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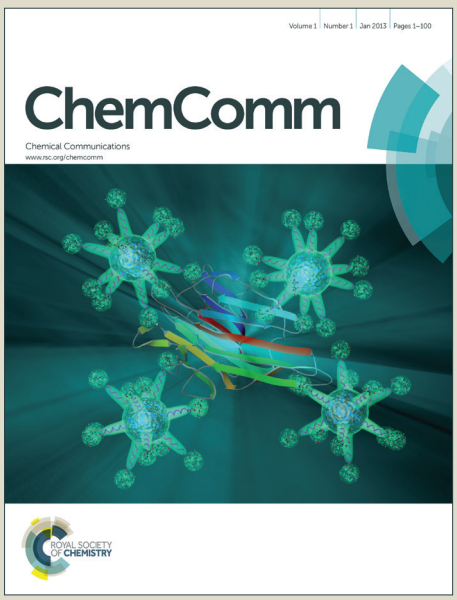

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Ratiometric fluorescent sensing of sugar with a reversible disassembly and assembly of the peptide aggregates mediated by sugar

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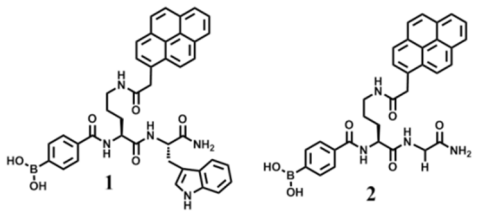
An amphiphilic dipeptide (1) bearing pyrene and phenylboronic acid was demonstrated as a unique example of a ratiometric sensing system for sugar by reversibly converting the peptide aggregates into the monomer form of the complex with sugar in aqueous solutions.

Carbohydrates are the major energy sources for living organisms and play an important role in a wide range of biological processes such as signal transduction, inflammation, cell–cell interactions, bacteria–host interactions, fertility, and development.[1] Thus, new methods of monitoring carbohydrates have received much attention in the field of biology and medicinal chemistry. As fluorescence is one of the most powerful and simple ways for detecting low concentrations of analytes, fluorescent chemosensors for sugars have received growing interest.

Fluorescent chemosensors for sugars have been used mainly with arylboronic acid as a receptor because arylboronic acid rapidly forms reversible covalent bonding with several diol compounds including sugar in aqueous solutions.[2,3] As it was believed that the covalent interactions of arylboronic acid with carbohydrates did not considerably affect the fluorescence of the neighboring fluorophore, most of the chemosensors based on arylboronic acid detected carbohydrates by a turn on response using a photoinduced electron transfer (PET) process.[2,3] Among various fluorophores, pyrene has received much attention in the design of the chemosensors because the unique monomer and excimer emissions with a distinctive different wavelength varied depending on the proximity between the pyrene fluorophores.[4] Until now, various pyrene systems capable of controlling the pyrene monomer and excimer emissions by external stimuli such as metal ions, anions, and pH have been reported.[5] Even though some sugar sensing systems involving a pyrene fluorophore have been reported,[6] they detected sugar by change of the excimer emissions without a change of monomer emissions. In general, a ratiometric response using two different emission bands was more ideal because a ratiometric response could measure analytes more accurately with a correction of environmental effects such as pH, temperature, and solvent media.[7]

In the present study, we synthesized a new ratiometric sensing system based on peptides for sugar including glucose capable of controlling the pyrene monomer and excimer emissions by sugar binding. Amphiphilic dipeptide **1** containing phenylboronic acid as a receptor was aggregated in the absence of sugar in aqueous solutions at pH 7.4, resulting in a considerable excimer emission. The covalent bonding of the phenylboronic acid of **1** with glucose converted the aggregates into a monomer form of the complex, resulting in a decrease of excimer emission and concomitant increase of monomer emission intensity. To the best of our knowledge, this is a unique ratiometric sensing system for sugars by changing the aggregates form into the monomer form in aqueous solutions after sugar binding.



Scheme 1. Structures of **1** and **2**.

