Sugar sensing based on induced pH changes

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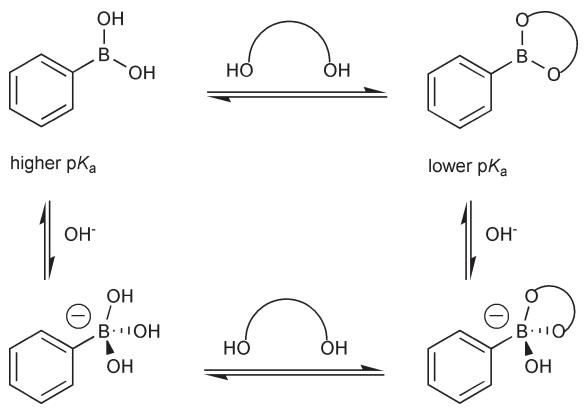
A sensory assembly consisting of a pH sensitive NIR dye and an arylboronic acid shows ratiometric absorption changes with increased fluorescence intensity upon addition of sugar in aqueous media; this demonstrates a new signal transduction mechanism for the detection of sugar based on pH changes induced in the microenvironment of the sensory assembly.

Glucose sensors are of particular interest for their potential application in monitoring of blood glucose levels for diabetes patients.[1] Arylboronic acids are widely used as the recognition motifs that bind preferentially to sugars and other vicinal diols.[2] The use of boronic acid groups is based on the formation of a tight complex between the boronic acid and a 1,2- or 1,3-diol (Scheme 1). Over the past decade, several fluorescence based probes functionalized with boronic acid derivatives have been developed using molecular rigidification,[3] photoinduced electron transfer (PET),[4] or excited-state charge transfer (CT)[5] signaling mechanisms. In such systems, the boronic acid is usually attached directly to a fluorophore and forms a cyclic boronate ester upon carbohydrate binding, resulting in modulation of the fluorescence emission.

Despite the promising results demonstrated by these systems, development of fluorescent probes is a complex process and optical responses are not easily predictable. A fluorescent sensor design typically includes three parts: fluorophore, linker, and reporter.[6] The use of such fluorophores frequently results in hydrophobic probes that require addition of an organic co-solvent to increase their solubility in aqueous media. Moreover, there are only a few reported examples of carbohydrate chemosensors emitting in the near infrared (NIR) region where there is minimal background signal from biomolecules and blood.[3a,7] Recently an indicator-displacement assay, which eliminates the need to covalently incorporate the fluorophore into the structure of the receptor, has been exploited to detect various analytes such as phosphate, α-hydroxycarboxylates, and diols.[8] Herein, we report a similar approach for sugar sensing using a pH sensitive NIR dye that is sensitive to pH changes induced by alterations in the pK_a of arylboronic acids after binding sugars in aqueous solution.



Scheme 1 Equilibrium between boronic acid and generic diols.

The pK_a responsive diol sensing assembly is prepared by mixing a pH sensitive NIR dye and an arylboronic acid in aqueous solution. The binding of sugars to the boronic acid derivatives in solution results in a decrease in the pK_a of the boronic acid moieties. This reduction in pK_a translates into increased acidity of the solution, which is monitored by a NIR pH sensitive dye.

The pH responsive reporter, dye **1**, is a water-soluble cyanine-based fluorochrome (Fig. 1).[9] Absorption spectra of dye **1** display spectral shifts and intensity changes in different pH media in a ratiometric manner. In acidic environments, a 640 nm peak is dominant whereas absorption spectra of dye **1** at neutral or basic pH are dominated by a peak at 484 nm. In addition, the fluorescence emission also varies in a pH dependent manner with strong emission at 666 nm under acidic conditions.

