

## Remarkable Color Changes Induced by Saccharide-responsive Sequential Release of Anionic Dyes

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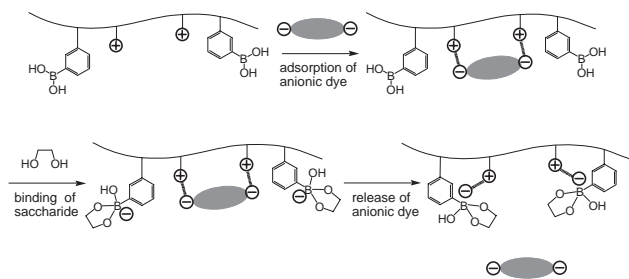
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A novel saccharide sensory system showing remarkable color changes has been developed. A copolymer containing boronic acid and cationic units was synthesized on which anionic dyes were adsorbed. By immersing the dye-adsorbed copolymer in aqueous saccharide solutions, blue and yellow dyes were sequentially released from the copolymer: with increasing saccharide concentration, the solution changed its color from colorless to blue, then changed into green.

Research concerning saccharide sensor has attracted much attention because saccharides have crucial role in the metabolic pathway of living organisms. In recent years, the number of diabetic patient has been significantly growing not only in developed but also in developing countries. It is, therefore, highly desired to realize a versatile glucose monitoring system for a diagnosis and treatment of patients. So far, most of glucose sensors are based on enzymatic reactions which are catalyzed by glucose oxidase or glucose hydrogenase. Even though the enzyme-based systems has high selectivity toward glucose, there are significant drawbacks: those are, 1) low durability and reproducibility due to changes in the enzymatic activity, 2) disturbance by co-existing chemicals, 3) complicated measurement procedure, etc.<sup>1</sup> Since 1990's, boronic acid-based sensory systems for saccharides have been extensively studied by the group of Shinkai and others.<sup>2</sup> These researches have proven that boronic acid is a powerful tool for the molecular recognition of saccharides in aqueous systems. However, a boronic acid-based sensor that shows easily observable color change has not been reported yet. For the development of practical saccharide sensors, it is necessary to introduce novel methodology.

We here report our preliminary result on the endeavor for realizing saccharide sensory systems showing remarkable color changes. The present system is based on the saccharide-responsive release of anionic dyes from a copolymer containing boronic acid and cationic units. The strategy is illustrated in Scheme 1.

We first synthesized the copolymer by a radical copolymerization of boronic acid monomer,<sup>3</sup> cation monomer,<sup>4</sup> acryl-

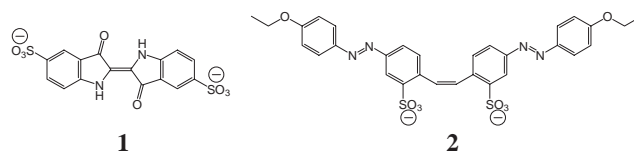


**Scheme 1.** Saccharide-responsive release of anionic dye from copolymer containing boronic acid and cationic units.

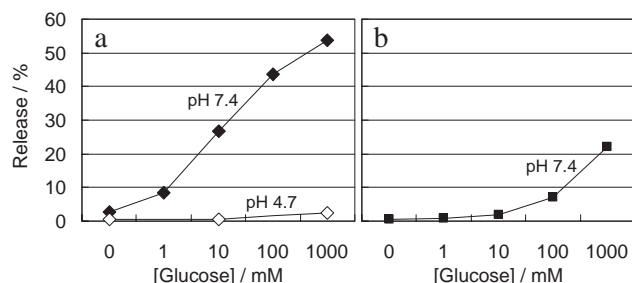
amide, and crosslinker that was initiated by an azo-bis compound. The molar ratio between boronic acid monomer and cation monomer was 1:1. The chemical structures of the monomers and the detailed synthetic condition are shown in Supporting Information.<sup>8</sup>

For the adsorption of anionic dyes, the copolymer was equilibrated with aqueous solutions containing divalent anionic dyes (shown in Figure 1), i.e. indigo carmine (**1**, blue dye) or chrisophenine (**2**, yellow dye). The amount of adsorbed **1** decreased with raising solution pH (see Table S2). The copolymer contains dimethylamino units so that the cationic charge density is expected to be pH-dependent due to protonation and deprotonation. Therefore, the significant decrease in dye adsorption at higher pH condition indicates that an electrostatic interaction between copolymer and dye is the main driving force for the dye adsorption.

