Selective Glucose Sensing Utilizing Complexation with Fluorescent Boronic Acid on Polycation

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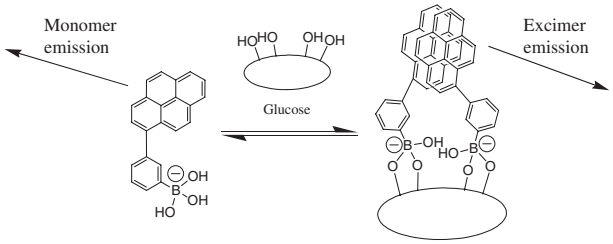
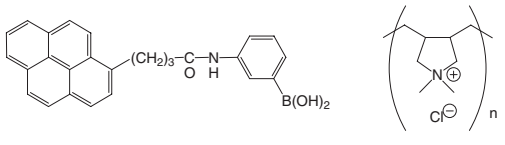
A novel sensing system for selective glucose detection has been established based on the 1:2 complex formation between glucose and fluorescent boronic acid in the presence of polycation which results in the emergence of excimer emission.

There is a great demand for sensitive and selective glucose sensing systems. Typically, glucose oxidase-based sensors have been used for medical diagnostics and bioprocessing. It is well recognized, however, that the enzyme-based systems have many problems such as instability, expensiveness, complexity, etc.[1] Alternatively, sensors based on the boronic acid–glucose interaction have been extensively studied by Shinkai et al.[2] The unique characteristic of glucose is its tendency to form 1:2 complexes in which two boronic acids are bound by one glucose.[3,4] By utilizing this characteristic, various types of diboronic acid receptors have been developed and shown that these receptors posses higher binding affinity toward glucose than for other saccharides.[5] Nevertheless, it seems to us that the sensing specificity of diboronic acid receptors are not satisfactory especially when saccharide concentration is high. Since the detection selectivity of diboronic acids are governed by the difference in binding constants for saccharides, non-selective response against saccharides having low binding affinities is inevitable when the saccharides concentrations are high enough to shift the equilibrium toward the complex formation side.



Scheme 1. Generation of excimer emission due to 1:2 complex formation between glucose and fluorescent boronic acid.



1 **2**

We here report a novel fluorometric detection strategy for the selective detection of glucose. As illustrated in Scheme 1, a 1:2 complex formation between glucose and fluorescent boronic acid (**1**)[6] is utilized to bring two pyrenyl moieties nearby and to create excimer emission. Since saccharides such as fructose and galactose have low tendency to form 1:2 complexes, the detection can be made selectively toward glucose.

In an aqueous glucose solution, **1** showed only monomer emissions in the range of 360–430 nm having maxima at 377 and 396 nm, and no excimer emission was observed (Figure 1, spectrum a). The spectrum was drastically changed by adding commercially available polycation (**2**)[7] in the solution (Figure 1, spectrum b): a broad excimer emission arose in the range of 430–600 nm. The observed spectral change indicates that the 1:2 complex between glucose and **1** was formed only in the presence of polycation. We then checked the effect of the concentration of **2** on the spectral behavior (Figure 2). In the presence of glucose, the excimer emission from **1** was intensified with increasing concentration of **2**, while excess addition of **2** resulted in a decrease of the excimer emission. It must be noted that the excimer emission was somewhat increased by the addition of **2** even in the absence of glucose. Since **1** exists as a univalent anion, this observation suggests that an electrostatic interaction exists between uncomplexed **1** and **2**.

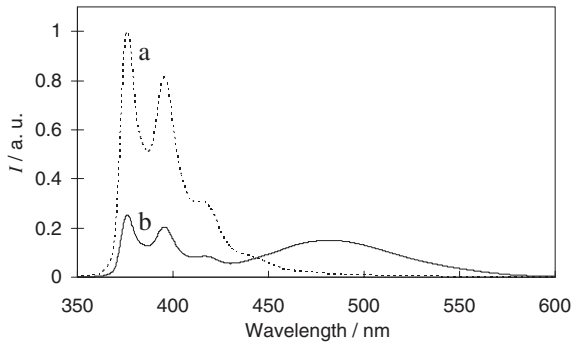


Figure 1. Fluorescence spectra of **1** ([**1**] = 1 μM) excited at 342 nm in aq solution containing 10 mM glucose buffered with 10 mM of NH_3 (pH = 10.2) in the absence (a) and presence (b) of **2** ([**2**] = 310 μM).[8]

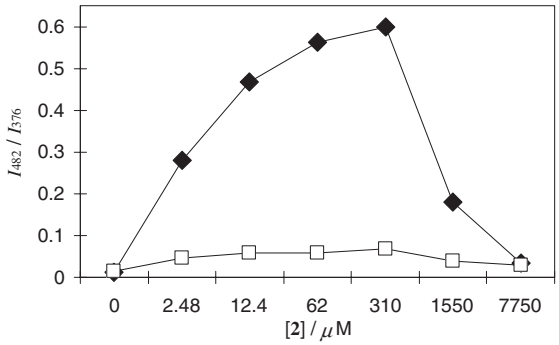


Figure 2. Effect of the concentration of **2** on the intensity ratio between excimer (482 nm) and monomer (376 nm) emissions in the presence (◆) and absence (□) of glucose. Excitation wavelength, concentrations of **1** and NH_3 are the same as those in Figure 1.

