doi:10.1246/cl.2010.1075

Stimuli-responsive Inclusion Complex of Boronic Acid-modified Amylose for Colorimetric Detection of Polyhydroxy Compounds

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A new stimuli-responsive supramolecular system has been established based on the inclusion complex of boronic acid-modified amylose with iodine. When iodine was mixed with the boronic acid-modified amylose in water, it encapsulated iodine within the helical cavity of amylose. The encapsulated iodine was dissociated from the amylose cavity by the addition of polyhydroxy compounds, and the process could be conveniently monitored by distinct color changes.

Amylose, a (1→4) α-D-glucan, is the main constituent of starch that is contained in plants such as grain, rice, and potatoes. It has a unique tendency to form inclusion complexes with hydrophobic guest molecules by encapsulating within a helical cavity.[1] When amylose is mixed with iodine in an aqueous solution, blue or purple color emerges upon encapsulation of iodine by amylose.[2] This phenomenon is known as an "iodo-starch reaction" and has long been utilized for the detection of starch and iodine. Another interesting feature of amylose is that it has hydroxy groups through which various kinds of functional groups can be introduced.[3] Thus, we believe that amylose is a natural polymer with a high potential for the development of novel functional materials having environment compatibility.

Our research group has already succeeded in introducing polymerizable moieties into amylose, and the modified amylose was utilized for developing amylose-based molecularly imprinted polymers.[4] To expand the possibility of amylose as a host matrix for molecular recognition, we have also been trying to introduce a variety of functional groups, and have been examining their molecular recognition characteristics. In this paper, we report a unique behavior of boronic acid-modified amylose. It is well-established that boronic acid can reversibly bind polyhydroxy compounds (polyols) such as saccharides via the boronate–diol interaction.[5] We expected that if a boronic acid moiety was introduced into amylose, the inclusion complex formation ability of amylose could be controlled by adding polyols as an external stimulus.

Introduction of a boronic acid moiety into amylose was conducted by reacting amylose with *p*-bromomethylphenylboronic acid in the presence of sodium methoxide (Figure 1). It is expected that the substitution was mainly taken place at C-2 position, since the hydroxy group at C-2 is most reactive in the presence of strong base.[6] The degree of substitution (DS), which is defined as the average number of boronic acid group per anhydroglucose unit, was determined by measuring ^{1}H NMR spectra of the products (Figure S3 in the Supporting Information[10]). DS value was controlled by changing the reactant ratio of amylose to *p*-bromomethylphenylboronic acid. In the present paper, we used amylose of DS = 0, 0.11, and 0.20.

As the first step, we examined whether boronic acid-modified amylose is capable of encapsulating iodine. An aqueous iodine solution (mixture of I_2 and KI) showed no absorption in the visible region, whereas absorption with a maximum at 570 nm emerged upon addition of boronic acid-modified amylose of DS = 0.20 (Figure S1[10]). This spectral change clearly indicated that boronic acid-modified amylose formed helical inclusion complexes with iodine.

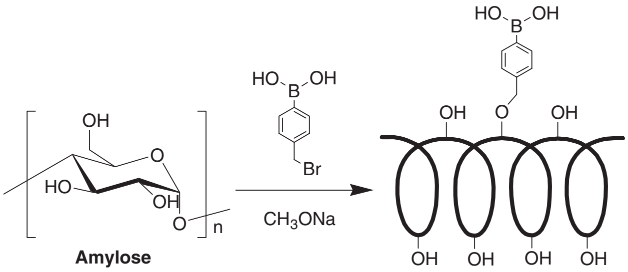

Figure 1. Synthesis of boronic acid-modified amylose.

