Supporting Information

Selective Glucose Recognition by Boronic Acid Azoprobe/γ-Cyclodextrin Complexes in Water

Chie Shimpuku, Rimiko Ozawa, Akira Sasaki, Fuyuki Sato, Takeshi Hashimoto, Akiyo Yamauchi, Iwao Suzuki, and Takashi Hayashita*

*Department of Material and Life Sciences, Faculty of Science and Technology, Sophia University, Chiyoda, Tokyo 102–8554, Japan
b Department of Biochemistry, Nara Medical University, Nara 634–8521, Japan.
c Graduate School of Pharmaceutical Science, Tohoku University, Sendai 980–8578, Japan

1. Apparatus

UV-vis absorption spectra were recorded on a Hitachi U-3000 spectrophotometer with a 1.0-cm quartz cell. The absorption spectra of each sample were obtained by subtraction of the spectra of γ-CD solution containing 1% DMSO-99% water (v/v) in the absence of probe. ICD spectra were obtained on a JASCO J-820 spectrophotometer with a 1.0-cm quartz cell. 1H NMR spectra were obtained using a JEOL-GX-500MHz.

2. Syntheses of BA-Azo and B-Azo

Synthesis of BA-Azo. Scheme 4.1 shows the synthesis procedure for BA-Azo. First, 1 was synthesized by diazo-coupling: 5.01 g (36.5 mmol) p-aminobenzoic acid was dissolved in 100 cm³ of water, and 10 cm³ of concentrated hydrochloric acid was added, and then the solution was stirred and cooled in the water bath with ice. 2.53 g (36.7 mmol) of sodium nitrite in 15 cm³ of water was slowly added into the cooled solution, and the mixture was stirred for an hour. The other mixture of 3.50 g (37.2 mmol) of phenol in 10 cm³ of water and 10 cm³ of 5 M sodium hydroxide was also
prepared and cooled. The latter solution was slowly added into the former solution and stirred for an hour (pH 2). The resultant precipitate was filtered. The filtrate was cooled to obtain the additional precipitate and the mixture of 3.5 g (37.2 mmol) of phenol in 10 cm$^3$ of water and 10 cm$^3$ of 5 M sodium hydroxide was added again. This mixture was stirred for 30 minutes (pH 9). The obtained precipitate was filtered. The two precipitates were recrystallized together from methanol with water. The yield of 1 was 77.3% (6.84 g, 28.2 mmol). The structure of 1 was confirmed by $^1$H NMR and EI-MS. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ (ppm) 6.93 (d, 2H, $H_a$, $J_{ab} = 9.0$), 7.87 (d, 2H, $H_b$, $J_{ab} = 9.0$), 7.89 (d, 2H, $H_c$, $J_{cd} = 9.0$), 8.16 (d, 2H, $H_d$, $J_{cd} = 9.0$). EI-MS, $m/z$ 242.

Second, 1 (0.500 g, 2.06 mmol) and 0.502 g (2.29 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) aniline were dissolved in 100 cm$^3$ of methanol and the mixture was stirred at room temperature. The 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl- morpholinium chloride (DMT-MM, 0.724 g, 2.30 mmol) in 25 cm$^3$ of methanol was added into the mixture and this solution was stirred for 6 hours at r.t. The reaction mixture was evaporated in vacuo. The residue was dissolved in chloroform, and washed with 1% acetic acid aqueous solution. The organic solution was dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was dissolved in chloroform and purified by SEC (size exclusion chromatography). The yield was 59.3% (0.543 g, 1.22 mmol) as orange crystal. The structure of the product was confirmed as pinacolyl ester of BA-Azo by $^1$H NMR, negative-ion ESI-MS, and elemental analyses. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ (ppm) 1.30 (s, 12H, $H_h$), 6.97 (d, 2H, $H_a$, $J_{ab} = 8.5$), 7.68 (d, 2H, $H_g$, $J_{fg} = 8.5$), 7.86 (m, 4H, $H_{hg}$), 7.93 (d, 2H, $H_c$, $J_{cd} = 8.5$), 8.14 (d, 2H, $H_d$, $J_{cd} = 8.5$), 10.49 (s, 1H, $H_e$). Negative-ion ESI-MS, $m/z$ 442. Anal. Calculated for C$_{25}$H$_{26}$BN$_3$O$_4$·0.7H$_2$O, C, 65.86; H, 6.06; N, 9.22, Found, C, 66.12; H, 6.17; N, 8.90.

