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Fluorescence sensors for monosaccharides based on the 6-methylquinolinium nucleus and boronic acid moiety: potential application to ophthalmic diagnostics

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Abstract

Continuous monitoring of glucose levels in human physiology is important for the long-term management of diabetes. New signaling methods/probes may provide an improved technology to monitor glucose and other physiologically important analytes. The glucose sensing probes, BMQBAs, fabricated using the 6-methylquinolinium moiety as a fluorescent indicator, and boronic acid as a chelating group, may have versatile applications in glucose sensing because of their unique properties. In this paper we discuss the design logic, synthesis, characterization and spectral properties of three new isomeric glucose sensors (BMQBAs), and a control compound (BMQ) in the presence and absence of sugars. The sensing ability of the new probes is based on a charge neutralization and stabilization mechanism upon sugar binding. The new probes have attractive fluorescence quantum yields, are highly water-soluble, and have spectral characteristics compatible with cheap and portable LEDs and LDs. One of the probes, *o*-BMQBA, has a sugar bound pK_a of 6.1, and a dissociation constant K_D of 100 mM glucose. These probes have been designed specifically to respond to tear glucose in a contact lens polymer for ophthalmic glucose monitoring, where the reduced sugar bound pK_a affords for sensing, in a lens environment that we have previously shown to be mildly acidic.

Keywords: Ophthalmic diagnostics; Fluorescent probes; Glucose sensing; Contact lens; Continuous and non-invasive glucose monitoring

1. Introduction

Diabetes results in long-term health disorders including cardiovascular disease, blindness and cancer [1,2]. To date, a wide variety of methods for glucose analysis have been reported in the research literature, including electrochemistry [3,4], near infrared spectroscopy [5,6], optical rotation [7,8], colorimetric [9,10] and fluorescence detection [11–15]. The most commonly used technology for blood glucose determination is an enzyme-based method [16], which requires frequent blood sampling and therefore drawing. Although frequent "finger pricking" with a small needle to obtain the blood sample is a relatively painless process, this method does suffer from a few practical problems. The first one is inconvenience and the required compliance by patients, while the second is that this is not a continuous monitoring method, with patients tolerating only a few glucose checks a day. Thus, there is a growing interest in the development of continuous non-invasive glucose sensing technologies. To this end, our laboratories have recently made significant progress towards the development of a non-invasive and continuous

Abbreviations: BA, boronic acid; BAFs, boronic acid containing fluorophores; BMQ, N-benzyl-6-methylquinolinium bromide; BMQBA, N-(boronobenzyl)-6-methylquinolinium bromide; LD, laser diode; LED, light emitting diode

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glucose sensing method using a daily disposable, plastic contact lens embedded with intelligent glucose sensitive boronic acid probes [17,18]. This new technology promises to alleviate many of the current problems associated with continuous glucose monitoring and the current invasive methods employed for glucose sensing. Subsequently in this paper, we report the design rationale, new signaling mechanism and synthesis of these new contact lens fluorescent probes.

The boronic acid group has been long known to have high affinity for diol-containing compounds such as carbohydrates [19–21], where the strong complexation has been used for the construction of carbohydrate sensors [22–29], transporters [30], and chromatographic materials [31]. Naturally, boronic acid compounds have been considered as a chelating group for the synthesis of glucose sensors [32–39], where we note the work of Shinkai [32,33], Norrild [34], Lakowicz [35–39] and Drueckhammer [25]. However, the published probes developed for solution (blood/serum)-based measurements are not compatible for glucose sensing within a contact lens, because of the different microenvironment within the lens, in particular, the local pH and polarity [17]. Based on our recent contact lens findings, the pH inside a contact lens is relatively acidic (≈6.0) and the local polarity of the lens is not indifferent than that of methanol. Subsequently, published boronic acid probes embedded within a contact lens typically show a significantly reduced response towards glucose [17]. Hence there is a need to develop suitable fluorescent probe molecules for use in the contact lens. In addition to the environmental parameters and constraints such as pH and polarity, the probes have to be additionally sensitive to the very low concentrations of tear glucose, ≈500 μM, recalling that the blood glucose levels for a healthy person are ≈10-fold higher.

To address the environmental constraints imposed by the contact lens for glucose sensing, we considered lowering the pK_a of the probe. The pK_a of phenyl boronic acid is known to be tunable with the appropriate substituents [39], for example, an electron withdrawing group reduces the pK_a while an electron donating group increases the pK_a of the sugar bound form. We therefore considered the interaction between the quaternary nitrogen of the 6-methylquinolinium moiety, and the boronic acid group, which reduces the pK_a of the probe. In this regard we have synthesized three isomeric boronic acid containing probes, *o*-, *m*- and *p*-BMQBA, where the spacing between the interacting moieties, quaternary nitrogen of the 6-methylquinolinium and boronic acid groups, enables an understanding of the sensing mechanism to be realized. Also, a control compound (BMQ), which does not contain the boronic acid moiety, and is therefore insensitive towards sugar, has been synthesized to understand the spectral properties of the probes (Fig. 1). A detailed photophysical aqueous study of the probes in the presence and in the absence of sugars is discussed in this paper; their response towards glucose within a contact lens is to be presented in a full paper elsewhere.

2. Experimental

2.1. Materials

All chemicals were purchased from Aldrich.

2.2. Methods

All steady-state fluorescence measurements were undertaken in 4 cm × 1 cm × 1 cm fluorometric plastic cuvettes (Sigma), using a Varian Cary Eclipse fluorometer, and all absorption measurements were performed using a Varian UV/VIS 50 spectrophotometer.

Time-resolved intensity decays were measured using reverse start–stop time-correlated single-photon timing (TCSPC), with a Becker and Hickl Gmbh 630 SPC PC card and unamplified MCP-PMT. Vertically polarized excitation at ≈372 nm was obtained using a pulsed LED source (1 MHz repetition rate) and a dichroic sheet polarizer. The instrumental response function was ≈1.1 ns fwhm. The emission was collected at the magic angle (54.7°), using a long pass filter (Edmund Scientific) which cut-off the excitation wavelengths.

2.3. Data analysis

Titration curves with pH were determined in buffer solution: pH 3 and 4 acetate buffer; pH 5–9 phosphate buffer and pH 10 and 11 carbonate buffer. Titration curves were fitted and pK_a (pK_a = $-\log_{10} K_a$) values were obtained using the relation:

$$I = \frac{10^{-\mathrm{pH}} I_{\mathrm{acid}} + K_a I_{\mathrm{base}}}{K_a + 10^{-\mathrm{pH}}} \quad (1)$$

where I_{acid} and I_{base} are the intensity limits in the acid and base regions, respectively.

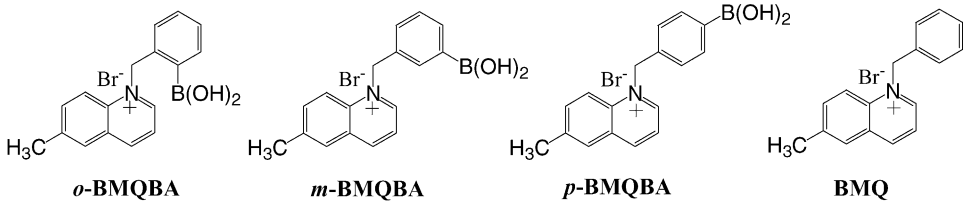

Fig. 1. Molecular structure of *ortho*, *meta* and *para*-BMQBA probes and the control compound BMQ. BMQBA: *N*-(boronobenzyl)-6-methoxyquinolinium bromide, BMQ: *N*-benzyl-6-methoxyquinolinium bromide.