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The effect of boronic acid acidity on performance of viologen-based boronic acids in a two-component optical glucose-sensing system

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Abstract—A two-component saccharide sensing system using the fluorescent dye, hydroxypyrene trisulfonic acid, combined with a boronic acid functional viologen as a receptor/quencher in pH 7.4 buffer solution has been further investigated. The effect of substituents on the acidity of the boronic acid was measured. The boronic acid pK_a changed in the expected manner when electron donating or withdrawing groups were present. The glucose binding constants were dependent on pK_a, but no simple correlation was observed for the Stern–Volmer quenching constants and the fluorescence signal modulation.
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The detection of glucose using boronic acid-based fluorescent chemosensors has been a major area of research in recent years.[1] This is driven in part by the need for better techniques for monitoring blood sugar concentrations in diabetics[2] and critically ill patients.[3] Boronic acid-based sensors can be utilized for this application because of their ability to bind reversibly to 1,2- and 1,3-diols in aqueous media.[4] In most of these systems, the boronic acid receptor is attached directly to a fluorophore, and the saccharide recognition event causes a change in fluorescence emission.[5]

In contrast to systems in which the boronic acid and fluorophore are incorporated into a single molecule, the study described in this Letter uses a two-component system, developed earlier,[6] comprising the anionic fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS), and a boronic acid-appended viologen. The latter serves both as a fluorescence quencher and a glucose receptor.

Though the detailed mechanism of glucose modulation in the two-component system has not been completely elucidated, it has been established that the quenching step involves ground-state charge-transfer complex formation between anionic dye and cationic quencher. The existence of complexes has been shown both in solution[6b,e] and in the solid state.[6h] The complex exhibits diminished fluorescence relative to that of the free dye. We propose that a signal is generated when the complex dissociates as a consequence of the reaction of the quencher component with glucose, resulting in an increase in fluorescence intensity related to glucose concentration.

The first step in the dissociation process involves reaction of the boronic acid receptor on the viologen with glucose. The conversion of the neutral boronic acid (trigonal boron) to a negatively charged boronate ester (tetrahedral boron) alters the properties of the viologen leading to a reduction in quenching efficacy. Though electronic and steric effects may play a role, at least part of the process is simple charge neutralization, which reduces the electrostatic attraction of dye for quencher and leads to weaker binding and therefore enhanced fluorescence.

The purpose of the present study was to explore the effect of boronic acid acidity on quenching and glucose response. This knowledge should lead to a better understanding of the sensing mechanism. The system studied combines benzyl viologens as the quenching component with HPTS as the fluorescent dye in aqueous media at physiological pH. The approach taken is to manipulate the pK_a of the boronic acid by adding

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electron donating and withdrawing groups in the *para* position and observing how such substituents affect the quenching ability and glucose response of the viologens. It has been shown that the pK_a values for arylboronic acids are dependent upon the substitution pattern and nature of the substituents on the aromatic ring.[7]

Three quencher/receptors, N,N'-4,4′-bis(benzyl-3-boronic acid)-bipyridinium dibromide (BBV), N,N'-4,4′-bis-(benzyl-5-fluoro-3-boronic acid)-bipyridinium dibromide (F-BBV), and N,N'-4,4′-bis(benzyl-5-methoxy-3-boronic acid)-bipyridinium dibromide (OMe-BBV), were prepared and used in this study (Fig. 1). The effect of an electron donating substituent (methoxy) and electron withdrawing substituent (fluoro) on the pK_a of the boronic acid, as well as on the quenching ability and glucose response of these compounds was investigated. Non-boronic acid analogs, benzyl viologen (BV), fluoro-benzyl viologen (F-BV), and methoxy-benzyl viologen (OMe-BV), were used as controls. The six compounds were synthesized as outlined in Scheme 1.

The apparent pK_a values of the boronic acid-containing compounds were determined using potentiometric titration and are listed in Table 1. As expected, the presence of an electron-donating methoxy group *para* to the boronic acid in OMe-BBV raised the apparent pK_a relative to that of the unsubstituted compound (BBV). Similarly, the apparent pK_a of F-BBV was lower than that of the unsubstituted compound, BBV, due to the electron-withdrawing fluoro group *para* to the boronic acid. As observed earlier,[6g] the value of BBV is substantially lower than that of phenyl boronic acid (pK_a = 8.8) and this is attributed to the effect of the positively charged nitrogen atom in the pyridinium ring. The change in pK_a observed by adding a second substituent to the aromatic ring is therefore, the net effect of both groups. The F-atom adds to the electron withdrawing effect of N^+; the OMe-group counteracts it.

