# Sugar-Sensitive Supramolecular Structures Based on Phenylboronic Acid-Modified Cyclodextrins

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**Supramolecular structures were developed from phenylboronic acid-modified cyclodextrins (PBA-CyDs). The intermolecular interaction between the PBA moiety and the CyD cavity was proved using two dimensional (2D)-NMR and powder X-ray diffraction techniques. PBA-*α*-CyD formed a head-to-tail supramolecular polymer, whereas PBA-*β*-CyD formed a head-to-head dimer. The supramolecular structures were disintegrated in the presence of sugars owing to the resulting boronate sugar interactions.**

**Key words** cyclodextrin; phenylboronic acid; supramolecular chemistry; sugar recognition

Cyclodextrins (CyDs) are cyclic oligosaccharides composed of six, seven, or eight glucopyranoside units and are named as *α*-, *β*-, or *γ*-CyD, respectively. CyDs consist of a hydrophobic cavity in which hydrophobic guest molecules could be enclosed to form an inclusion complex. When such a guest molecule is covalently attached to the CyD, the guest-modified CyD forms an intermolecular complex resulting in supramolecular assemblies, thus attracting great interest because of their unique structures.[1,2]

Harada *et al.* have reported chemical-responsive supramolecular structures based on guest-modified CyD derivatives.[3–6] The supramolecular structure became disintegrated when an excess amount of another type of guest was added to it. This chemical stimuli-sensitivity could be explained based on a competition between the covalently attached guest moiety and the other type of guest for the CyD cavity.

In this study, a new type of chemical stimuli-sensitive guest-modified CyD was developed based on a structural change of guest moiety, where phenylboronic acid (PBA) was used as the guest molecule. PBA reacts with *cis*-diol functional groups of sugars to form a five- or six-membered ring through an ester bond.[7,8] Consequently, PBA derivatives have been widely investigated as sugar-recognition motifs in chemical probes for sugar analysis[9] and sugar-sensitive insulin release systems.[10–12] If the PBA-modified CyDs (PBA-CyDs) form a supramolecular structure, then the structure will be transformed by a chemical stimulus caused by varying the sugar concentration. This stimuli-responsiveness is derived from the structural change of the guest moiety rather than owing to the competition between the two types of guests for the CyD cavity.[3–6]

First, we confirmed that PBA acts as a guest for CyDs by monitoring the p$K_a$ of PBA, which is a Lewis acid with a p$K_a$ to coordinate with hydroxide ion. The p$K_a$ of PBA was estimated by monitoring the change in absorbance at 267 nm, corresponding to an $n \rightarrow \pi^*$ transition. By using curve fitting analysis, the p$K_a$ of PBA was calculated to be 8.8.[9] In the presence of 100 mM *α*-CyD, the p$K_a$ of PBA increased to 9.1. This result is interpreted by the formation of a host–guest complex between the molecular form of PBA and *α*-CyD.[13] There is an acid-base equilibrium between the molecular form and the ionic form of PBA at a certain pH. The addition of *α*-CyD induces a complexation between *α*-CyD and the molecular form of PBA because it is more hydrophobic than the ionic form. The molecular form of PBA is consumed by the complexation. In order to maintain the acid-base equilibrium, the ionic form of PBA transforms to the molecular form. Accordingly, the total amount of the non-charged form of PBA and included PBA increase, which results in the rise of apparent p$K_a$. The increase in the p$K_a$ also proves that ester bonds are not formed between PBA and *α*-CyD. In the same procedure, the p$K_a$ of PBA was decreased to 7.8 in the presence of 100 mM D-glucose (Glu), which is known to make ester bonds with PBA.

