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Wavelength-ratiometric and colorimetric probes for glucose determination

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Abstract

We characterize two new water-soluble probes, *m*- and *p*-NBDPBA, which show notable changes in their absorption spectra in the presence of monosaccharides such as glucose and fructose. Subsequently glucose concentrations can readily be determined in the physiological glucose concentration range in a wavelength-ratiometric and colorimetric manner. *m*-NBDPBA has a dissociation constant of 204 mM for glucose, which is not unlike other mono-phenyl boronic acid derived probes. In addition we have also synthesized a control compound, NBDP, which does not contain the boronic acid moiety and is therefore insensitive to sugars, to help rationale the spectral changes observed.

Keywords: Glucose; Fructose; Monosaccharides; Wavelength-ratiometric transduction

1. Introduction

It is well-known that the continuous monitoring of blood glucose in diabetics is essential to avoid the long term health effects of elevated blood glucose, such as blindness and sequella [1,2]. Over the past decade we have seen the development of many colorimetric and fluorescence based probes based on boronic acid, which is known to have a high affinity for diol-containing compounds, such as for monosaccharides [3–10]. In addition it is also widely accepted that ratiometric or lifetime based methods offer intrinsic advantages for biomedical sensing, yet there are relatively few probes based on boronic acid with a wavelength-ratiometric signal transduction [3–10].

In this paper we have fashioned two new glucose probes using the widely used NBD nucleus and both *m*- and *p*-phenyl boronic acid. The *o*-derivative shows evidence of a unique time-dependent intermolecular hydrogen bond interaction, and will be the subject of a detailed report elsewhere in due course. The two new probes reported here show changes in their absorption spectra as a function of glucose addition, enabling sensing in a wavelength-ratiometric manner.

The signal transduction is based on the ability of the boronic acid moiety to change its electron accepting and

Abbreviations: BA, boronic acid; ICT, intramolecular charge transfer; NBD, 7-nitrobenz-2-oxa-1,3-diazole; NBDP, 4-(*N*-phenyl)-amino-7-nitrobenz-2-oxa-1,3-diazole; *m*-NBDPBA, 4-[*N*-(3-borono-phenyl)amino-7-nitrobenz-2-oxa-1,3-diazole; *p*-NBDPBA, 4-[*N*-(4-boronophenyl)amino-7-nitrobenz-2-oxa-1,3-diazole.

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donating capabilities in the absence and presence of glucose, respectively, a ground-state binding interaction influencing the ICT (intramolecular charge transfer) within the molecule [11].

In these new probes, Fig. 1, the BA group [$-B(OH)_2$] acts as an electron withdrawing group. However, in the presence of sugar and at an appropriate pH, the boronic acid group is present in its anionic form, namely [$-B^-(OH)$(sugar)] and is no longer an electron withdrawing group. These changes directly influence the electron donating ability of the amino group towards the nitro group, which is an acceptor. Hence spectral changes can be readily observed by modulating the electron density on the boronic acid, in both the presence and absence of sugars. In addition we have also synthesized a control compound, NBDP – 4-(*N*-phenyl)amino-7-nitrobenz-2-oxa-1,3-diazole, which does not contain the boronic acid group and is therefore unperturbed by sugars, in an attempt to understand the signaling mechanism and pH dependence of the probes.

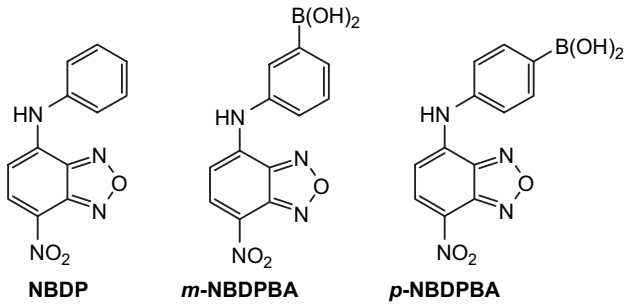

Fig. 1. Molecular structure of the new probes (NBDP: 4-*N*-phenyl-amino-7-nitrobenz-2-oxa-1,3-diazole, *m*-NBDPBA: 4-*N*-(3-borono-phenyl)amino-7-nitrobenz-2-oxa-1,3-diazole and *p*-NBDPBA: 4-*N*-(4-boronophenyl)amino-7-nitrobenz-2-oxa-1,3-diazole).

