

## Photo-isomerization of a phenylboronic acid bearing an azo group to switch sugar recognition

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Phenylboronic acid in water exists as an equilibrium mixture of the nonionic trigonal boronic form and the ionic tetrahedral monoboronate form. It is known that the tetrahedral-formed phenylboronate can form significantly stable covalent bonds with polyol compounds including glucose in aqueous solution. Therefore, the binding/dissociation equilibrium between boronic acids and sugar molecules can be controlled with pH change. However, there has been no report on controlling this equilibrium by the external stimulus such as light and magnetic field. Thus, we tried to create a novel photo-switching compound which can control the binding/dissociation with sugars by photoirradiation as an external stimulus at a constant pH condition. In our strategy, an azobenzene moiety which gives the geometrical change of photoisomerization (*cis-trans* isomerization) is employed as a photo-switching moiety, which leads the change of the acidity of boronic acid with the electronic and steric effects. Therefore, we synthesized *o*-, *m*-, *p*- azophenylboronic acids (APB) which have phenylboronic acid and azo groups. It was confirmed that the isomerization of synthesized compounds was certainly induced, which was measured by UV-vis and <sup>1</sup>H/<sup>13</sup>C NMR. Furthermore, it was confirmed using <sup>11</sup>B NMR measurement that the binding/dissociation ratio to fructose was changed before and after photoirradiation. In this study, it was proved that newly synthesized boronic acid derivatives can control the binding/dissociation to sugar by photo-stimulation at a constant pH condition. In near future, this new sugar-recognition system is expected to be utilized in medical field as a biosensor and so on.

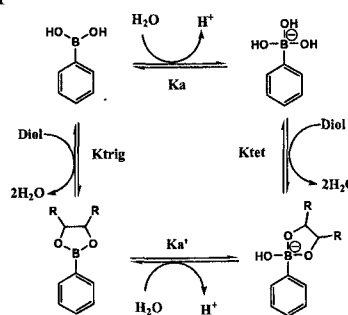
key words: boronic acid, polyol, photoswitching, azobenzene, sugar recognition

### 1. Introduction

Phenylboronic acid in water exists as an equilibrium mixture of the nonionic trigonal boronic form and the ionic tetrahedral monoboronate form (Scheme 1).<sup>1-4</sup> It is known that the tetrahedral-formed phenylboronate can form significantly stable covalent bonds with polyol compounds including glucose in aqueous solution<sup>5</sup>. On the other hand, complex of trigonal boronic acid is easy to hydrolyze, and can't exist stably in aqueous solution. Therefore, complexation equilibrium between boronic acid and polyol compounds could be controlled by pH change. When boronic acids are used in biosystem, especially in human body, the binding/dissociation reaction with polyol compounds should be conducted at a constant pH under the physiological condition. However, there has been no report controlling this equilibrium by light or magnetic field as an external stimuli. Therefore, we study for the purpose of creating compounds whose binding/dissociation reaction with polyol compounds is

reversibly controllable at a certain pH value using photo-stimulation.

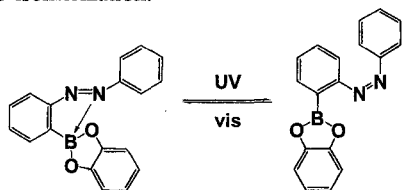
Scheme 1



In order to control the binding/dissociation reaction, we chose an azobenzene moiety that could be isomerized reversibly by photo-irradiation. Azobenzene derivatives usually exist as a *trans* form. It