After confirming that the molecular form of PBA acts as a guest for the CyD cavity, the PBA moiety was covalently attached to the CyD through a carboxylic ester linkage by the reaction of one hydroxyl group of CyD with *p*-chlorocarbonyl phenylboronic acid (Chart 1). PBA-*α*-CyD and PBA-*β*-CyD were successfully synthesized, and their solubilities were determined (Table 1). The lower solubility of PBA-*α*-CyD and PBA-*β*-CyD than that of the parent CyDs strongly indicated the intermolecular interactions of PBA-CyDs and the formation of supramolecular structures.[3–5]

In order to confirm the presence of intermolecular interactions in the dissolved state, two dimensional (2D)-NMR techniques were used. The rotating Overhauser enhancement and exchange spectroscopy (ROESY) spectrum of PBA-CyDs showed the nuclear Overhauser effect (NOE) interaction between the protons of the phenyl group and H-3 of the CyD (Fig. S1), indicating that the PBA moiety was shallowly included from the wide secondary hydroxyl group side of CyDs. The diffusion ordered spectroscopy (DOSY) spectrum of PBA-*α*-CyD shows the presence of three species in a $D_2O$ solution (Fig. S2), corresponding to the monomer, dimer, and trimer of PBA-*α*-CyD.[14] The powder X-ray diffraction (PXRD) analyses were carried out to investigate the supramolecular structure of PBA-CyDs in the solid state. The PXRD of PBA-*α*-CyD shows a similar pattern as that of mono-6-azide-6-deoxy-*α*-CyD, which is known to form a helical supramolecular polymer through the head-to-tail intermolecular interactions[15] (Fig. S5). From these results, we propose that PBA-*α*-CyD forms an oligomer through the head-to-tail

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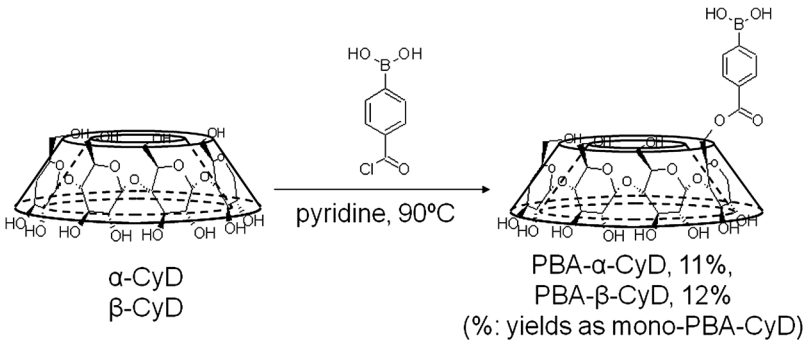
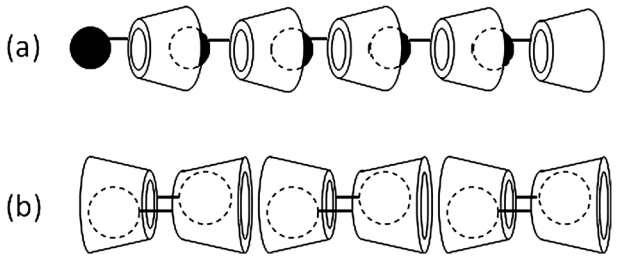



Chart 1. Synthesis of PBA-CyDs

Table 1. Solubility (mM) of CyDs in the Presence and Absence of Sugar (10 mM) at 37°C

| CyD | No sugar | With Glu | With Fru |
|---|---|---|---|
| $\alpha$-CyD | 270 | — | — |
| $\beta$-CyD | 37 | — | — |
| PBA-$\alpha$-CyD | 3.0 | 3.8 | 6.3 |
| PBA-$\beta$-CyD | 0.23 | 0.26 | 1.2 |




(a) Head-to-tail supramolecular polymer based on PBA-$\alpha$-CyD, (b) head-to-head dimer based on PBA-$\beta$-CyD.