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma-Aldrich at the highest purity available. The preparation of *o*-, *m*-, *p*-NBDPBA will be published elsewhere in due course as will a report on the anomalous behavior of the *o*-NBDPBA isomer.

2.2. Methods

All solution absorption measurements were performed in $4 \times 1 \times 1$ cm quartz cuvettes (Starna) using a Cary 50 Spectrophotometer from Varian.

Stability (K_S/units mM^{-1}) and dissociation constants (K_D) were obtained by fitting the titration curves with sugar to the relation:

$$I = \frac{I_{min} + I_{max} K_S[\text{sugar}]}{1 + K_S[\text{sugar}]} \quad (1)$$

where I_{min} and I_{max} are the initial (no sugar) and final (plateau) fluorescence intensities of the titration curves, respectively, and $K_D = (1/K_S)$.

3. Results and discussion

The boronic acid moiety is well-known to be pH sensitive, readily forming the $B^-(OH_3)$ at suitable pHs and can thus directly influence ICT transduction [3–11]. In an attempt to understand the pH dependence of the new isomeric probes we considered their response in different pH media, Figs. 2–4.

The control compound, NBDP, shows a pH dependence in absorbance, Fig. 2, which we have attributed to the deprotonation of the –NH group above pH 7. Fig. 2A shows only very slight changes in absorbance below pH 7, evident by the band at 490 nm, whereas above pH 7, a new broad and blue shifted band

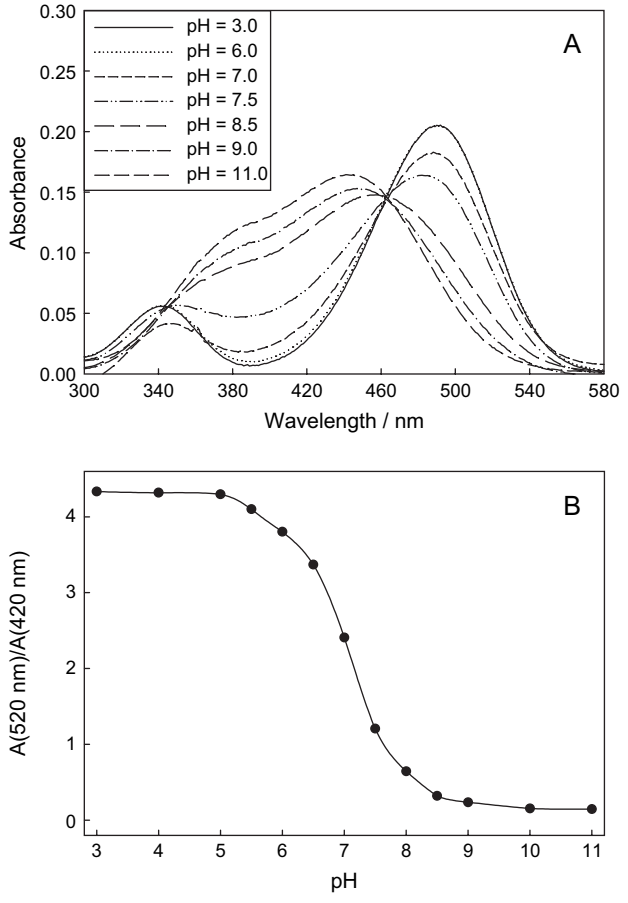

Fig. 2. Absorption spectra of NBDP as a function of pH (A), and the wavelength-ratiometric plot, constructed from the A_{520} and A_{420} absorption values vs solution pH (B).