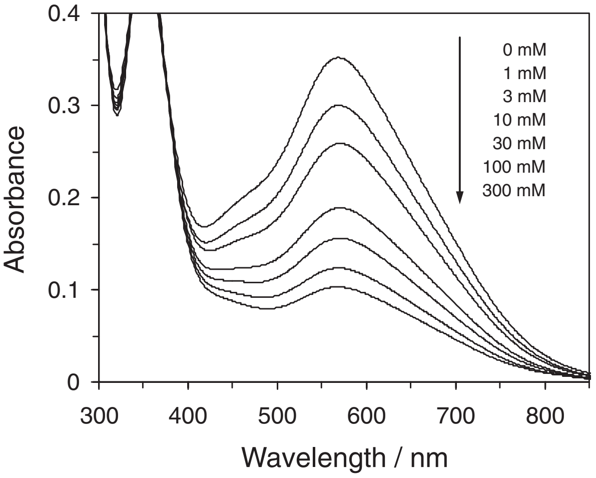

Figure 2. Effect of sorbitol on the absorption spectrum of iodine ($[I_2] = 14\,mg\,L^{-1}$, $[KI] = 140\,mg\,L^{-1}$). Measured in the presence of boronic acid-modified amylose (DS = 0.20, $90\,mg\,L^{-1}$) at pH 7.4 (4 mM phosphate) and 20 °C.

Sorbitol, a sugar alcohol bearing six hydroxy groups, was then added to the aqueous solution containing boronic acid-modified amylose and iodine at pH 7.4. As shown in Figure 2, the absorption band at 570 nm decreased with increasing sorbitol concentration. We observed a sorbitol-dependent color change from bluish-purple to almost colorless (Figure S2[10]). This observation suggests that the addition of sorbitol induced dissociation of iodine from the helical cavity of amylose. To evaluate the responsiveness against other polyols, we conducted studies using xylitol, erythritol, and glycerol bearing five, four,

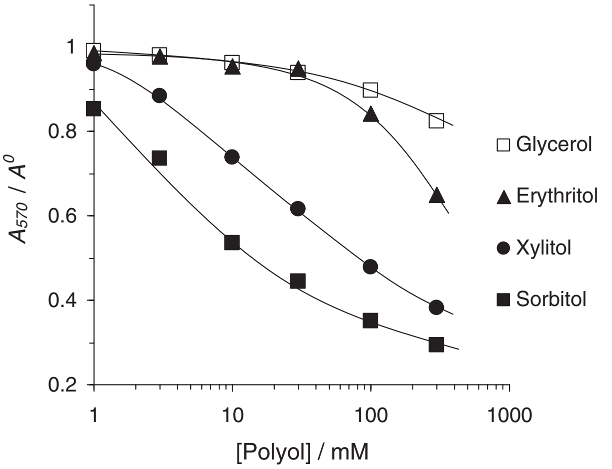

Figure 3. Detection curves against polyols: decrease in absorbance at 570 nm is plotted against polyol concentration. Measurement conditions are the same as those in Figure 2.

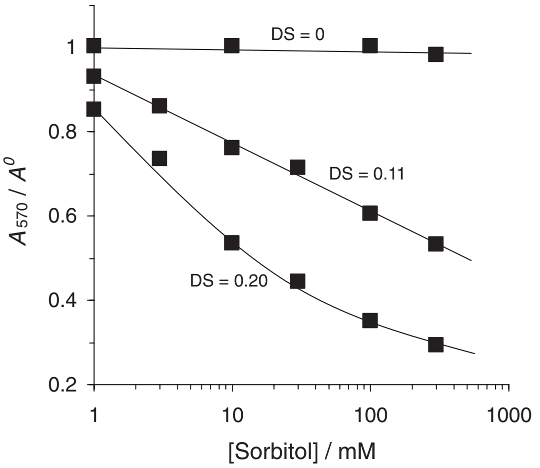

Figure 4. Effect of degree of substitution (DS) on the response against sorbitol. Measurement conditions are the same as those in Figure 2.

and three hydroxy groups, respectively. The results are shown in Figure 3. It was shown that xylitol caused a smaller decrease of absorption at 570 nm compared with sorbitol. The response against erythritol was largely diminished, and glycerol showed only a small response. This response selectivity agreed with the order of binding affinity of boronic acid with polyols, namely stability of phenylboronic acid–polyol complex increases with increasing the number of hydroxy group in polyol.[7] Therefore, it is reasonable to assume that the boronic acid moiety introduced into amylose plays an important role in the occurrence of the polyol-responsive color changes.

To clarify the response mechanism, we evaluated the effect of degree of substitution (DS) on the response against sorbitol. The results are shown in Figure 4. When boronic acid-modified amylose of DS = 0.11 was used, the color change was considerably diminished compared with the case when amylose of DS = 0.20 was used. Furthermore, there was almost no response when unmodified amylose (DS = 0) was used. The observed DS dependency clearly supports the view that the boronic acid moiety is indispensable for the response against polyols.