Chart 2. Proposed Supramolecular Structures of PBA-CyDs in Solid State

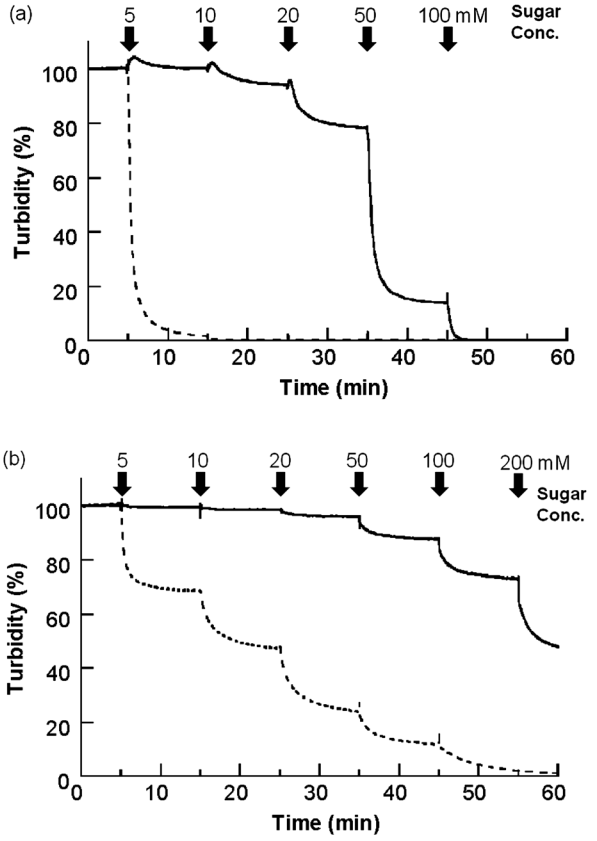



Fig. 1. Sugar-Dependent Turbidity Change of PBA-CyDs in PBS (pH 7.4) at 37°C

(a) PBA-$\alpha$-CyD, (b) PBA-$\beta$-CyD; solid line (Glu), dashed line (Fru).

interactions in the dissolved state, which is demonstrated the results of the ROESY and DOSY spectra. Possibly, more continuous interactions lead to the formation of insoluble supramolecular polymer of PBA$\alpha$-CyD (Chart 2a).

A nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum of PBA-$\beta$-CyD shows the NOE interactions between each of the two kinds of protons of PBA moiety and each of the interior H-3 and H-5 protons of $\beta$-CyD (Fig. S3), indicating that the PBA moiety was deeply included into the $\beta$-CyD cavity. The DOSY spectrum shows the existence of two species (Fig. S4), which corresponds to the monomer and dimer of PBA-$\beta$-CyD. The PXRD patterns of PBA-$\beta$-CyD closely resembled that of the inclusion structures, *i.e.*, a dimer structure formed through a head-to-head interaction (Fig. S6).[16] From these results, we propose that PBA-$\beta$-CyD forms a dimer through the head-to-head interactions in the dissolved state, which is demonstrated the results of the NOESY and DOSY spectra, and the dimer forms the channel crystalline structures thorough hydrogen bonding of the secondary hydroxyl groups (C2b).

Next, we investigated the sugar response of PBA-CyDs. A solid state of PBA-CyD (PBA-$\alpha$-CyD: 11 $\mu$mol, PBA-$\beta$-CyD: 3.9 $\mu$mol) was suspended in the Dulbecco's phosphate buffered saline (PBS) solution (pH 7.4, 2.0 mL) at 37°C and the resulting turbidity was studied by monitoring the absorbance at 700 nm wavelength. After the turbidity became constant, a stock sugar solution (1.00 M) was added to the suspended solution. The turbidity decreased with increasing concentration of sugar (Fig. 1), indicating that the solid form of the supramolecular structure was disintegrated and dissolved in water owing to the effect of sugar. To achieve 100 mM sugar solution, 222 $\mu$L of the sugar stock solution was used. When 222 $\mu$L of buffer solution without sugar was added to the PBA-$\alpha$-CyD suspended solution, the turbidity was decreased