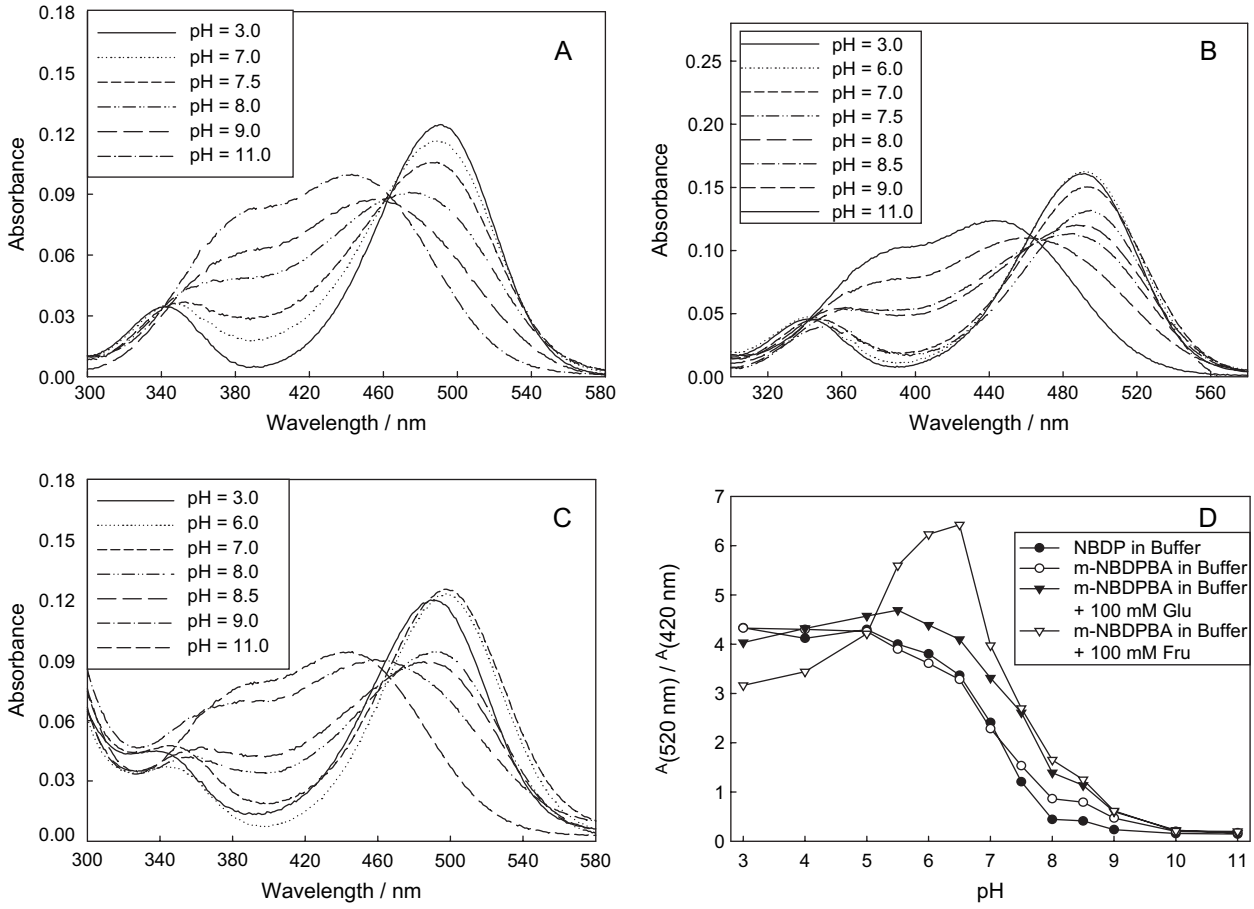

Fig. 3. Absorption spectra of *m*-NBDPBA in the absence (A) and in the presence of 100 mM glucose and fructose (B and C, respectively) as a function of pH, and the corresponding wavelength-ratiometric plots, constructed from the A_{520} and A_{420} absorption values vs solution pH (D).

