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Boronic acid substituted viologen based optical sugar sensors: modulated quenching with viologen as a method for monosaccharide detection†

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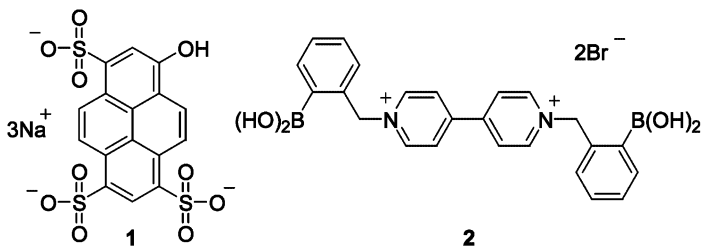
Abstract—In our search for an optical glucose sensor we have found that the combination of pyranine **1** and a boronic acid substituted benzyl viologen (*o*-BBV, **2**) signals monosaccharides in the range of 0–1800 mg/dL. The quenching ability of *o*-BBV is modulated upon sugar binding, which in turn alters the fluorescence of pyranine. The system utilizes a dye that is active in the visible spectrum, operates in aqueous solution at pH 7.4, and is highly sensitive to glucose in the physiological range. © 2002 Elsevier Science Ltd. All rights reserved.

There exists today an increasing interest in the development of a viable optical glucose sensor to assist in the management of diabetes.[1] Among the various techniques employed, fluorescence sensing utilizing boronic acids has received a considerable amount of attention.[2] In these systems signal transduction is derived from the ability of a dye functionalized with boronic acid to reversibly bind to monosaccharides, with the dye properties being altered upon binding. As a result, the concentration of sugars in solution can be detected and quantified via fluorescence. Inherent in this methodology is the necessity of a boronic acid to be connected to a fluorophore. In effect, the system is limited by the photochemical properties of the dye, such as UV excitation and photostability. An alternative approach being pursued in this laboratory is to develop saccharide sensitive boronic acid functionalized quenchers that can be used to modulate the emission of a variety of dyes. These quencher/dye combinations would thus serve as signal transducers for the detection and quantification of sugars.

Along these lines we began screening various quenchers with pyranine **1** (trisodium 8-hydroxy-1,3,6-pyrenetrisulfonate), a commercially available, water soluble, anionic dye that can be excited by visible light and fluoresces with high quantum yield.[3] Recently, it was reported that 4,4′-*N*,*N*′-dimethylbipyridinium dichloride (methyl viologen) quenched the fluorescence of MPS-PPV[4] with extreme efficiency.[5,6] This prompted us to check the ability of other viologens to quench the fluorescence of pyranine. We found that benzyl viologen also quenched pyranine effectively.[7] This result encouraged us to synthesize boronic acid substituted viologens and study their ability to quench pyranine. We now disclose our findings on optical sugar detection utilizing pyranine and 4,4′-*N*,*N*′-bis(benzyl-2-boronic acid)-bipyridinium dibromide (*o*-BBV **2**), a boronic acid-modified quencher.



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† This paper is cordially dedicated to Professor Herbert C. Brown on the occasion of his ninetieth birthday.

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Synthetically, the preparation of *o*-BBV is very similar to that of benzyl viologen. Because neither the mononor the bis-boronic acid substituted viologen was expected to demonstrate selectivity for glucose we chose to study the bis- due to its expected ease of preparation and enhanced water solubility. Thus, reaction of dimethyl-(2-bromomethyl)-benzeneboronate with 4,4′-dipyridyl in dry methanol gave *o*-BBV **2** in 70% yield.[8] Its ability to quench pyranine fluorescence was then investigated and found to be comparable to that of benzyl viologen (BV^{2+}).[9] Having established the quenching ability of *o*-BBV, monosaccharide sensing was examined utilizing the *o*-BBV/pyranine system.