isomerizes to a *cis* form by UV irradiation, and it is got back to the *trans* form by irradiation with visible light. Additionally, many physical properties of azobenzene (absorbance, dipole moment, and refractive index, etc) change between *trans* and *cis* forms. Therefore, the compounds containing both azobenzene and boronic acid can change such physical properties by photo-irradiation. A change of dipole moment may result in the change of electron density around boron atom. Consequently, for this effect, it can be controlled the binding/dissociation reaction of boronic acid with diol compounds by photo-irradiation. On the contrary, it is known that boronic acid which has amino group adjacent to boron atom is promoted the boron-nitrogen (B-N) interaction caused by the increasing acidity of boronic acid. Using this B-N interaction system, Wulff made the boronic acid derivatives binding to diol compounds at neutral pH<sup>6</sup>. Shinkai et al. developed an artificial carbohydrate receptor using various boronic acid derivatives<sup>2,7-9</sup>. Recently, it have been studied about switching coordinate bond using stereo-isomerization (Scheme 2)<sup>10</sup>. In this study, using a change of B-N interaction and/or electron density on boron atom by isomerization of azobenzene, we tried to control the binding/dissociation reaction between boronic acid and diol compounds, especially sugars, reversibly. Azophenylboronic acids containing both boronic acid and azo groups were synthesized. Binding properties of these boronic acids with sugar were evaluated before and after photoirradiation.

Scheme 2 Switching of B-N interaction by stereo-isomerization.



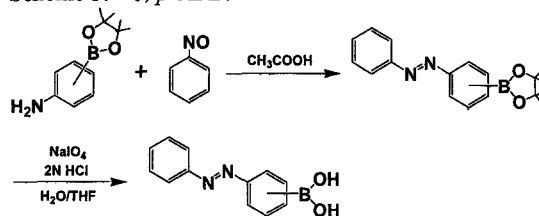
## 2. Experimental Section

**Preparation of *o*, *m*, *p*-azophenylboronic acid (*o*, *m*, *p*-APB).** All azophenylboronic acids (APB) were prepared by coupling reaction between aminophenylboronic acid or its pinacol esters and nitrosobenzene. Pinacol ester of *o*-aminophenylboronic acid **1** (0.456 mmol; ALDRICH) and nitrosobenzene **2** (0.912 mmol; TCI) were added to acetic acid (7.3 ml) and stirred for 3 h at room temperature. The reaction mixture was extracted with ethyl acetate, and the organic layer was concentrated under a reduced pressure. The residue was purified with column chromatography yielding orange solid of **3** (0.16 mmol). Then, **3** and sodium periodate (0.50 mmol; Wako) were added to water/tetrahydrofuran = 1/4 solution (1.32 ml), and the mixture was stirred for 30 min. After stirring, 2N hydrochloric acid was added to the mixture dropwise, and the mixture was stirred for overnight at room temperature. The reaction mixture was filtered with celite, and filtrate was extracted with ethyl acetate, concentrated under a reduced pressure. The residue was further purified with silica gel chromatography giving

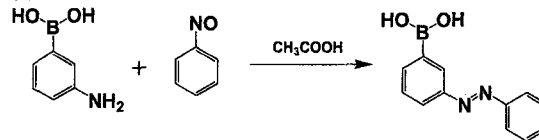
50 mg (30%) of an orange powder (Scheme 3).

*p*-APB was prepared with a reaction process similar to *o*-APB. The total yield was 41 %. *m*-APB was prepared in similar manner. Crude compound was purified with column chromatography. The yield was 30 % (Scheme 4). The structure of APBs were confirmed with a <sup>1</sup>H NMR (300 MHz JEOL AL-300) spectrometer in DMSO-d<sub>6</sub> [*o*-APB: <sup>1</sup>H NMR δ = 7.88-7.85 (m, 3H, arom.H), 7.77 (s, 2H, OH), 7.64-7.47 (m, 6H, arom.H); Uv-vis spectrum in methanol, λ<sub>max</sub> in *trans* form = 320 nm and 430 nm. *m*-APB: <sup>1</sup>H NMR δ = 8.33 (s, 1H, arom.H), 8.27 (s, 2H, OH), 7.98-7.88 (m, 4H, arom.H), 7.61-7.57 (m, 4H, arom.H); Uv-vis spectrum in methanol, λ<sub>max</sub> in *trans* form = 320 nm and 430 nm. *p*-APB: <sup>1</sup>H NMR δ = 8.24 (s, 2H, OH), 8.00 (d, 2H, arom.H), 7.91 (q, 2H, arom.H), 7.85 (d, 2H, arom.H), 7.62-7.59 (m, 3H, arom.H)]; Uv-vis spectrum in methanol, λ<sub>max</sub> in *trans* form = 320 nm and 430 nm.. All other chemicals were of reagent grade and were used as received. Water was purified with an ion-exchange column, followed by distillation.