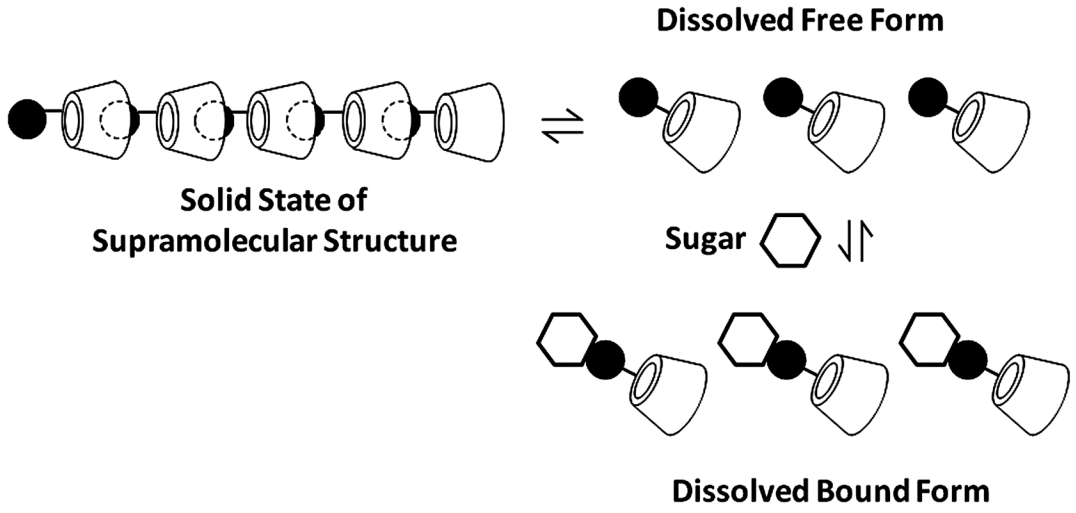



Chart 3. Proposed Mechanism of Sugar-Induced Disintegration of Supramolecular Polymer Structure of PBA-$\alpha$-CyD

Table 2. Binding Constants ($M^{-1}$) of PBA-CyDs (60 $\mu$M) in PBS (pH 7.4) at 37°C

| CyD | Glu | Fru |
|---|---|---|
| PBA-$\alpha$-CyD | 22 | 740 |
| PBA-$\beta$-CyD | 29 | 720 |

to 86%. These results show that the turbidity decrease in the presence of sugar was not only due to the simple dilution effect but also the effect of sugar. The effect of D-fructose (Fru) was found to be larger than that of Glu. This difference in response demonstrated that the PBA moiety acts as a sugar-recognition motif because PBA is known to strongly react with Fru than with Glu.[7–9] The solubilities of PBA-CyDs in the presence of sugar were measured, and the increase in the solubility of PBA-CyDs with increasing sugar concentration was confirmed (Table 1).

To better understand the mechanism of the sugar response of PBA-CyDs, the binding constants between PBA-CyDs and sugars were measured. The change in the UV-visible absorption spectra was applied to a curve fitting analysis.[9] PBA-$\alpha$-CyD and PBA-$\beta$-CyD show similar binding constants to sugar (Table 2). In order to estimate the strength of intermolecular interactions of PBA-CyDs, binding constants between PBA-CyD and parent CyD were determined by circular dichroic spectra (Fig. S7). The binding constant of the combination between PBA-$\beta$-CyD and $\beta$-CyD was calculated to be 258 $M^{-1}$, which is higher than that of the combination of PBA-$\alpha$-CyD and $\alpha$-CyD (58 $M^{-1}$). Hence, the difference in the sugar response between PBA-$\alpha$-CyD and PBA-$\beta$-CyD were derived from the strength of the host-guest interactions between the PBA moiety and CyD cavity. It is reasonable to assume that the quite low solubility of PBA-$\beta$-CyD is because of the head-to-head bidentate interactions.

A sugar-response mechanism is proposed as shown in Chart 3. When PBA-CyDs are suspended in an aqueous solution, the solid state of the supramolecular structure and the dissolved form are in equilibrium with each other. The dissolved form of PBA-CyDs is responsible for the binding to sugar because the inserted PBA moiety in the supramolecular structures cannot interact with sugar. The sugar-bound PBA-CyD becomes too bulky to be included in the cavity of other CyDs. Thus, the consumption of the dissolved free form of PBA-CyDs induces the further disintegration of the supramolecular structures to compensate for the lack of free PBA-CyDs. The mechanism is applicable to both PBA-$\alpha$-CyD and PBA-$\beta$-CyD structures.

In conclusion, we have demonstrated the formation of supramolecular structures of PBA-CyDs. PBA-$\alpha$-CyD formed the supramolecular polymer structures through the head-to-tail interactions, whereas PBA-$\beta$-CyD formed a dimer through the head-to-head interactions. The resulting supramolecular structures showed a low solubility in water; however, the addition of sugar induced an increase in their solubility. This is a new concept for chemical-responsive materials based on the use of guest-modified CyDs, and it has potential applications in drug delivery systems.

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