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Specific Detection of D-Glucose by a Tetraphenylethene-Based Fluorescent Sensor

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Supporting Information

ABSTRACT: A conceptually new "light-up" biosensor with a high specificity for D-glucose (Glu) in aqueous media has been developed. The emission from a tetraphenylethene (TPE)-cored diboronic acid (**1**) was greatly boosted when the fluorogen was oligomerized with Glu because of restriction of the intramolecular rotations of the aryl rotors of TPE by formation of the oligomer. Little change in the light emission was observed when **1** was mixed with D-fructose, D-galactose, or D-mannose, as these saccharides are unable to oligomerize with the fluorogen.

D-Glucose (Glu) is a basic necessity of living organisms and a ubiquitous fuel for biological processes. An unusual Glu level in the body fluid is a warning signal of a medical condition: for example, an irregular concentration of Glu in human blood or urine implies a biological dysfunction. Glu detection is therefore of biomedical importance. However, the development of a selective Glu assay has been hampered by the fact that there are many saccharides with subtle structural differences in nature. D-Fructose (Fru), D-galactose (Gal), and D-mannose (Man), for instance, are naturally occurring stereoisomers of Glu. Early studies of alcohol-affinitive molecules revealed that phenylboronic acid (PBA) rapidly and reversibly reacts with diols in aqueous media.[1] PBA and its derivatives have thus been widely used as synthetic receptors for carbohydrates[2] and as building blocks in self-assembling systems.[3] PBA-based biosensors have also been developed, many of which, however, exhibit high selectivity for Fru because of the high binding affinity of Fru to boronic acids.[4]

Through elaborate molecular structural designs,[5,6] fluorescence (FL) sensors with preferential binding to Glu have been developed in the past decade.[7] In a typical example, two PBA units were attached to a fluorophoric molecule at the "correct" positions to ensure the formation of 1:1 complex between the PBA probe and Glu analyte.[8] A photoinduced electron transfer process was utilized to incite an FL turn-on response to the Glu binding. The Glu selectivity of such an affinity-dependent FL sensor, however, is rather limited because the PBA unit has a stronger affinity to other saccharides (e.g., Fru, Gal, and Man) than to Glu.[4] To develop new FL sensors with improved Glu selectivity, new approaches based on new concepts need to be devised to exclude these non-Glu saccharides from participating in the FL turn-on processes.

We have embarked on the development of Glu-specific biosensors operating in conceptually new mechanisms. We have recently discovered an "abnormal" phenomenon of aggregation-induced emission (AIE), in which a group of nonemissive fluorogenic molecules, such as tetraphenylethene (TPE), are induced to fluoresce efficiently by aggregate formation.[9,10] Both experimental data and theoretical calculations support the rationale that the unusual AIE effect is caused by restriction of the intramolecular rotations (RIR) of the phenyl rotors in the aggregate state.[9−11] It is envisioned that the RIR mechanism may be utilized to generate new FL biosensors for Glu detection. A straightforward target fluorogen for this purpose is the simple TPE derivative **1** containing two boronic acid units, whose structure is shown in Figure 1. It was anticipated that **1** would become fluorescent when the RIR process was activated (i.e., when the intramolecular rotations of its phenyl rotors were restricted) by its reactions with Glu.

To explore this attractive possibility, we functionalized TPE with two boronic acid units using the synthetic route[12] shown in Scheme S1.[13] The obtained TPE−diboronic acid adduct **1** was immiscible with water, and when its aggregates were suspended in an acidic buffer (pH 4.34), they emitted a strong blue light (Figure 1). The light emission of **1** started to decrease in intensity when the pH of the buffer was raised to >8.61 and became very weak at pH ≥ 9.74. Since the pK_a of PBA is ∼9, **1** was ionized by the alkaline medium and became soluble in the buffers with high pH. The solutions of **1** were virtually nonfluorescent, while its aggregates were highly emissive, proving that **1**, like its parent form TPE, is AIE-active.