As seen in Fig. 1, addition of glucose to an aqueous solution of *o*-BBV (3×10^{-4} M) and pyranine (1×10^{-5} M) led to a significant increase in fluorescence intensity, whereas BV^{2+}/pyranine showed no response. Thus, addition of 360 mg/dL glucose resulted in approximately a twofold signal increase.[10] Fructose and galactose gave a fivefold and three- and one-half-fold increase in signal, respectively. The observed selectivity for fructose is in line with the general observation for other monoboronic acid derivatives reported in the literature.

The signal transduction in this system is thought to be derived from two separate reversible complexation reactions: *o*-BBV/pyranine and *o*-BBV/monosaccharide.[11] In the absence of sugar *o*-BBV and pyranine form a photo-inactive complex, which may be similar to that proposed by Baptista et al.[7] In the presence of sugar *o*-BBV forms a boronate ester-complex.[12] Through boronate ester formation *o*-BBV is converted from the dicationic viologen to a neutral zwitterionic species. Neutralization of charge then results in a loss of electrostatic attraction and subsequent dissociation of the dye/quencher complex. Evidence for this mechanism is seen in the UV–vis spectra where addition of fructose results in regeneration of the free pyranine (Fig. 2). As a result of this dissociation, nonradiative deactivation pathways are removed leading to a relative increase in fluorescence emission with increasing sugar concentration. It is apparent from the data shown in Fig. 1 that even with 1800 mg/dL of glucose the boronic acid is not saturated. Consequently, the pyranine UV–vis spectrum is only partially restored with glucose (Fig. 3).

For existing systems signal transduction is derived from a single reversible complexation reaction: boronic acid with monosaccharides. These systems fall primarily under two categories. In the first case, where a boronic acid is directly attached to a dye, sugar binding influences fluorescent emission by altering the electronic properties of the dye.[13] In the second case, where the boronic acid is attached to the dye through a secondary or tertiary alkyl amine connecting group, a Lewis acid/base interaction is involved, and photo induced electron transfer responsible for quenching is disrupted.[14] In contrast to those described above, our system involves a bimolecular dye/quencher complex that is nonfluorescent; when quencher binds to sugar, the complex dissociates, resulting in a relative increase in fluorescence.

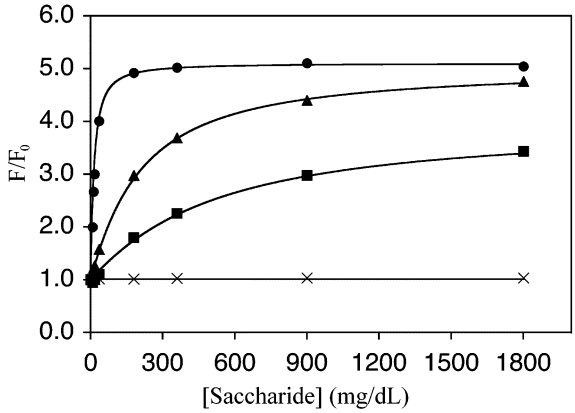

Figure 1. Relative fluorescence emission of pyranine (1×10^{-5} M, excitation = 461.8 nm, emission λ_{max} = 511 nm)/*o*-BBV (3×10^{-4} M) system as a function of saccharide concentration in 0.1 ionic strength aqueous phosphate buffer pH 7.4 (●, fructose; ▲, galactose; ■ glucose; ×, glucose (*o*-BBV replaced with BV^{2+} (3×10^{-4} M) to demonstrate lack of sensitivity without boronic acid functionality)).

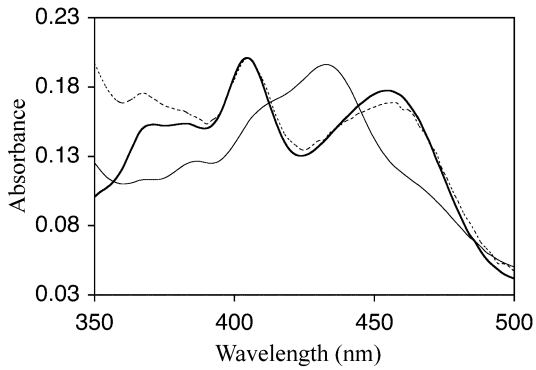

Figure 2. UV–vis absorption spectra of pyranine (1×10^{-5} M) —; pyranine (1×10^{-5} M) with *o*-BBV (3×10^{-4} M) —; pyranine (1×10^{-5} M) with *o*-BBV (3×10^{-4} M) and fructose (1800 mg/dL) --.