Scheme 3. *o*, *p*-APB.



Scheme 4. *m*-APB.



**UV-vis spectroscopy.** First, UV-vis spectra before photoirradiation were measured using a spectrophotometer (Jasco, J-725). Next, UV-vis spectra after photoirradiation (365 nm, 3.3 mW/cm<sup>2</sup>, 1 h) were measured. The concentration was 0.30 mM, and the solvent used the methanol (Wako optical grade).

**<sup>1</sup>H · <sup>13</sup>C NMR spectroscopy.** <sup>1</sup>H and <sup>13</sup>C NMR spectra were measured before and after UV-irradiation (365 nm, 3.3 mW/cm<sup>2</sup>, 1 h).

**Acid-base titration.** Acid-base titration were conducted in order to measure acidity of APBs in the presence of sugar before and after irradiation. First, APBs (15 mg) were dissolved in methanol (20 ml), then diluted with 0.1M aqueous NaCl solution (10 ml), and then 2 molar eq. of fructose was dissolved in APBs solution. Titration was performed with 0.1 M sodium hydroxide controlling with automated titrator. Next, APBs (15 mg) were dissolved in methanol (1 ml), after UV irradiation (365 nm, 3.3 mW/cm<sup>2</sup>, 3 h), resulting solution was diluted with methanol (19 ml) and 0.1 M NaCl aq. Titration was performed with same method..

**Measurement of binding/dissociation ratio with sugar.** <sup>11</sup>B NMR spectra were measured in order to compare the binding ability of APBs before and after

UV-irradiation. Boron trifluoride diethyl ether complex (Wako Pure Chemical Industries Co., Ltd) was used as a reference. In measurement before UV-irradiation, APBs were dissolved in methanol- $d_4$  : buffer solution = 8 : 2, and added to fructose as sugar molecules. Then  $^{11}\text{B}$  NMR spectra were measured. In measurement after photoirradiation,  $^{11}\text{B}$  NMR spectra were measured after UV-irradiation (365 nm, 3.3 mW/cm $^2$ , 1 h). Buffer solutions were used phosphate buffer solution (pH 7.82) and  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  buffer solution (pH 9.10).

### 3. RESULTS AND DISCUSSION

#### 3.1 Isomerization

UV-vis spectra were measured before and after irradiation to confirm that synthesized samples were isomerized. UV-vis spectra of APBs before and after photoirradiation were shown in Figure 1. For all samples, it was considered that the absorption of  $n\text{-}\pi^*$  transition at 440 nm increased and the absorption of

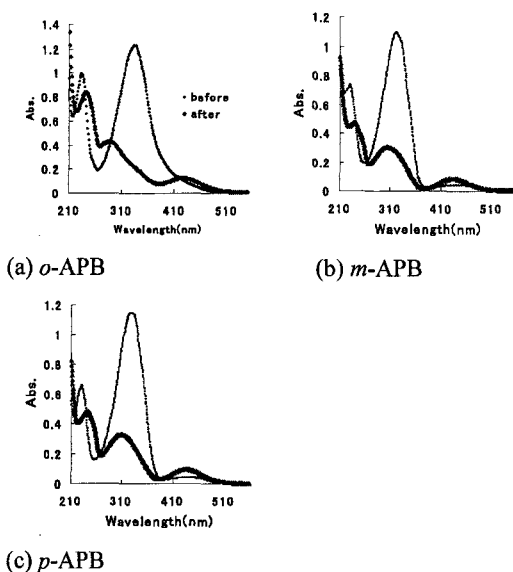


Figure 1. UV-vis spectral change of APBs before and after UV-irradiation.

$\pi\text{-}\pi^*$  transition at 320 nm decreased after UV irradiation for 2 h. These results were similar to the UV-vis spectral change of the azobenzene. Therefore, these results indicate that the APBs were isomerized by UV-irradiation. Irradiation ( $\lambda_{\text{max}} = 431$  nm) of APBs in *cis* form for 1 h caused recovery of APBs in *trans* form in 98 % yield.