Table 1. pK_a values determined by potentiometric titration for the BBVs (4 mM) in the absence and presence of glucose (0.1 M)

	pK_a without glucose	pK_a with 0.1 M glucose	ΔpK_a
BBV	7.7	6.8	0.9
F-BBV	7.5	6.7	0.8
OMe-BBV	8.2	7.4	0.8

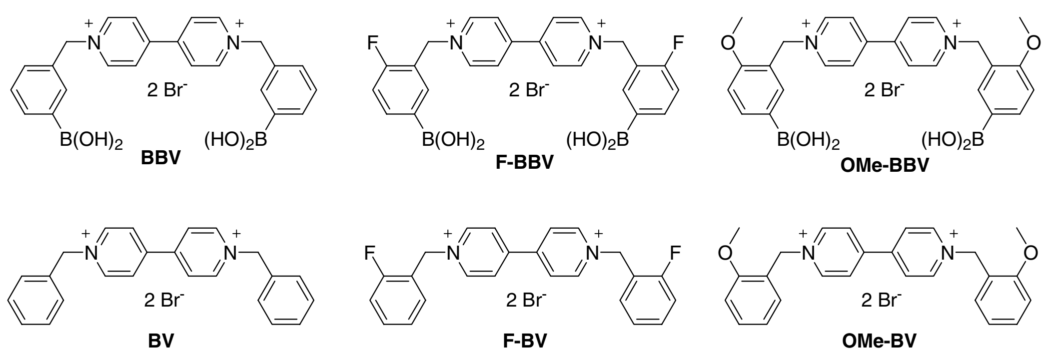
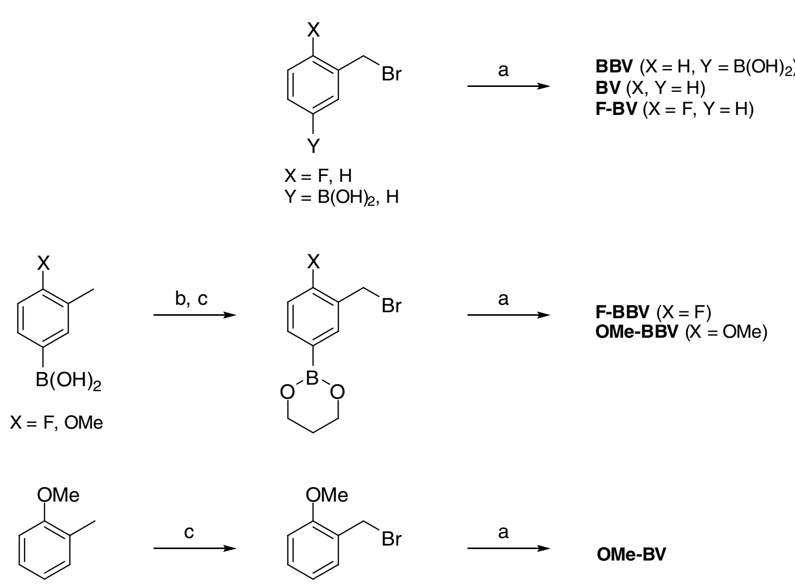


Figure 1. Structures of boronic acid-substituted benzyl viologens and benzyl viologens.



Scheme 1. Synthesis of viologen-based quenchers. Reagents and conditions: (a) 4,4′-dipyridyl, DMF, 60 °C, 48 h; (b) 1,3-propanediol, CaH_2, DCE, 88 °C, 1 h; (c) *N*-bromo succinimide, 2,2′-azobisisobutyronitrile, DCE, 88 °C, 3 h.

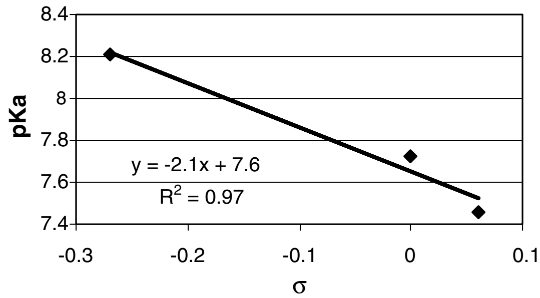


Figure 2. Hammet plot for BBVs.

The pK_a values of these compounds were also determined in the presence of glucose (Table 1). Consistent with typical boronic acids, all of the compounds displayed lower pK_a values with glucose present, versus those measured in the absence of glucose.