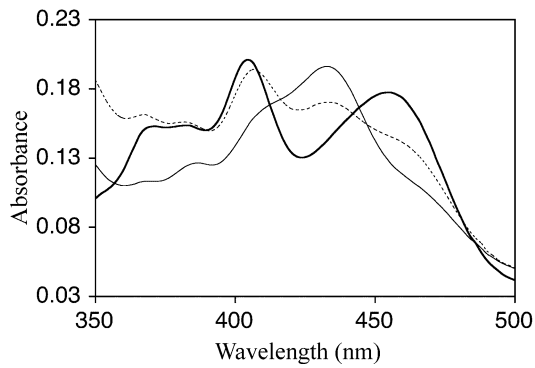

Figure 3. UV–vis absorption spectra of pyranine (1×10^{-5} M) —; pyranine (1×10^{-5} M) with *o*-BBV (3×10^{-4} M) —; pyranine (1×10^{-5} M) with *o*-BBV (3×10^{-4} M) and glucose (1800 mg/dL) --.

The system utilizes a dye that is active in the visible spectrum, operates in aqueous solution at pH 7.4, and is highly sensitive to glucose in the physiological range. Consequently, we believe our system shows promise as the basis for a viable optical glucose sensor. We are currently exploring the scope of the concept, including variations in dye and quencher structure, methods of achieving glucose selectivity, and monosaccharide binding stoichiometry.

Acknowledgements

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8. 4,4′-*N*,*N*′-bis(Benzyl-2-boronic acid)-bipyridinium dibromide (*o*-BBV **2**) was prepared as follows. An oven-dried, 50 mL centrifuge tube was cooled under argon, equipped with a magnetic stir bar, and charged with 4,4′-dipyridyl (0.469 g, 3 mmols). The tube was sealed with a septum and charged with anhydrous CH_3OH (7 mL). The homogenous solution was stirred at room temperature while freshly prepared dimethyl-(2-bromomethyl)-benzeneboronate (1.82 g, 7.5 mmols) was added via syringe. After stirring the solution for 15 h, the reaction vessel was centrifuged and the CH_3OH cannulated to a separate flask. The remaining yellow solid was triturated with acetone:water (24:1, v/v, 25 mL), stirred vigorously on a vortex mixer, and centrifuged. The acetone solution was removed by cannula and the process repeated two more times. The solid was then triturated with diethyl ether using the same process. The remaining pale yellow solid was dried under reduced pressure (0.6 torr, 2 hr). Yield: 1.23 g, 1.63 mmols (70%), mp: >230°C (decomposition). ^{1}H NMR (250 MHz, D_2O, δ): 6.21 (s, 2H), 7.72, (m, 3H), 7.91 (d, 1H), 8.60 (d, 2H), 9.18 (d, 2H). ^{13}C NMR (125 MHz, D_2O, δ): 66.20, 127.96, 131.02, 132.50, 132.77, 136.45, 136.83, 147.04, 151.56. ^{11}B NMR (80 MHz, D_2O, δ relative to $BF_3{\cdot}Et_2O$): +30.2 (broad s). HRMS-ESI (m/z): $[M-2Br^-]^{2+}$ calcd for $C_{24}H_{24}B_2N_2O_4$, 213.0956; found, 213.0977.
9. All studies were conducted in pH 7.4 aqueous phosphate buffer of 0.1 ionic strength. Pyranine concentration was chosen to be 1×10^{-5} M and quencher concentration was varied between zero and 3×10^{-4} M. The excitation wavelength was 461.8 nm and the fluorescence emission spectrum was recorded between 470 and 630 nm. The total fluorescence emission of each sample was quantified as the area under the emission curve (data not shown).
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