From  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, for all samples, it was confirmed that all peaks of APBs shifted after UV-irradiation. (Fig. 2, Table 1). From these results, it

is expected that the electron density of APBs changed *via* photoisomerization. Therefore, it was expected that the boron chemical shift is also changed by

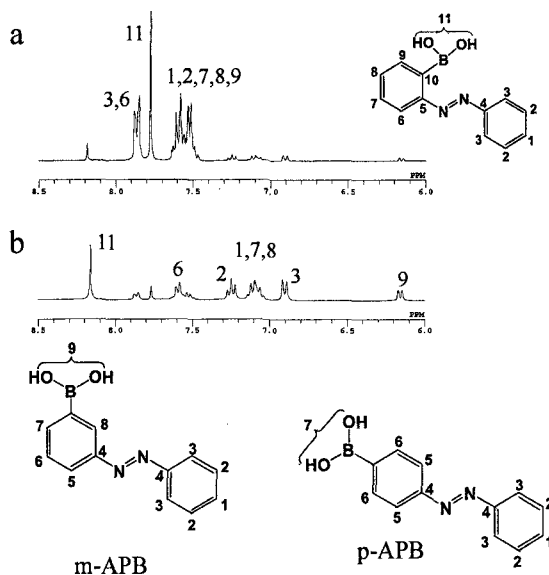


Figure 2  $^1\text{H}$ NMR spectra of *o*-APB. a : before UV irradiation, b : after UV irradiation. *m*- and *p*-APBs are also shown for the reference.

UV-irradiation. If electron density on boron atom changed, it is suggested that acidity of boronic acid change.

#### 3.2 Acid-base titration

Since the change of electron density on boron atom by UV-irradiation was suggested, acidity of APBs were measured before and after UV-irradiation using acid-base titration. In *o*-APB, a titration curve largely changed after UV-exposure, when compared with *m*-*p*-APB (Figure 3). It was considered that this change was due to the switching of B-N interaction (lone pair at nitrogen atom of azo group adjacent to boronic acids) by isomerization. The *trans* form is considered to have strong B-N interaction, but the *cis* form had weak B-N interaction or didn't have B-N interaction by stereo-isomerization. Therefore, acidity of boronic acid largely changed before and after UV-irradiation. In *m*- and *p*-APB, change in acidity of boronic acids was smaller than that in *o*-APB. It was considered that there was no B-N interaction for these compounds, and the changes were caused only by the effect of intramolecular electron density change by isomerization. It was revealed that acidity of boronic acids increased after photoirradiation in all APBs. It was indicated that UV-irradiation increased the binding ability of APBs to fructose, especially for *o*-APB.

Table 1 Chemical shift of APBs from  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra before and after UV irradiation  $\Delta\delta(\text{ppm})$

	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪
<i>o</i> -	0.46	0.3	0.96	—	—	0.27	0.46	0.46	1.39	—	0.39
	5.9	0.8	2.4	2.9	4.2	11.7	2.2	1.1	16.5	2.8	—
<i>m</i> -	0.32	0.78	0.66	—	0.66	0.78	0.66	0.78	0.18	—	—
	2.8	1.7	2.5	1.6	2.9	2.8	4.4	1.8	—	—	—
<i>p</i> -	0.44	0.32	1.08	—	1.07	0.32	0.18	—	—	—	—
	4.3	0.6	3.9	2	1.4	0.6	—	—	—	—	—

### 3.3 Change of binding/dissociation ratio with sugar before and after photoirradiation

Using the result from acid-base titration,  $^{11}\text{B}$  NMR spectra were measured at pH 9.10 (Fig.4). It was expected that the extent of change in binding/dissociation ratio is biggest before and after photoirradiation for *o*-APB.