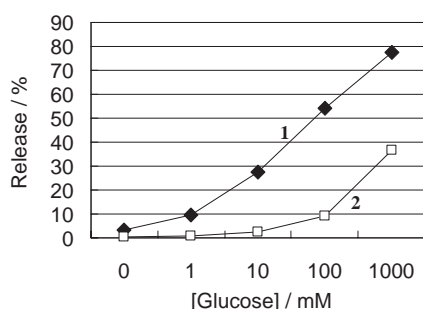
After the dye was adsorbed at pH 7.4, the copolymer was immersed in aqueous saccharide solutions. The supernatant solutions gradually changed its color from colorless to blue for **1**-adsorbed copolymer<sup>4</sup> and yellow for **2**-adsorbed copolymer. Photographs of these solutions are shown in Figure S1. After immersing for 3 h, the amounts of released dyes were determined by measuring absorption spectra of the supernatant solutions. As shown in Figure 2a, **1** (blue dye) was gradually released with increasing glucose concentration at pH 7.4, whereas it was scarcely released at pH 4.7. It is known that the complexation between boronic acid and diol takes place in neutral and alkaline solutions to form anionic species, and the complexation is disfavored in acidic conditions (see equilibria shown in Scheme S1). Therefore, the suppression of dye-release at pH 4.7 indicates that the complexation between glucose and boronic acid unit in the copolymer is responsible for the release of dye.<sup>5</sup> By binding with glucose, the boronic acid units become negatively charged to form boronate groups that electrostatically interact with cationic units. Accordingly anionic dyes lose their binding site in the copolymer. We also prepared a copolymer containing trimethylammonium units, however, it showed only small responsiveness (data not shown). Compared to dimethylammonium unit, trimethylammonium unit would be less favorable for the electrostatic interaction with boronate group because a steric hindrance imposed by the additional methyl group makes it get closer to an anionic site. By comparing Figures 2a and 2b, it is



**Figure 1.** Chemical structure of anionic dyes; **1**: indigo carmine (blue), **2**: chrisophenine (yellow).



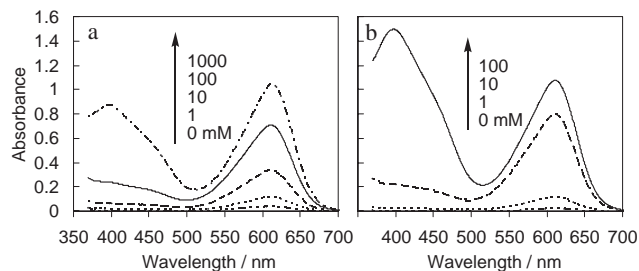
**Figure 2.** Glucose-responsive release of dyes: (a) copolymer loaded with **1**, (b) copolymer loaded with **2**.



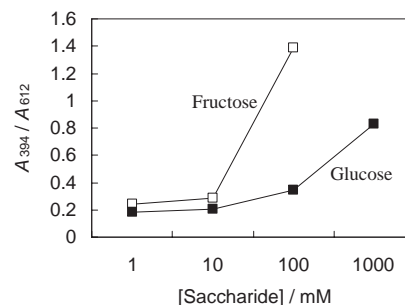
**Figure 3.** Amount of released dye from copolymer loaded with **1** and **2** in response to glucose concentration at pH 7.4.

clear that percentages of release for **2** are smaller than those for **1**. For example, when the copolymers are immersed in 10 mM glucose solutions at pH 7.4, nearly 30% of **1** is released while release for **2** is only 2%. We speculate that **2** is more tightly bound by the copolymer according to an additional hydrophobic interaction since **2** is more hydrophobic than **1**.<sup>6</sup> Then, it occurred to us that the difference in the releasing profiles for the blue and yellow dyes can be utilized for “one-by-one” sequential release of dyes: with increasing glucose concentration, **1** would be preferentially released then **2** should be released afterward.

In order to verify the above-mentioned hypothesis, we simultaneously adsorbed **1** and **2** onto the copolymer, then examined a releasing profile against glucose. As shown in Figure 3, the amount of released **1** gradually increased with increasing glucose concentration, while release of **2** was suppressed when glucose concentration was low. The release of **2** gradually increased at higher glucose concentration. As the result of this sequential release of dyes, the supernatant solution firstly became blue then turned to be green (for photographs, see Figure S3). The corresponding spectral change is shown in Figure 4a. We also tested response against fructose. It is clearly seen from Figure 4 and Figure S3 that the color change for fructose occurs at lower concentration compared with the case for glucose. When saccharide concentration is 100 mM, the color is already turned into green for fructose solution, whereas the color is still remained as blue for glucose solution (refer to solid lines in Figure 4). The higher sensitivity against fructose agrees with the tendency of boronic acids to form more stable complex with fructose than with glucose.<sup>7</sup> The response profile is compared in Figure 5 by plotting absorbance ratio at 394 and 612 nm. When saccharide concentrations are low, absorbance ratio ( $A_{394}/A_{612}$ ) is small so that the solution is blue. With increasing saccharide



**Figure 4.** Absorption spectra of (a) glucose and (b) fructose solutions containing copolymer loaded with **1** and **2**.



**Figure 5.** Plot of absorbance ratio at 394 and 612 nm against saccharide concentration.

concentration, absorbance ratio becomes larger leading to the color change of the solution to be green.

In conclusion, we have developed a novel saccharide-responsive sensory system by adsorbing blue and yellow anionic dyes onto a copolymer containing boronic acid and cationic units. We could demonstrate that the dyes are sequentially released with increasing saccharide concentrations, which lead to the distinct color changes in the solution. We are now trying to enhance the sensitivity against glucose, and also processing the copolymer into a thin film for the development of a non-enzymatic glucose sensing chip.

#### References and Notes

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- 4 The time course of the dye release is plotted in Figure S2.
- 5 As a control experiment, we synthesized a copolymer not containing boronic acid unit. It was confirmed that the copolymer does not show saccharide-responsive release of anionic dyes.
- 6 When the dyes were equilibrated in water/ethyl acetate binary system, **2** was extracted into organic phase, whereas **1** was remained in water phase.
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- 8 Supporting Information is also available electronically on the CSJ-Journal Web site, <http://www.csj.jp/journals/chem-lett/index.html>.