The sugar detection study was performed in aqueous media at multiple initial pH values with different boronic acid derivatives and a variety of sugar analytes. The pH changes of solutions containing various boronic acid derivatives upon addition of sugar analytes were investigated. In the absence of the boronic acid derivatives, addition of sugars (100 mM) did not change the pH values of the test solutions. This indicates that the observed pH changes in the presence of the arylboronic acids, after the addition of sugar, are a consequence of boronate ester formation. Several arylboronic acids were screened: phenylboronic acid (PBA) (pK_a 8.8), 3-nitrophenylboronic acid (3-NPBA, pK_a 7.1), *ortho*-dimethylaminomethylphenylboronic acid (*o*-DMAPBA, pK_a 6.7),

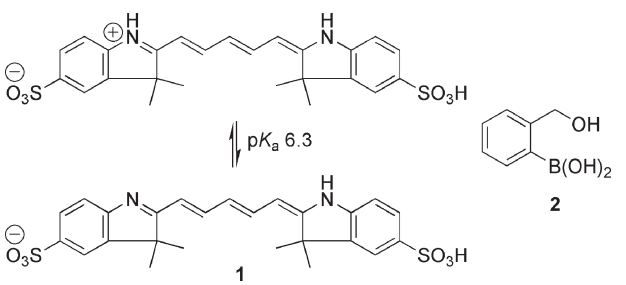


Fig. 1 Structure of the pH sensitive NIR dye **1** (ε: 141 000 M^{-1} cm^{-1} at 640 nm, quantum yield: 0.13 in aqueous solution) and *ortho*-hydroxyalkyl arylboronic acid **2** (*o*-HABA).

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the established standard for the recognition of simple sugars,[10] and water-soluble *ortho*-hydroxyalkyl arylboronic acid **2** (*o*-HABA, pK_a 7.2, Fig. 1). The sensing assembly was prepared by mixing dye **1** (5 μM) and an arylboronic acid (10 mM) in a pH 7.0 aqueous solution.† All of the arylboronic acids displayed good pH response upon addition of sugar molecules to the sensing assembly. Since PBA, 3-NPBA, and *o*-DMAPBA require an organic co-solvent for solubilization in aqueous media, we focus on *o*-HABA, which was recently reported to bind monosaccharides, such as glucose and fructose, with high affinity in neutral water.[11]

Increasing sugar concentrations results in absorption spectral shifts: the peak at 484 nm decreases in intensity with a concomitant increase in absorption at 640 nm (Fig. 2A). The pH change of the sensing assembly after addition of sugar is summarized in Table 1. As shown in Fig. 2B, the absorption ratio (640 nm/484 nm) increases with increasing sugar concentration, suggesting that the concentration of sugars can be determined in a probe concentration independent manner. The absorbance ratio changes from 0.6 in the absence of fructose to 9.4 at 100 mM fructose. In the presence of glucose, the ratio changes from 0.6 (0 mM glucose) to 2.8 (100 mM glucose).

Addition of sugar causes a clearly visible color change in the sensor solutions (Fig. 3A). Furthermore, semi-qualitative detection of D-fructose was demonstrated using test strips, allowing naked eye detection (Fig. 3B). The test strip was prepared by spotting