is present at 400 nm. A plot of A_{520}/A_{420} vs pH gives the amino group p*K*a ≈ 7, Fig. 2B. We speculate that the deprotonation of the amino group above pH 7, further facilitates the intramolecular charge transfer (ICT) to the nitro group. Further it is known that by methylating this nitrogen, the pH dependence is not observed [12]. Hence, the pH dependence can clearly be assigned to the presence of the amino group. It is interesting, however, to comment on the fact that an N^-, present after deprotonation above pH 7, would be a more effective donor, and one would therefore expect that an increased efficiency in ICT would be manifested by a red shifted absorption, and not the blue shift observed. While not directly relevant to this manuscript, as physiologies do not experience any notable changes in pH, this observation is none the least interesting, and we are sure will be the subject of future debate.

Similarly, both *m*- and *p*-NBDPBA show pH dependent absorption spectra, Figs. 3A and 4A. In the presence of 100 mM glucose both probes show an almost identical response to pH, Figs. 3B and 4B, respectively.

By again plotting the ratiometric plots from the A_{520} and A_{420} bands for changes in pH, in the presence of both 100 mM glucose and fructose, we can clearly see a more pronounced effect for fructose, Figs. 3D and 4D. This we attribute to the higher binding affinity (lower K_D) of boronic acid for fructose as compared to glucose [13], Table 1, and the subsequent greater electron density on the boron atom, which in turn influences the extent of interaction of the protonated/deprotonated amino group with the nitro group. Interestingly, by additionally plotting the ratiometric pH response of the control compound NBDP, we can see that the presence of 100 mM glucose has little effect on the spectral changes observed, indicating that the electronic contribution from the [$-B^-$(OH)(glucose)] is minor as compared to that from [$-B^-$(OH)(fructose)].

Given that physiologies do not experience notable changes in pH, we measured the response of both the *m*- and *p*-probes in pH 7 phosphate buffer, Fig. 5. By comparing Fig. 5A and B, we can clearly see the greater response of the mono-phenyl boronic acid derived probes for fructose as compared to glucose. While this