Since the FL of an alkaline solution of **1** was very weak, we checked whether the emission could be enhanced by its reaction with Glu. When a tiny amount (<0.2 mM) of an aqueous solution of Glu was added to a solution of **1** in a carbonate buffer (pH 10.5), almost no change in the FL spectrum of **1** was observed (Figure S1[13]). When 0.2 mM Glu was added, however, the FL spectrum of **1** was intensified. The FL intensity increased with an increase in the amount of Glu (Figure 2A). At [Glu] = 5 mM, the solution of **1** became very emissive, with an intensity ∼5.4-fold higher than that in the absence of Glu. Interestingly, however, further increases in [Glu] decreased the FL intensity. In comparison with the big changes caused by Glu, the emission spectrum of **1** was only slightly intensified (Figure S2[13]) even when a large amount (10 mM) of Fru was added to the buffer solution

Received: August 7, 2010
Published: December 20, 2010

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dx.doi.org/10.1021/ja107086y | J. Am. Chem. Soc. 2011, 133, 660–663

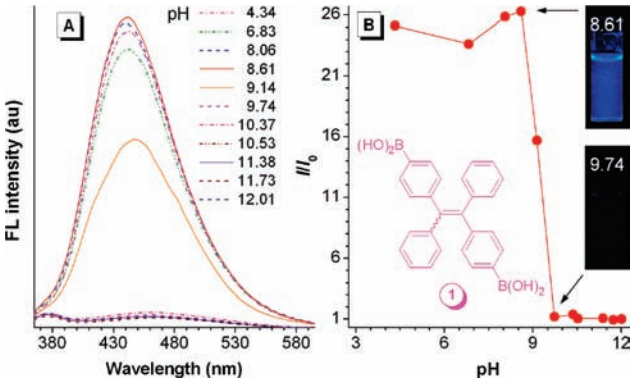


Figure 1. (A) FL spectra of **1** (10 μM) in Britton−Robinson buffers containing 2 vol % DMSO at different pH values. (B) Change in the FL intensity (I) of **1** at 440 nm with pH; I_0 is the intensity at pH 12.01. Inset: photographs of the mixtures of **1** in the aqueous buffers at pH 8.6 and 9.7 taken under the illumination of a hand-held UV lamp (λ_{ex} = 365 nm).

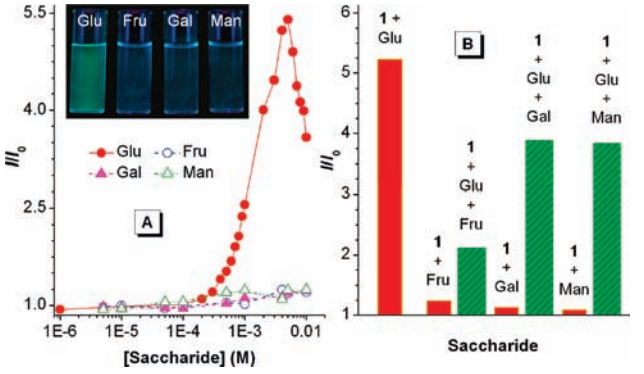


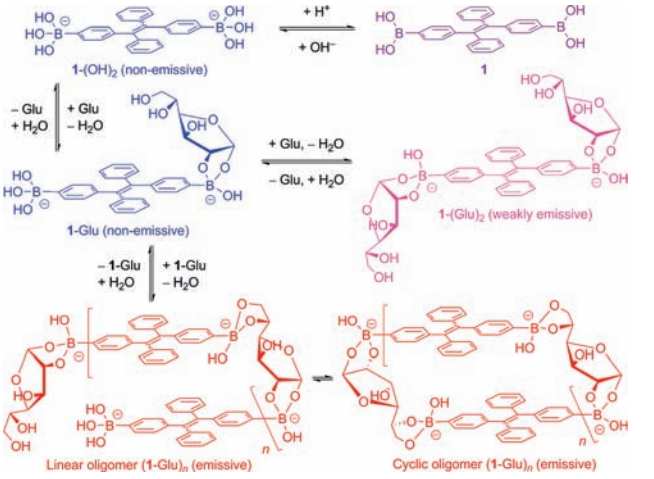
Figure 2. (A) Variation in the FL intensity (I) of **1** (50 μM) at 485 nm as a function of the concentration of saccharide in a carbonate buffer containing 2 vol % DMSO (pH 10.5). I_0 is the intensity in the absence of a saccharide. Inset: photographs of solutions of **1** in carbonate buffers containing 5 mM saccharide taken under UV illumination. (B) FL responses of **1** (50 μM) to saccharides (4 mM; red solid bars) or to Glu in the presence of another saccharide interferent (0.1 mM; green shaded bars).

of **1** (Figure 2A), and similar results were observed in the cases of Gal and Man.

Glu, Fru, and Gal are important dietary saccharides, while Glu, Gal, and Man are essential glyconutrients. The four saccharides coexist in body fluids (e.g., blood). Although [Glu] in the blood (3.6−5.8 mM) is much higher than [Fru], [Gal], and [Man] (all <0.1 mM), the affinity constants (K_a) for binding of the latter three saccharides to PBA are all larger than that of Glu.[4] It is thus of great interest to learn how these isomeric saccharides interfere with the FL response of **1** to Glu.