A Hammett plot was generated using this data (Fig. 2).[8] The data in the Hammet plot were fitted with a straight line with a slope of 2.1. This value is consistent with the value previously reported for phenyl boronic acids.[7a]

To determine the relative fluorescence quenching ability of each viologen with HPTS, Stern–Volmer plots were generated (Fig. 3). A solution of HPTS (4×10^{-6} M in pH 7.4 phosphate buffer) was titrated with increasing amounts of the viologens, and the fluorescence emission at 510 nm ($\lambda_{ex} = 460$ nm) was recorded after each addition. Values for K_s and V, which represent the static and dynamic quenching constants, respectively, were determined from these data (Table 2).[9]

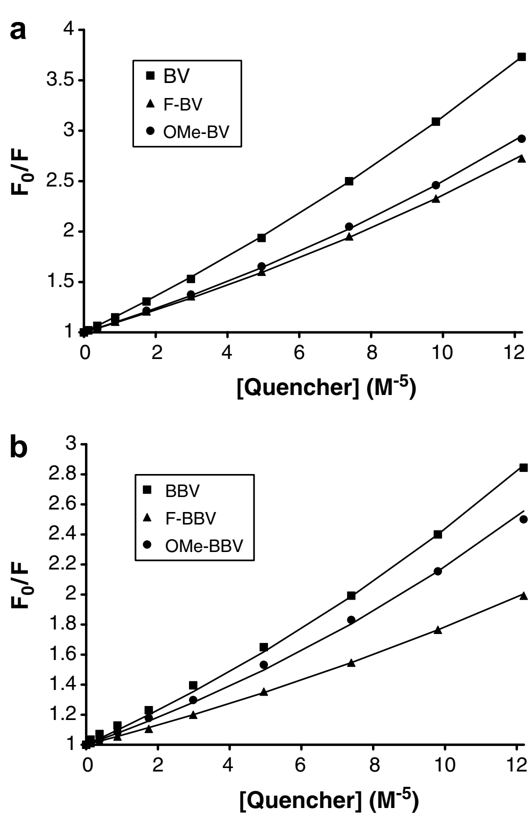


Figure 3. (a) Stern–Volmer plots of HPTS (4×10^{-6} M) with increasing concentrations of (■) BV, (▲) F-BV, and (●) OMe-BV at pH 7.4 $\lambda_{ex} = 460$ nm, $\lambda_{em} = 510$ nm. (b) Stern–Volmer plots of HPTS (4×10^{-6} M) with increasing concentrations of (■) BBV, (▲) F-BBV, and (●) OMe-BBV at pH 7.4 $\lambda_{ex} = 460$ nm, $\lambda_{em} = 510$ nm.

Table 2. Static (K_s) and dynamic (V) quenching components of pyranine (4×10^{-6} M in pH 7.4 phosphate buffer, $\lambda_{ex} = 460$ nm and $\lambda_{em} = 510$ nm) for the BBV and BV quenchers

	K_s (M^{-1})	V (M^{-1})
BBV	7.8×10^3	3.1×10^3
F-BBV	3.5×10^3	2.8×10^3
OMe-BBV	5.8×10^3	3.2×10^3
BV	1.5×10^4	2.3×10^3
F-BV	7.7×10^3	2.9×10^3
OMe-BV	7.9×10^3	3.3×10^3

A comparison of F-BV, OMe-BV, and BV is given in Figure. 3a.

From the Stern–Volmer plot and the K_s values, it is clear that each ring substitution decreases the quenching ability of benzyl viologen to nearly the same extent. One possible explanation of this diminished quenching is a weak intramolecular charge-transfer between the substituent heteroatom and the quaternized pyridinium nitrogen (Fig. 4). Because the quenching mechanism depends on the transfer of an electron from the dye to the viologen quencher in the ground-state complex,[6a] any change in the viologen that reduces its electron affinity would make it a less effective quencher. It is also possible that the substituents interfere sterically with the complex formation.

When analyzing the relative quenching characteristics of the three boronic acid-based viologens, two main factors have to be considered as noted above. One is the possible steric implications of the fluoro and methoxy substituents. As was observed with the non-boronic acid containing viologens (BVs), this could explain why both OMe-BBV and F-BBV are weaker quenchers than BBV (Fig. 3b, Table 2). The second factor is the charge-neutralization mechanism. In further support of this mechanism of diminished quenching, it should be noted that F-BBV is the poorest quencher of all. The reason why the quenching strengths of OMe-BBV and F-BBV differ is likely due to the electronic effects that the *para* substituent exert on the boronic acid. As evidenced by the apparent pK_a value of F-BBV (Table 1), the electron withdrawing fluoro group causes the boronic acid to be partially in the anionic boronate form at pH 7.4, thus decreasing its ability to complex with HPTS. The same rationale can be used to explain the quenching behavior

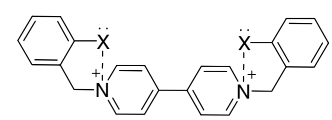


Figure 4. Proposed charge interaction resulting in diminished quenching ability.

of OMe-BBV, relative to F-BBV. That is, the boronic acid is mainly in the trigonal neutral state at this pH. The observation that the quenching by OMe-BBV is poorer than BBV indicates that other factors are involved which are not forthcoming at present.