As the peptide was biologically compatible and highly water soluble and acted as ligands for specific analytes,[8] the amphiphilic dipeptide (**1**) was designed to contain pyrene as a fluorophore, and phenylboronic acid as a receptor, respectively. A Trp amino acid was selected because Trp was reported to act as a ligand for several analytes including sugar (Scheme 1).[9] **1** was easily synthesized to a 79% yield using solid phase synthesis. Details on the synthesis and characterization of **1** are described in the Supporting Information (Figures S1-S5).

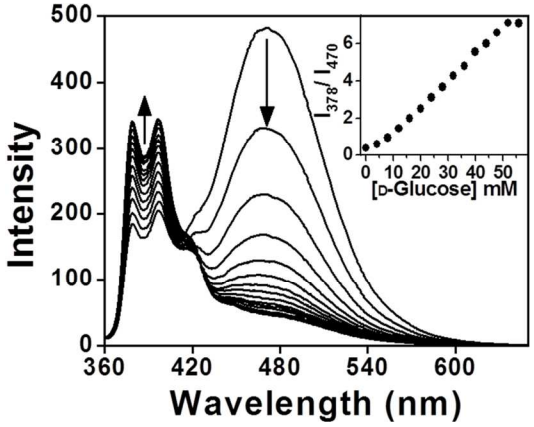


Fig. 1 Fluorescence emission spectra of **1** (10 μM) upon addition of D-glucose (0 – 0.056 M) (λ_{ex} = 342 nm) in 50 mM phosphate buffer solution containing 1% DMSO at pH 7.4.

We investigated the fluorescence response of **1** to glucose in an aqueous solution containing 1% DMSO. As shown in Fig. 1, in the absence of glucose, significant excimer emission at 470 nm with weak typical monomer emission bands at 378 and 395 nm were observed. This indicated that **1** might form self-assembled aggregates and two pyrene fluorophores were stacked even in the absence of glucose. Interestingly, upon the addition of glucose, a

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significant decrease of the excimer emission and a concomitant increase of the monomer emission with an isoemissive point at 417 nm were observed. **1** exhibited a ratiometric response to glucose in an aqueous solution at a neutral pH. The intensity ratio (I_{378}/I_{470}) at 378 and 470 nm changed significantly from 0 to 7 by adding glucose. About 0.05 M of glucose was enough for the saturation of the emission intensity change. During the UV-visible titration of **1** to glucose, the increase of the absorbance at 343 nm was observed (Figure S6), which indicated a decrease of pyrene-pyrene interactions in the presence of glucose. The covalent bonding between **1** and glucose was analyzed by ESI mass spectrum (Figure S7). A new peak corresponding to $[\mathbf{1}\cdot\text{glucose} - 2H_2O - H^+]^-$ appeared at 850.46 (m/z), which reveals that the covalent complex between **1** and glucose was formed by a boronate ester group and **1** formed a 1:1 complex with glucose. The covalent bonding of **1** with glucose might induce the formation of the monomer form from the aggregates of **1** in aqueous solutions, resulting in the decrease of excimer emission and concomitant increase of monomer emission intensity.

To investigate the role of phenylboronic acid for the binding mode, the fluorescence spectra of **1** were measured at different pHs. Fig 2a showed the fluorescence spectra of **1** in the absence of glucose at different pH. As the neutral pH of the solution was changed to an acidic pH, the excimer increased and a small decrease of monomer emissions were observed. However, as the neutral pH increased to a basic pH, the excimer emission significantly decreased and the monomer emission significantly increased, which indicates that the two pyrene fluorophores were less overlapped in basic pHs. As shown in Fig 2b, the monomer emission intensity, depending on pH in the absence of sugar, indicated that the pK_a value of phenylboronic acid played a critical role in the ratio between excimer and monomer emissions. As the pH is higher than the pK_a value of phenylboronic acid, the boronic acid with sp^2 hybridization was converted to the tetrahedral boronate form with sp^3 hybridization. The anionic boronate form of **1** preferred the monomer form rather than the aggregates may be due to the charge–charge repulsion and increase of hydrophilicity, which induced the increase of monomer emission intensity and decrease of excimer emission. UV-visible titration of **1** at different pHs showed that as pH increased, a considerable increase of the absorbance at 343 nm was observed (Fig. S8), which revealed that pyrene-pyrene interactions decreased as pH increased.