The pinacol ester of BA-Azo was used for the measurement in this study, because the pinacol protection group is known to be easily deprotected in water.
**Scheme S1** Synthesis procedure for BA-Azo.

**Synthesis of B-Azo.** Scheme S2 shows the synthesis procedure for B-Azo. A mixture of 1.00 g (4.56 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, 30 cm³ of water, and 0.86 cm³ of concentrated hydrochloric acid were stirred and cooled in a water bath with ice. Sodium nitrite (0.315 g, 4.57 mmol) in 10 cm³ of water was slowly added into the cooled solution, and the mixture was stirred for an hour. The other mixture of 0.429 g (4.56 mmol) of phenol in 10 cm³ of water and 1 cm³ of 5 M sodium hydroxide was also prepared and cooled. The latter solution was
slowly added into the former solution and the combined mixture was stirred for an hour (pH 4). The red-brown precipitate was filtered. The filtrate was cooled to obtain additional precipitate, and the mixture of 0.425 g (4.52 mmol) of phenol in 10 cm$^3$ of water and 0.5 cm$^3$ of 5 M sodium hydroxide were added again, and this mixture was stirred for 30 minutes (pH 8). The obtained precipitate was filtered. The two precipitates were recrystallized together from methanol with water. The product yield was 36.7% (0.405 g, 1.67 mmol). The structure was confirmed as **B-Azo** by $^1$H NMR, EI-MS, and elemental analyses. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ (ppm) 6.92 (d, 2H, $H_a$, $J_{ab} = 8.5$), 7.82 (m, 6H, $H_{bc,cd}$). EI-MS, $m/z$ 242. Anal. Calculated for C$_{12}$H$_{11}$BN$_2$O$_3$$\cdot$0.14H$_2$O, C, 58.92; H, 4.65; N, 11.45, Found, C, 59.00; H, 4.42; N, 11.59.

**Scheme S2** Synthesis procedure for **B-Azo**
3. ICD and UV-Vis spectral response of B-Azo and BA-Azo

Figure S1 shows the ICD spectra (a and c) and the UV-Vis spectra (b and d) of the B-Azo and the BA-Azo. No significant spectral responses were noted for both azoprobes upon addition of 30 mM D-fructose and D-glucose.

Fig. S1 (a) ICD and (b) UV-Vis spectra of B-Azo, (c) ICD and (d) UV-Vis spectra of BA-Azo in 1% DMSO - 99% water (v/v). [Azoprobe] = 5.0 x 10^{-5} M (a, c), 2.5 x 10^{-5} M (b, d), [saccharide] = 30 mM, \( I = 0.1 \) M with NaCl, pH 10.0 adjusted by Na\(_2\)CO\(_3\)/HCl buffer, 25°C.

4. ICD and UV-Vis spectral response of B-Azo/\(\beta\)-CD and BA-Azo/\(\beta\)-CD complexes

Figure S2 shows the ICD spectra (a and c) and the UV-Vis spectra (b and d) of the B-Azo/\(\beta\)-CD and the BA-Azo/\(\beta\)-CD complexes. Similar to Fig. S1, no significant spectral responses were noted for both azoprobes upon addition of 30 mM D-fructose and D-glucose.
Fig. S2  (a) ICD and (b) UV-Vis spectra of B-Azo/β-CD complex, (c) ICD and (d) UV-Vis spectra of BA-Azo/β-CD complex in 1% DMSO - 99% water (v/v) containing 3.0 mM β-CD. [Azoprobe] = 5.0 x 10^{-5} M (a, c), 2.5 x 10^{-5} M (b, d), [ saccharide] = 30 mM, I = 0.1 M with NaCl, pH 10.0 adjusted by Na₂CO₃/HCl buffer, 25°C.

5. Inclusion complex formation of BA-Azo with γ-CD

To clarify the 2:1 inclusion complex formation of BA-Azo with γ-CD in the presence of D-glucose, the effect of γ-CD concentration on the absorbance from the UV-vis spectra for BA-Azo was examined. With an increase in γ-CD concentration, the absorbance ratio (A₄₃₆/A₄₄₆) for BA-Azo increased in the presence of D-glucose (Fig. 2). When it is assumed that the absorbance change is only induced by the formation of a 2:1 complex (L₂CD) between BA-Azo (L) and γ-CD (CD), the absorbance ratio is expressed by the following equations:

\[
\frac{A_{436}}{A_{446}} = \frac{4 \frac{\varepsilon_{L436}}{\varepsilon_{L446}} + \varepsilon_{(L,CD)436} (1 + \sqrt{1 + 8K[CD][L]})}{4 + \frac{\varepsilon_{(L,CD)446}}{\varepsilon_{L446}} (1 + \sqrt{1 + 8K[CD][L]})}
\]  