The use of BBV and HPTS as a viable saccharide sensing system has been previously demonstrated.[6] In this system, the amount of fluorescence signal modulation is dependant on the amount of saccharide that is titrated into a solution of HPTS quenched by BBV. When glucose binds to the boronic acid on BBV, the Lewis acidity of boron is increased, resulting in tetrahedral anionic glucoboronate formation at pH 7.4. This zwitterionic form of the BBV molecule is a less effective quencher and thus an increase in the fluorescence intensity of HPTS is observed.

The apparent binding affinities of the boronic acid-based viologens for glucose were determined by measuring the relative fluorescence intensity (F/F_0) ($\lambda_{ex} = 460$ nm, $\lambda_{em} = 510$ nm) when increasing amounts of glucose (0–30 mM) were added to an aqueous solution of HPTS (4×10^{-6} M, pH 7.4) and a BBV (1.2×10^{-4} M). The binding curves generated from the titration are shown in Figure 5, and the calculated apparent binding constants (K_b) are listed in Table 3. It is known that the binding constants of boronic acids with diols increase with decreasing boronic acid pK_a.[7] The BBV compounds display a behavior that is consistent with this trend. The compound with the lowest pK_a, F-BBV, produced the highest binding constant and OMe-BBV, which has the highest pK_a, gave the lowest binding constant.

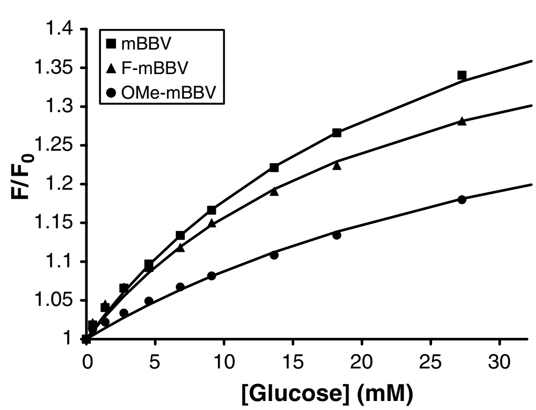


Figure 5. Fluorescence increase of HPTS (4×10^{-6} M in phosphate buffer, $\lambda_{ex} = 460$ nm and $\lambda_{em} = 510$ nm) in the presence of BBV, F-BBV, and OMe-BBV when titrated with glucose.

Table 3. Apparent saccharide binding constants (K_b) determined for the BBVs (1.2×10^{-4} M) with HPTS (4×10^{-6} M in phosphate buffer, $\lambda_{ex} = 460$ nm and $\lambda_{em} = 510$ nm)

	Glucose K_b (M^{-1})	F/F_0 @ 10 mM
BBV	37 ± 5	1.17
F-BBV	44 ± 5	1.14
OMe-BBV	23 ± 5	1.09

Besides binding constants, another metric that can be used to evaluate these compounds is the degree of fluorescence recovery (F/F_0) obtained upon glucose addition. In this case, HPTS/BBV produced the largest degree of fluorescence enhancement, followed by HPTS/F-BBV, then HPTS/OMe-BBV. In spite of a higher apparent binding constant, F-BBV did not produce a larger F/F_0 value relative to BBV. This could be the result of F-BBV existing more in the boronate form due to its lower apparent pK_a. As pointed out earlier, this would weaken the F-BBV/HPTS interaction thereby decreasing the overall modulation.

The BBV quenchers used in this study, when combined with the dye HPTS in pH 7.4 buffer, modulated the fluorescence of HPTS when titrated with physiological amounts (2.5–20 mM) of glucose. This study showed the expected substituent effect on the pK_a of boronic acid-substituted viologens. A clear correlation was shown between apparent pK_a and the binding constants of the boronic acids with glucose. The addition of either an electron withdrawing group or an electron donating group to the benzyl ring of boronic acid-substituted benzyl viologens also affected the quenching and glucose sensing behavior of the viologens relative to the unsubstituted derivatives; however the relationship is more complex. The addition of the fluoro group increased the apparent binding constant but slightly decreased the fluorescence modulation. The methoxy group unexpectedly produced a slight decrease in quenching efficacy, and caused a drop in both the binding constant and modulation. Further research will be required to establish the exact relationship between the apparent pK_a of the boronic acids and the performance of the HPTS/BBV sensing system, though the correlation with glucose binding is clear.

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