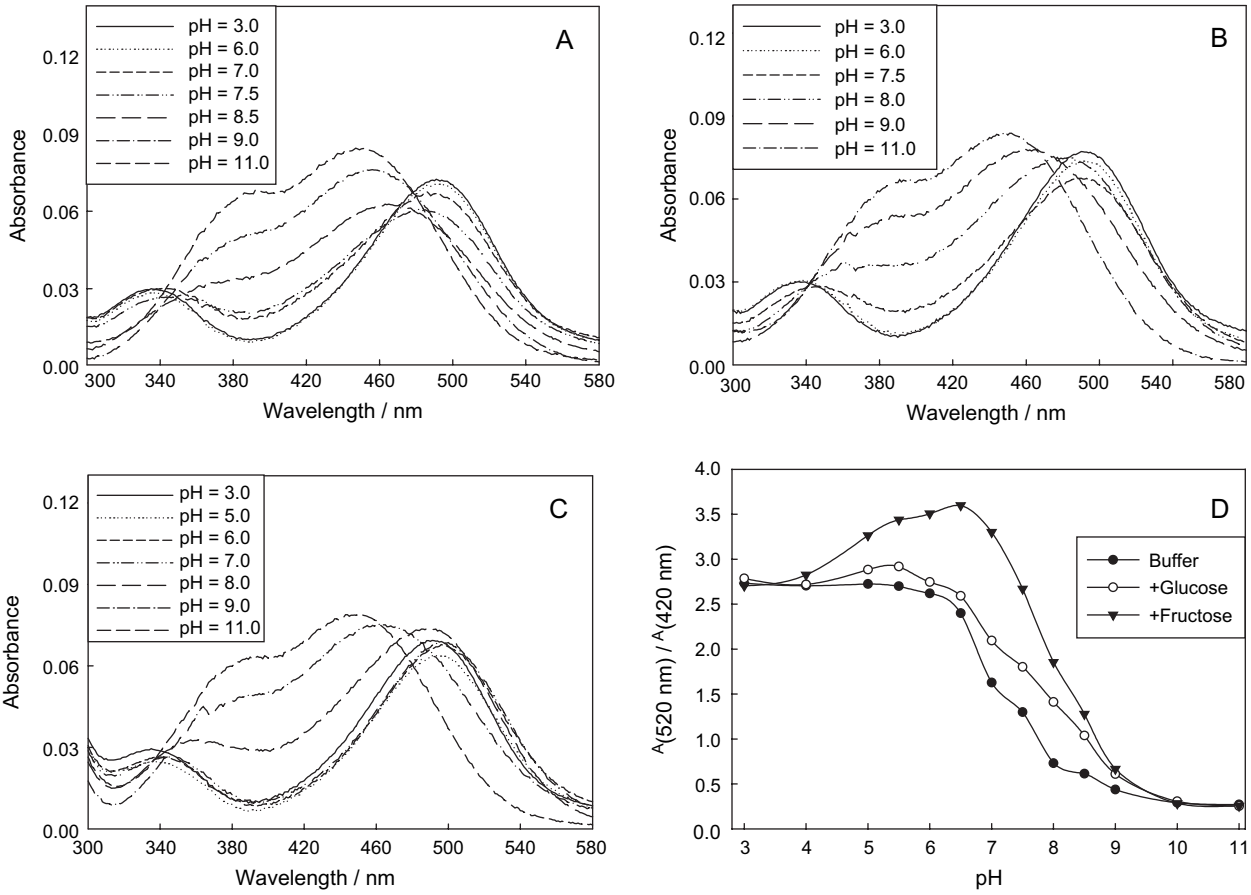

Fig. 4. Absorption spectra of *p*-NBDPBA in the absence (A) and in the presence of 100 mM glucose (B) and fructose (C) as a function of pH, and the corresponding wavelength-ratiometric plots, constructed from the A_{520} and A_{420} absorption values vs solution pH (D).

greater affinity for fructose is well-known [13], it should be noted that the concentration of fructose in physiological fluids, such as in tears or blood, is significantly lower than for glucose [14].

From the sugar binding isotherms, constructed by ratiometrically plotting A_{520}/A_{420} as a function of sugar concentration (Fig. 5C), we were able to determine the disassociation constants, Table 1, using Eq. (1). *m*-NBDPBA shows lower K_Ds (higher affinity) for both sugars suggesting that in these probes the *meta* derivative is much more effective (greater than 2-fold) at influencing the amino group electron density at physiological pH.

Table 1
Dissociation constants for *m*- and *p*-NBDPBA in pH 7.0 phosphate buffer with glucose and fructose

Probe	pH 7.0 buffer with	K_D/mM
m-NBDPBA	Glucose	204
	Fructose	2.12
p-NBDPBA	Glucose	434
	Fructose	8.14

Finally, the changes in the absorption spectra in the presence of sugars, readily lead to visual solution color changes, suggesting the use of these probes for glucose determination in a colorimetric manner, Fig. 6. In closing it is also worth noting that the fluorescence quantum yields of the probes were found to be very low, eliminating their potential use for fluorescence based wavelength-ratiometric measurements.

4. Conclusions

We have developed two new water-soluble probes that show changes in their absorption spectra as a function of [sugar]. These changes readily allow for the wavelength-ratiometric sensing of glucose up to ≈40 mM, which directly corresponds to the blood hyperglycemic range, 2–40 mM glucose [14]. In addition, the new probes readily show colorimetric behavior, making them ideal candidates for simple visual based sensing. The probes' spectral responses are, however, strongly influenced by changes in pH, limiting the utility of the probes to sensing in buffered systems.