Fig 2b showed the emission intensity at 378 nm as a function of pH in the presence of glucose in aqueous buffer solutions. When glucose interacted with the phenylboronic acid of **1** to form boronate-ester, the pK_a value of the phenylboronic acid decreased from 8.36 to 7.62. As a result, **1** was converted to an anionic boronate form at neutral pH and this complex preferred the monomer form rather than the aggregates, resulting in the decrease of excimer emission and concomitant increase of monomer emissions. The shift of pK_a value by the binding of glucose was in agreement with the previous reported results. [4a-c,10] We also investigated the fluorescent response of **1** to D-fructose, D-galactose, and D-mannose, respectively (Fig. S9). **1** showed a ratiometric response to the sugars at a neutral pH by increasing monomer emission intensity and decreasing excimer emission. Assuming a 1:1 complex formation based on ESI mass spectrum, the association constants of **1** for the sugars were calculated by fitting the emission intensity at 378 nm versus concentrations of the sugars (Fig. 3).[11] The association constants for D-fructose, D-galactose, D-mannose, and D-glucose were calculated as 1199.72 M^{-1}, 105.08 M^{-1}, 90.13M^{-1} and 42.91 M^{-1}, respectively. The order of the binding affinity of **1** for sugar was consistent with that of the reported sugar sensor using phenylboronic acid.[4a-c, 10,12]

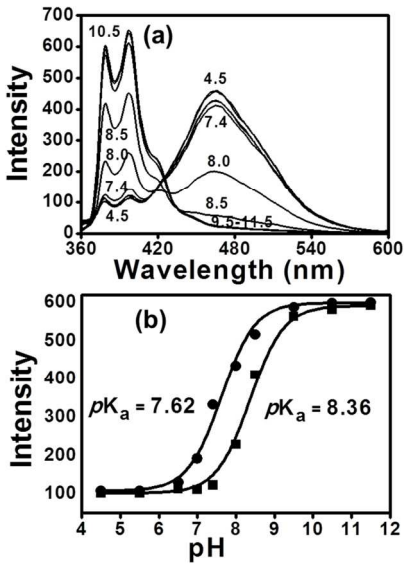

Fig. 2 (a) Fluorescence response of **1** (10 μM) in the absence of carbohydrate at different pHs and (b) the emission intensity at 378 nm as a function of pH in the presence (●) and absence (■) of glucose (0.05M) in a 50 mM phosphate buffer solution containing 1% DMSO.

Interestingly, the excimer emission was completely decreased by adding the sugar, whereas the enhancement of monomer emission intensity depended on the kind of the binding sugar. The enhancement of monomer emission intensity of **1** correlates well with the association constants for the sugar.

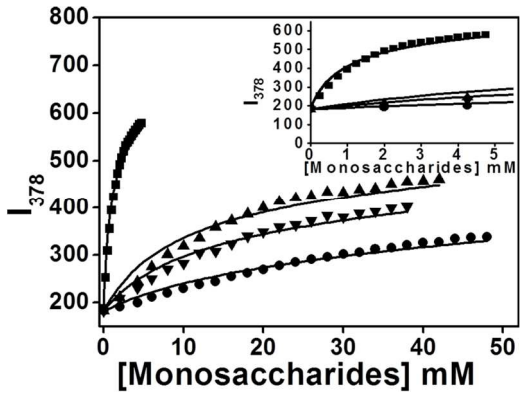

Fig. 3 Emission intensity of **1** (10 μM) upon addition of D-glucose (●), D-galactose (▲), D-mannose (▼) and D-fructose (■) in 50 mM phosphate buffer solution containing 1% DMSO at pH 7.4.