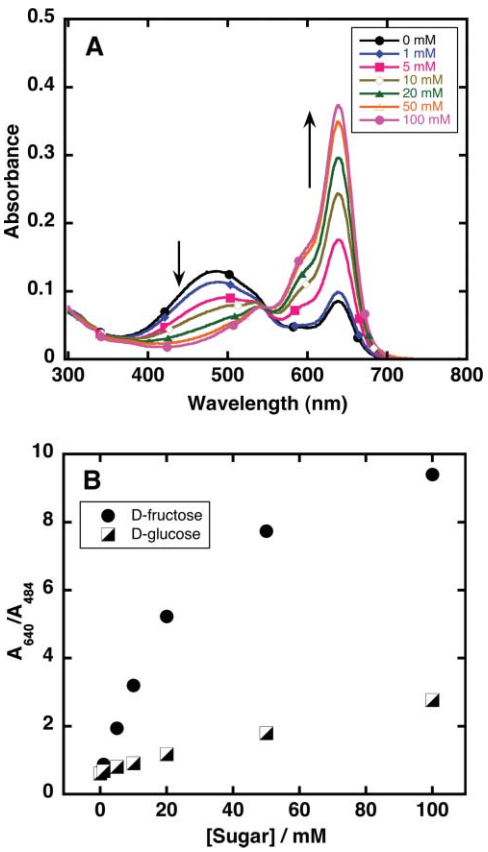

Fig. 2 (A) Absorption changes of the sensing assembly with different concentrations (0–100 mM) of D-fructose. (B) Absorption ratiometric plots based on A_{640}/A_{484} as a function of added sugar concentration ([D-fructose or D-glucose] = 0–100 mM). All measurements were acquired with 5 μM dye **1** and 10 mM *o*-HABA in a pH 7.0 aqueous solution.

Table 1 pH change of sensing assembly depending on sugar concentration[a]

[D-fructose] mM	0	1	5	10	20	50	100
ΔpH^b	0	0.07	0.46	0.76	1.04	1.53	1.81
ΔpH^c	0	0.05	0.27	0.48	0.71	1.21	1.45

[a] The sensing assembly was prepared with 5 μM dye **1** and 10 mM *o*-HABA. After addition of D-fructose to each sensing assembly, pH was determined by pH meter at 25 °C. [b,c]ΔpH is the decrease of pH of the sensing assembly in the absence and the presence of D-fructose. *b* and *c* are prepared in water and 10 mM HEPES buffer, respectively. The initial pHs are adjusted to 7.00 for *b* and 6.85 for *c*.

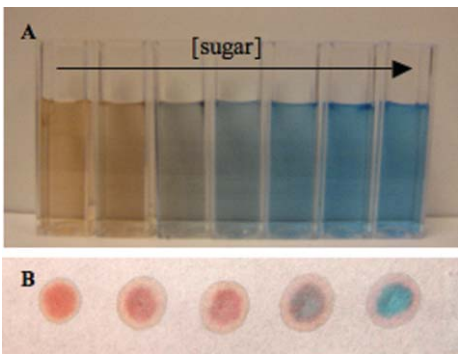

Fig. 3 (A) The color responses of the sensory system in the absence and presence of D-fructose (from left to right: 0, 1, 5, 10, 20, 50, 100 mM) in aqueous media (initial pH 7.0). Each solution contains 5 μM dye **1** and 10 mM *o*-HABA. (B) Test strip with spots of different concentrations of D-fructose on each spot prepared from a mixture solution of 1 mM dye **1** and 10 mM *o*-HABA in water (pH 7.4) (from left to right: no analyte, water, 1, 10, 100 mM D-fructose).

with the solution consisting of dye **1** (1 mM) and boronic acid (10 mM) on filter paper and air-dried. The initial color of each spot was reddish pink given by dye **1** at pH 7.4. After applying different concentrations of D-fructose solution, the spots applied with 10–100 mM D-fructose turned from reddish pink to blue immediately. As control, the spot with only water did not show any color change.

The fluorescence emission (λ_{em} = 666 nm) intensity of dye **1** also increases after addition of sugar and shows a three-fold increase in the emission intensity at 100 mM fructose (Fig. 4). However, the fluorescence response is not as significant as that observed for the ratiometric absorption measurements.

The sensing assembly (5 μM dye **1** and 10 mM *o*-HABA) was also prepared in HEPES buffer solutions of various buffering capacities (1 to 100 mM). Upon addition of sugar, similar colorimetric and fluorometric changes were observed in the buffered solution (for example, 10 mM HEPES buffer solution in Table 1), however, the magnitudes of the responses were attenuated at higher buffer concentrations.