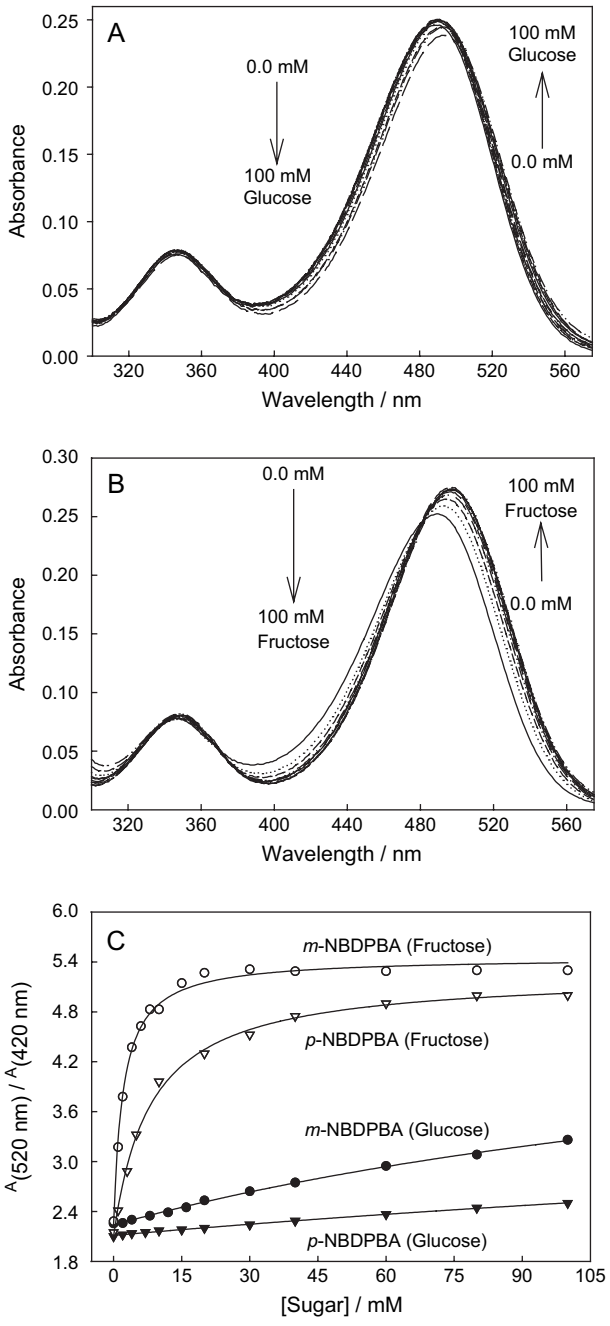

Fig. 5. Absorption spectra of *m*-NBDPBA in pH 7 buffer as a function of both glucose and fructose addition (A and B, respectively), and the corresponding wavelength-ratiometric plots for both probes, constructed from the A_{520} and A_{420} absorption values vs [sugar] (C).

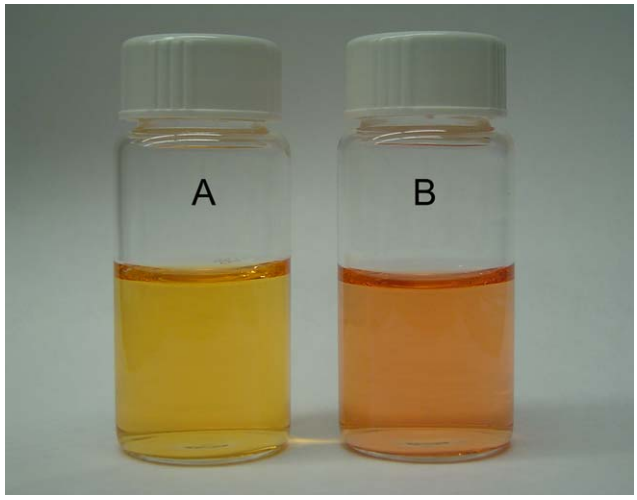

Fig. 6. A photograph of two viols containing equal concentrations of *m*-NBDPBA in pH 7.0 phosphate buffer with 0 (yellow color) and 100 mM fructose (orange color) (A and B, respectively).

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