To assess the sensing selectivity of this system, we conduct-

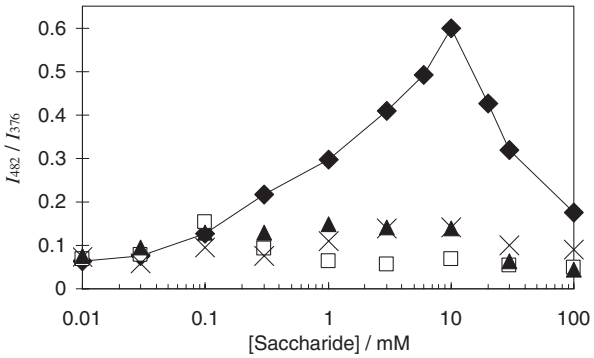


Figure 3. Dependence of I_{482}/I_{377} on the concentration of saccharides (◆: glucose, □: fructose, ×: galactose, ▲: ribose) in the presence of **2** ([**2**] = 310 μM). Excitation wavelength, concentrations of **1** and NH_3 are the same as those in Figure 1.

ed a series of fluorescence studies against saccharides. As shown in Figure 3, the excimer emission intensity showed the maximum value when glucose concentration was 10 mM. In contrast, other saccharides induced quite week excimer emissions. The observed sensing selectivity can be attributed to the difference in the tendency to form 1:2 complexes: glucose binds two molecules of **1** so that two pyrenyl moieties are brought close together and thus the strong excimer emission is observed, whereas saccharides having low tendency to form 1:2 complexes cannot gather two pyrenyl moieties.

Now, why polycation is necessary for the generation of excimer emission? As mentioned above, **1** is supposed to be enriched along the polycation chain via electrostatic interaction. If the local concentration of **1** becomes higher, the formation of 1:2 complex with glucose would be thermodynamically more favorable. This hypothesis is supported by the fact that the excimer emission is decreased when a large excess of **2** is added (see Figure 2), because it should lower the local concentration of **1**. To further confirm the validity of the hypothesis, we made a model calculation for the equilibrium mole fraction of 1:2 complex (X value[9] in Figure 4) using the stability constants for glucose–phenylboronic acid complexes.[3] When the total boronic acid concentration is 1 μM, which is the same condition for the fluorescence measurements, formation of 1:2 complex is negligibly small. The formation of 1:2 complex becomes significant when the total boronic acid concentration is increased three or four orders of magnitude. This result clearly supports the above mentioned hypothesis that the increase in the local concentration of **1** is the key factor for the formation of 1:2 complex and hence for the creation of excimer emission. In all the cases, formation of 1:2 complex is maximal when glucose concentration is around 9 mM, which is in accord with the experimental observations shown in Figure 3. This is explained by the fact that 1:1 complex becomes dominant over 1:2 complex in the presence of large excess of glucose. The importance of the electrostatic enrichment of **1** was additionally confirmed by the observation that the excimer emission was totally disappeared by adding 10 mM of NaCl into the solution (data not shown). We expect that the sensing behavior would be affected by changing the properties of polycation, namely charge density, conformational flexibility, etc. The investigation on that aspect is currently underway in our laboratory.

In conclusion, we have developed a glucose-specific fluorescent sensing system. The interaction between glucose and a fluorescent boronic acid to form 1:2 complex has been utilized. It has been found that the enrichment of fluorescent boronic acids along a polycation promotes the 1:2 complex formation that results in the creation of excimer emission. The present system provides an alternative strategy for the selective sensing of glucose without necessity of complicated and costly organic synthesis.

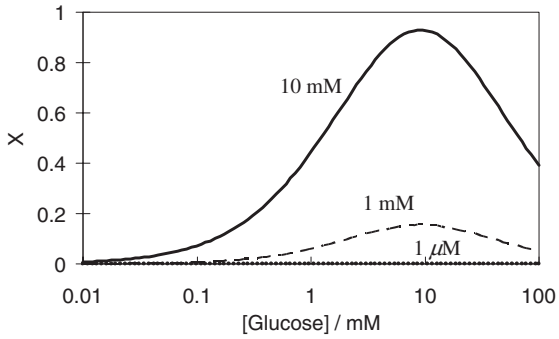


Figure 4. Calculated mole fractions of 1:2 complex formed between glucose and phenylboronic acid plotted against glucose concentrations at various total boronic acid concentrations.

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References and Notes

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6 **1** was synthesized by reacting 3-aminophenylboronic acid with 1-pyrenebutyric acid *N*-hydroxysuccinimide ester in DMF at 80 °C for 24 h.
7 **2**: Polydiallyldimethylammonium chloride (Aldrich), M_w = 400000–500000.
8 Concentration of **2** is defined as the concentration of cationic unit.
9 X = 2 × [1:2 complex]/{[1:1 complex] + [free boronate]}

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