Table 1. Effect of pH on the response of boronic acid-modified amylose (DS = 0.20) against sorbitol

pH[a]		A_{570}/A^0		
	[Sorbitol] =	1 mM	10 mM	300 mM
7.4		0.85	0.54	0.29
6.2		0.97	0.92	0.74
4.7		1.03	1.02	1.06

[a] 4 mM phosphate for pH 7.4 and 6.2, 4 mM acetate for pH 4.7.

This view is also supported by the observation that the response is highly dependent on pH of the solution. Table 1 shows the sorbitol-dependent changes in absorption at 570 nm at each pH conditions. It is clearly seen from the table that the response against sorbitol diminished with changing pH toward acidic conditions. When pH was changed from 7.4 to 6.2, the response against sorbitol was drastically suppressed. Virtually no response was observed when pH 4.7. It is well known that the complexation between boronic acids and diol compounds favorably takes place in neutral and alkaline solutions to form anionic species, and the complexation is disfavored in acidic conditions (see equilibrium shown in Scheme S1). The observed pH dependency in the present system is in agreement with the general tendency of boronic acid–diol complexation.

We also evaluated the effect of ionic strength on the responsiveness by means of changing phosphate buffer concentration. It is obvious from Table 2 that the response against sorbitol was drastically suppressed at higher phosphate concentrations. When phosphate concentration was 40 mM, the sorbitol-dependent change in absorption at 570 nm was greatly reduced. It scarcely responded against sorbitol when phosphate concentration was raised to 400 mM. These observations mean that some sort of electrostatic interaction played an important role in the response against polyols. It is reported that iodine is dissolved in water as anionic species such as tri-iodide (I_3^-) and penta-iodide (I_5^-).[8] In the absence of polyols, the boronic acid moiety is uncharged, thus no electrostatic interaction exist between the boronic acid-modified amylose and iodine. On the other hand, in the presence of polyols, the boronic acid moiety is negatively charged due to complexation with polyols, so the existence of electrostatic repulsion between the boronic acid-modified amylose and iodine would be expected. Therefore, we speculate that the polyol-responsive dissociation of iodine was triggered by the complexation of the boronic acid moiety with polyols and the encapsulated iodine was expelled from the helical cavity due to electrostatic repulsion with the negatively charged boronate groups. Another possible mechanism for the response is that the conformation of amylose was changed by the intramolecular electrostatic repulsion between boronate groups. In order to assess the conformational change in amylose, we measured specific rotation of boronic acid-modified amylose. It was found that the value[9] was not dependent on pH in the range where ionization of boronic acid group takes place (pH 8–10). This result implies the absence of conformational change in amylose. Probably, average distance between boronate groups was too far for inducing conformational change.

In summary, a new stimuli-responsive supramolecular system has been established based on the inclusion complex

Table 2. Effect of phosphate concentration on the response of boronic acid-modified amylose (DS = 0.20) against sorbitol at pH 7.4

[Phosphate]		A_{570}/A^0		
	[Sorbitol] =	1 mM	10 mM	300 mM
4 mM		0.85	0.54	0.29
40 mM		0.92	0.77	0.63
400 mM		1.06	1.07	1.03

formation of boronic acid-modified amylose with iodine. The encapsulated iodine was dissociated from the helical cavity of amylose by the addition of polyhydroxy compounds, and the process was conveniently monitored by distinct color changes. The response selectivity against polyols was governed by the complexation affinity of the boronic acid moiety. It was clarified that the origin of the responsiveness is the electrostatic interaction between boronic acid-modified amylose and iodine. By utilizing the present system, one can construct a sensing method for sorbitol that has a detection limit of as low as 1 mM. We are now trying to confirm if the present strategy is applicable not only for iodine but also for other guest molecules such as fluorescent dyes. We are also trying to prepare thin films that consist of the boronic acid-modified amylose. We believe that the present system will lead to the development of novel sensors, drag delivery systems, functional surfaces, etc, with practical applicability, in the near future.

This work was supported through JGC-S Scholarship Foundation.

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9 $[\alpha]_D$ +147°.

10 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.