To investigate the solvent effect on the response to sugar, we measured fluorescence of **1** in an aqueous solution containing different volumes of DMSO (Fig. S10). As the percent of DMSO in the solvent increased from 1 to 10 %, excimer emission significantly decreased and few excimer emissions were observed, whereas the monomer emission was enhanced less than 2 times. This indicates that the increase of volume percent of hydrophobic solvent, DMSO in solution may weaken the interactions between the compounds and stabilize the monomer form of **1**. Interestingly, the enhancement of the monomer emission intensity of **1** by binding with glucose in aqueous solution containing 1% DMSO was much higher than that measured in aqueous solution containing 10% DMSO without glucose. This may be due to the quenching effect of phenylboronic acid on the fluorescence of pyrene.[13] It was believed that the covalent interactions of arylboronic acid with

the sugars did not considerably change the fluorescence of the neighboring fluorophore. However, we have found that the phenylboronic acid with sp^2 hybridization showed a more potent quenching effect on the fluorescence of pyrene than the tetrahedral boronate form with sp^3 hybridization.[13] Even though the aggregates of **1** were disassembled in aqueous solution by increasing the percentage of DMSO, the phenylboronic acid still acted as a quencher for the fluorescence of the pyrene to induce a weak monomer emission. The solvent effect on the fluorescence of **1** suggested that the hydrophobic interactions between the compounds play an important role in the aggregation in an aqueous solution. To confirm this, we synthesized compound **2** in which hydrophobic Trp amino acid was replaced with Gly (Fig. S11-15). **2** showed only weak monomer emission in the absence of sugar in aqueous solutions containing 1% DMSO and showed a turn on response to glucose by the enhancement of monomer emissions (Fig. S16). This result confirms that the hydrophobicity of **1** is likely to be a major driving force for the aggregation of **1** in aqueous solutions.

To invesitgate the relationship between the fluorescene and the aggregates, we investigated the aggregates by dynamic light scattering (DLS) and by fluorescence, respectively. As shown in Fig. S17, the amphiphilic dipeptide **1** formed the aggregates of diameters ca. 100 nm and resulted in excimer emissions in aqueous solutions. The addition of glucose to the solution induced the disassembly of the aggregates with increasing monomer emissions and a concomitant decrease of the excimer emissions. At pH > 10, when the phenylboronic acid was converted into the boronate anion, no aggregate and no excimer emissions were observed either, whereas at pH ≤ 7.4, the aggregates of diameters ca. 100 nm and excimer emission were observed. This result indicated that the excimer emission was due to the formation of the aggregation of **1**. DLS measuments of **2** indicated that **2** did not form aggregates in aqueous solutions.

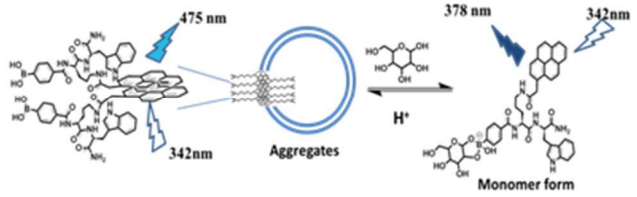

Fig. 4 The proposed binding mode of **1** with sugar.

The binding mode of **1** with sugar as proposed, is shown in Fig. 4. When the sugar covalently interacted with the phenylboronic acid of **1** to form boronate ester, the pK_a value of the boronic acid of the complex decreased. The resulting anionic boronate form of the complex with sugar preferred to the monomer form rather than the aggregates by charge-charge repulsions and an increase of the hydrophilicity of the complex. The sugar induced disassembly of the aggregates of **1** resulted in an increase of monomer emissions and a concomitant decrease of excimer emissions.

In summary, we report a unique ratiometric sensing system based on peptide containing phenylboronic acid for sugars by changing the aggregates into the monomer form in aqueous solutions after sugar binding. The covalent bonding of the phenylboronic acid of **1** with sugar induced, disassembled the aggregates into the monomer form of the complex and resulted in the decrease of excimer emissions and a concomitant increase of monomer emissions in aqueous solutions. To the best of our knowledge, this may be the first example for ratiometric sugar monitoring via a reversible disassembly of the aggregates into the monomer form in aqueous solutions by sugar binding.

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