In the presence of Fru, the FL spectrum of **1** was gradually intensified by the addition of Glu (Figure S3A[13]), but the rate of increase in the intensity was lower (Figure S3D[13]) because of the competition between Glu and Fru to bind with **1**. Similarly, Gal and Man interfered with the FL response of **1** to Glu, though the effects were smaller (Figure 2B). These data suggest the following affinity order of saccharide to **1**: Fru > Gal Man > Glu, which agrees with the trend in K_a reported for saccharide binding with PBA: e.g., $K_{a,sugar}/K_{a,Glu}$ = 34.8 for Fru, 3.3 for Gal, and 2.8 for Man.[4] The data in Figure 2 indicate that **1** works well as a Glu-specific FL biosensor in both the absence and the presence of a saccharide interferent and suggest that the binding affinity is not a decisive parameter in determining the specific FL response of **1** to Glu.

Scheme 1. Proposed Mechanism for the Process of Glucose-Specific Sensing by AIE-Active Bioprobe 1



On the basis of the above experimental data and enlightened by previous studies of the chemical reactions of arylboronic acids with saccharides,[14,15] we propose a working mechanism for the specific FL response of **1** to Glu as shown in Scheme 1. Under alkaline conditions, **1** is transformed to **1**-$(OH)_2$, which is nonemissive because of its dissolution in the aqueous medium aided by its charged boronate groups. At low [Glu] (<0.2 mM), the reaction of the boronate unit of **1** with the *cis*-1,2-diol units of Glu predominates, giving rise to a 1:1 monoadduct of **1** and Glu, i.e., **1**-Glu.[5] The aryl rotors of the resultant **1**-Glu can still undergo active intramolecular rotations, so it remains nonemissive in the aqueous buffer.

At higher [Glu] (~0.2 mM), two Glu units may end-cap a TPE core, resulting in the formation of the bisadduct **1**-Glu_2. The intramolecular rotations of the phenyl rings in **1**-Glu_2 should be less active than its parent form, thus making it somewhat emissive. At an even higher [Glu], oligomerization may occur, thanks to the accessibility of the *cis*-5,6-diol units of Glu by the boronate group of the fluorogen. In the oligomer $(\mathbf{1}\text{-Glu})_n$, two phenyl rings in adjacent TPE units are fastened by a Glu linker. The intramolecular rotation of one phenyl ring in the oligomer involves simultaneous movements of the Glu linker and another phenyl ring in the neighboring TPE repeat unit. The high energy barrier to such molecular motions stiffens the oligomer structure, activates the RIR process, and opens the radiative decay channel.

The possibility of oligomer formation increases with increasing [Glu], with the FL intensity reaching its maximum at [Glu] = 5 mM. As the boronate−saccharide complexation is a reversible equilibrium process, the end-capping reaction leading to the formation of adduct **1**-$(Glu)_2$ becomes prevalent over the oligomerization reaction at very high [Glu]. As discussed above, the intramolecular rotations in **1**-$(Glu)_2$ are only partly restricted, which explains the observed intensity decrease for [Glu] > 5 mM (see Figure 2A). As [Glu] is further increased, the **1**-$(Glu)_2$ bisadduct is further populated because of the increased prevalence of the end-capping reaction in its competition with the oligomerization process.

dx.doi.org/10.1021/ja107086y | J. Am. Chem. Soc. 2011, 133, 660–663

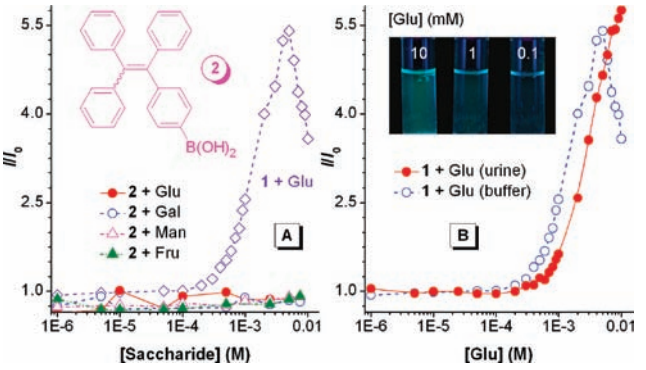


Figure 3. Variations in the FL intensities (I) of mixtures of (A) **2** (10 μM) with various concentrations of saccharides in the carbonate buffer containing 10 vol % DMSO and (B) **1** (50 μM) with Glu in artificial urine. I_0 is the intensity in the absence of saccharide. In each panel, FL data for **1** (50 μM) in the carbonate buffer containing Glu is shown for comparison. The inset in panel B shows photographs of mixtures of **1** (50 μM) in the artificial urines containing different amounts of Glu taken under UV illumination.