Absorption spectra of the sensing assembly in the absence and presence of sugar (sorbitol, fructose, galactose, and glucose) are shown in Fig. 5. The sensitivity of the system is dependent on the sugar with increasing sensitivity from left to right: galactose ≈ glucose < fructose < sorbitol. As expected, the monoarylboronic acids used in the sensor assembly show a higher affinity toward fructose over glucose, which is in agreement

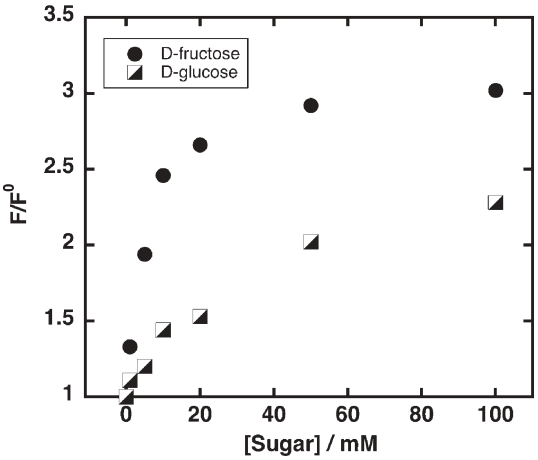

Fig. 4 Fluorescence intensity changes (F/F^0) of the sensing assembly as a function of sugar concentration (0–100 mM). The sensing assembly was prepared with 5 μM dye **1** and 10 mM *o*-HABA in a pH 7.0 aqueous solution: λ_{ex} = 640 nm, λ_{em} = 666 nm, F^0 = fluorescence intensity of the sensing assembly without sugar.

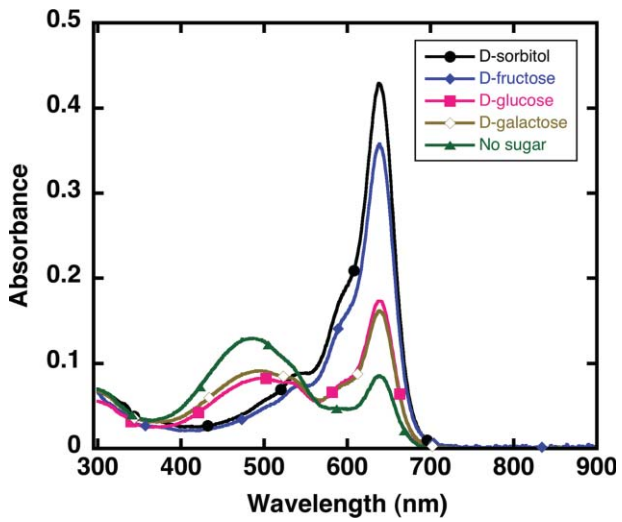

Fig. 5 Absorption spectra of the sensing assembly in the absence and presence of sugar (50 mM) in a pH 7.0 aqueous solution. All measurements were acquired with 5 μM dye **1** and 10 mM *o*-HABA.

with reported observations suggesting a stronger binding interaction with sorbitol and fructose compared with other monosaccharides.[12]

In summary, we detail a new paradigm for the detection of sugars based on changes in the pK_a of arylboronic acids on binding to diols. These pK_a alterations translate into altered optical properties of the pH-sensitive reporter dye. While the concept of pH change induced by binding of diols with boronic acids has been known for a long time[2] and pH indicator dyes have recently been used to discriminate mono- and disaccharides,[13] the NIR pH indicator approach in this report is more compatible to application in biological systems, such as *in vivo* sugar sensing. In addition to quantifiable absorption changes, fluorescence intensity increases are also observed upon sugar addition. The sensing assembly is water compatible, easy to assemble, and simple to use.

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Notes and references

† Preparation of the sensing assembly: The sensing assembly was prepared by dissolving 5 μM dye **1** and 10 mM *o*-HABA in distilled water and the pH was adjusted to 7.0 with 0.1 M NaOH or 0.1 M HCl solution. Sugar analyte was added to the sensing assembly solution.

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