herein that pseudorotaxanes formed by reacting **C7SP** (rod) with **1** (bead) are excellent hosts for saccharides and show favorable fluorescence emission in the presence of D-glc. We used a phenylboronic acid derivative and a styrylpyridinium dye as molecular recognition and fluorescent signaling elements, respectively. In a similar manner, CD derivatives bearing other moieties such as crown ether[11] and polyamine[12] can be used as a molecular recognition element, and other fluorescence dyes can be used as a fluorescent signaling element. Therefore, our approach is expected to be useful for the construction of chemical sensing systems for various target compounds by changing the beads and rods of the pseudorotaxanes.

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Notes and references

‡ Selected data: **1**: *m/z* (FAB, matrix thioglycerol (TG)) 1376 ([M + TG − $2H_2O$ + Na]$^+$); Found: C, 44.0; H, 6.1; N, 1.0. $C_{49}H_{76}BNO_{37}\cdot 3H_2O$ requires C, 44.1; H, 6.2; N, 1.1%. **C7SP**: *m/z* (FAB) 323.2472 (M^+, $C_{22}H_{31}N_2$ requires 323.2486); Found: C, 63.1; H, 7.5; N, 6.6. $C_{22}H_{31}BrN_2$ requires C, 65.5; H, 7.8; N, 6.9%. The results of the elemental analyses indicate approximately 96% dye content.

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