The two peaks derived from the trigonal boronic acid (28 ppm) and tetrahedral-formed phenylboronate (9 ppm) where the peak at 9 ppm suggest the complex with fructose with the ratio of 1 : 0.59 before UV irradiation. On the other hand, after UV irradiation, the ratio became 1 : 13.73. The binding/dissociation ratio increased significantly after UV irradiation as expected. It was suggested that boronic acid became to be almost ionized and bind to fructose after UV-irradiation. It was considered that newly synthesized *o*-APBs could control the binding/dissociation ratio with sugar by photo-irradiation at pH = 9.10.

In *m*- and *p*-APB,  $^{11}\text{B}$  NMR spectra were also measured at pH 7.82, using the result from acid-base titration (Table 2).

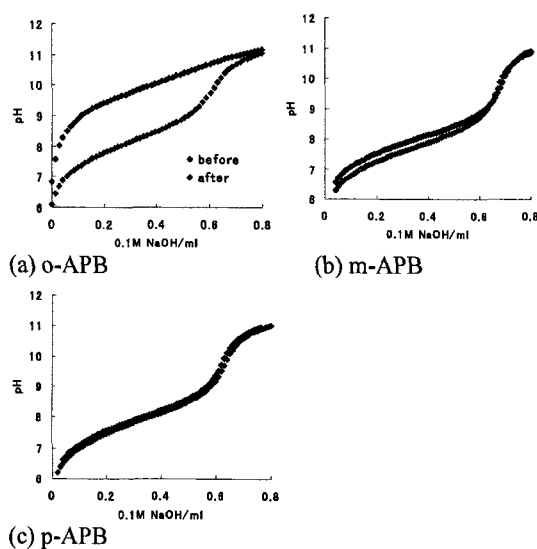


Figure 3 Acid-base titration of APBs before and after photoirradiation

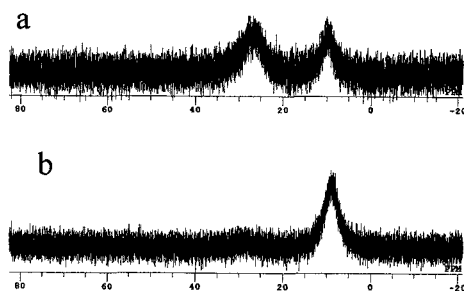


Figure 4 Binding/dissociation ratio of *o*-APB to fructose before and after UV-irradiation. a : before irradiation, b : after irradiation.

Table 2  $^{11}\text{B}$  NMR chemical shift of APBs

APB	before	afetr	pH
<i>o</i> -	0.59	13.73	9.10
<i>m</i> -	0.4	0.45	7.82
<i>p</i> -	0.38	0.43	

Integral value of tetrahedral-formed phenylboronate when that of trigonal boronic acid is 1.00.

In *m*-APB, the binding/dissociation ratio changed from 0.40 to 0.45 after UV-irradiation. In *p*-APB, the ratio changed from 0.38 to 0.43 after UV-irradiation. In *m*- and *p*-APB, the binding/dissociation ratio increased a little. Changing amounts were smaller than in *o*-APB, but it was confirmed that the binding/dissociation ratio with sugar were changed using intramolecular density change by isomerization. Comparing the effect of B-N interaction with intramolecular electron density, it was considered that the B-N interaction affected mainly the change of acidity of APBs. Therefore, it was confirmed that *o*-APB including the B-N interaction was the best compound in three APBs as a photoswitching molecule.

### 4. Conclusion

New photo-switchable molecules containing phenylboronic acid and azo groups, which bind to diol compounds including glucose, were synthesized. It was confirmed that the azo compound showed *trans*→*cis* photoisomerization, inducing change of intramolecular electron density by UV-irradiation. Acid-base titration experiments suggested that acidity of APBs was changed by isomerization. The binding/dissociation ratio of APBs with fructose was confirmed to change before and after UV-irradiation from  $^{11}\text{B}$  NMR spectra. *o*-APB including B-N interaction was the best molecule among three APBs as a photo-switching molecule. It was proved that the new system could control the binding/dissociation ratio at a constant pH by photo-stimulation. This system is expected to have high utility as a biosensor in the biomedical field.

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