(1)
where $[L]_t$ is the total concentration of **BA-Azo**, and $\epsilon_{L436}$ and $\epsilon_{L446}$ are the molar absorptivity for **BA-Azo** at 436 and 466 nm, respectively. Similarly, $\epsilon_{(L,2CD)436}$ and $\epsilon_{(L,2CD)436}$ are the molar absorptivity for the 2:1 complex at 436 and 466 nm, respectively. The observed results in Fig. 2 were fitted well with eq. 1 (solid line), and the binding constant ($K$) for the 2:1 inclusion complex of **BA-Azo** with $\gamma$-CD was calculated as $(2.7 \pm 0.4) \times 10^7 \text{M}^{-2}$. The binding constants without saccharide and D-fructose were not calculated due to their low spectral responses.

### 6. Binding constant of **BA-Azo**/$\gamma$-CD complex for D-glucose.

The apparent 2:1 binding constant ($K_{21}$) of **BA-Azo** with D-glucose in the presence of 3.0 mM $\gamma$-CD was determined from the changes in UV-vis spectra. With an increase in D-glucose concentration, the absorbance ratio ($A_{436}/A_{446}$) for **BA-Azo** increased (Fig. S3). On the assumption that the absorbance change is only induced by the formation of a 2:1 complex (L$_2$CD) between **BA-Azo** (L) and D-glucose (S), the absorbance ratio can be expressed by the following equations:

\[
A_{436} = \frac{4 \frac{\varepsilon_{L436}}{\varepsilon_{L446}} + \frac{\varepsilon_{(L,S)436}}{\varepsilon_{L446}} (-1 + \sqrt{1 + 8K_{21}}} \frac{[S][L]}{[L]_t} \) 

\]

(3)

\]

\[
A_{446} = \frac{4 + \frac{\varepsilon_{(L,S)446}}{\varepsilon_{L446}} (-1 + \sqrt{1 + 8K_{21}}} \frac{[S][L]}{[L]_t} \) 

\]

(4)

\]

where $[L]_t$ is the total concentration of **BA-Azo**, and $\epsilon_{L436}$ and $\epsilon_{L446}$ are the molar absorptivity for **BA-Azo** at 436 and 466 nm, respectively. Similarly, $\epsilon_{(L,S)436}$ and $\epsilon_{(L,S)436}$ are the molar absorptivity for the 2:1 complex at 436 and 466 nm respectively.
From eq. (3), the binding constant for the 2:1 inclusion complex of BA-Azo with 
D-glucose was calculated as $(3.0 \pm 0.5) \times 10^7$ M$^{-2}$.

![Absorbance ratio (A<sub>436</sub>/A<sub>446</sub>) of BA-Azo](image)

**Fig. S3** Absorbance ratio ($A_{436}/A_{446}$) of BA-Azo as a function of saccharide 
concentration of D-glucose and D-fructose in the presence of 3.0 mM γ-CD. 
$[\text{BA-Azo}] = 2.5 \times 10^{-5}$ M in 1% DMSO–99% water (v/v), 
$I = 0.1$ M with NaCl, pH 10.0 adjusted by Na$_2$CO$_3$/HCl buffer, 25°C.

7. **1H NMR analysis**

$^1$H NMR analysis was carried out for BA-Azo and BA-Azo/γ-CD complex. 
To enhance BA-Azo concentration at 2.0 mM for $^1$H NMR analysis, 80% D$_2$O-20% 
DMSO-d$_6$ (v/v) was used as solvent. Although the response efficiency was 
significantly reduced in 80% D$_2$O-20% DMSO-d$_6$ (v/v) solution, the additional peaks of 
phenyl protons appeared at the upfield region of each peaks for BA-Azo/γ-CD complex 
in the presence of D-glucose. This result supports the dimer formation of BA-Azo 
with D-glucose inside γ-CD.
Fig. S4  $^1$H NMR spectra of BA-Azo and BA-Azo/$\gamma$-CD complex in 80% D$_2$O-20% DMSO-d$_6$ (v/v). [BA-Azo] = 2.0 mM, [saccharide] = 1.0 mM, and [$\gamma$-CD] = 4.0 mM. pD = 11.4 adjusted by Na$_2$CO$_3$ buffer, 25°C.