Similar to Glu, Fru can react with **1** to furnish the monoadduct (**1**-Fru)[6] at low [Fru] (Scheme S2[13]). At high [Fru], the end-capping bisadduct **1**-$(Fru)_2$ is formed, which accounts for the weak FL in the high concentration region (see Figure 2A). Once reacted with a boronic acid unit, Fru has no additional diol unit with a cis conformation to further react with **1** to form oligomeric species.[6,8,16] This is why no big change in FL was observed at any concentration of Fru. As in the case of Fru, Gal and Man have no *cis*-diol moieties that would allow them to undergo the oligomerization reactions. Clearly, the existence of a pair of *cis*-diol units in the 1,2- and 5,6-positions in Glu is the key structural feature that confers a Glu-specific response on **1**. In other words, it is the oligomerization of **1** with Glu that boosts the emission of the AIE fluorogen.

To examine whether the oligomerization reaction indeed plays an essential role in the Glu-selective sensing process, we prepared 4-(1,2,2-triphenylvinyl)phenylboronic acid (**2**; Scheme S1[13]), a monosubstituted TPE derivative with one boronic acid unit that is incapable of undergoing the oligomerization reaction. When Glu was added into an aqueous solution of **2**, little change in the FL spectrum of **2** was observed over a wide [Glu] range (Figure S4[13]). The mono- and bisadducts **2**-Glu and $\mathbf{2}_2$-Glu may be formed, but neither of these can emit efficiently because the intramolecular rotations of the phenyl rotors in these adducts are hardly impeded. Expectedly and understandably, **2** remained nonemissive, no matter what amounts of Fru, Gal, and Man were added into its solution (Figure 3A). The sharp contrast between the FL data for the systems (**2** + Glu) and (**1** + Glu) offers strong support to the proposed working mechanism shown in Scheme 1.

To explore the potential of **1** for real-world applications, we checked the possibility of using **1** for detection of Glu in urine. Addition of Glu in artificial urine into a solution of **1** augmented its FL spectrum (Figure S5[13]). The profile of the I/I_0 versus [Glu] plot for the urine Glu, however, was different from that for the "pure" Glu (in the carbonate buffer without urine) in the high-[Glu] region (Figure 3B). Although the exact reason for this is unclear at the present time, it may be caused by the interactions of urea, α-hydroxycarboxylic acids, and salts in the urine with the boronate units of **1**.[17] Nevertheless, **1** is a promising bioprobe for the specific detection of urine Glu.

In summary, we have developed a new Glu-specific FL biosensing system in this study. It works on the basis of a conceptually new mechanism: the FL of an AIE fluorogen (**1**) is enhanced by the RIR process activated by the peculiar probe−analyte interactions. The probe has a simple structure and is easy to prepare,[12] in contrast to its conventional counterparts, which are often rather complicated in molecular structure and require elaborate synthetic efforts.[7] The oligomerization reaction of the two *cis*-diol units in Glu with the two boronic acid groups in **1** creates structurally rigid oligomers (**1**-Glu)$_n$, resulting in a great boost in the FL of their TPE repeat units. Such a sensing process excludes any saccharide with only one *cis*-diol unit, as manifested by the high specificity of **1** for Glu over its close isomeric cousins Fru, Gal, and Man.

We are currently designing and synthesizing new AIE-active fluorogens with water solubility over a wide pH range, in an effort to develop new Glu-specific FL biosensors with improved performances and to gain more mechanistic insights into the FL sensing processes. We are also working on expanding the scope of applications of **1** into such areas as detection and quantitation of cyclodextrin, polysaccharide, and other polyols of biological importance. Promising results have been obtained and will be published in due course.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, crystal structure of **8** (CCDC 801326) (CIF), characterization data and FL spectra for **1** and **2**, and proposed structures for the products from the reactions of **2** with Fru, Gal, and Man. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We are grateful for the support from the National Science Foundation of China (50873086 and 20974028), the Ministry of Science and Technology of China (2009CB623605), the Research Grants Council of Hong Kong (603008), and the University Grants Committee of Hong Kong (AoE/P-03/08). B.Z.T. is grateful for the support from the Cao Guangbiao Foundation of Zhejiang University.

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