Carbohydrate Sensors

Continuous Glucose Sensing with a Fluorescent Thin-Film Hydrogel**

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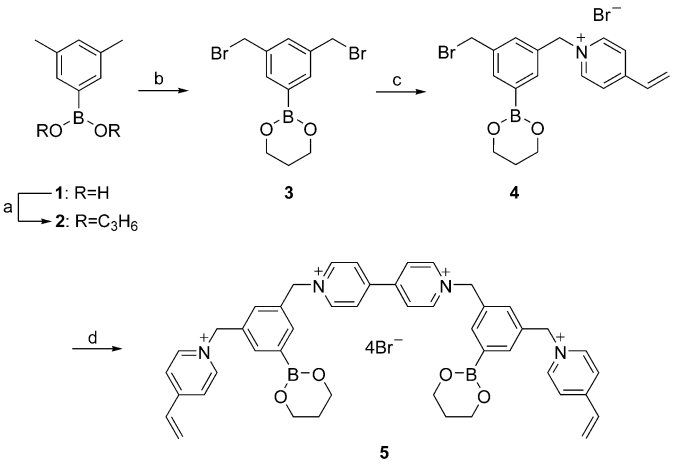
Diabetes mellitus is a chronic disease that impairs the ability of the body to manufacture or use insulin, a hormone necessary to metabolize glucose. Although there is no cure for the disease, tight glucose control substantially reduces morbidity and mortality among diabetes patients.[1] Consequently, there is a consensus that continuous glucose monitoring would be highly beneficial for the diabetic community.[2]

Many different approaches to developing a continuous glucose monitor (CGM) are being pursued.[3] Among them, the enzymatic method, which uses fluorescence-based biosensors, has attracted much attention.[3b,c] In contrast, our approach involves the use of a chemosensor that utilizes the interaction between a negatively charged fluorescent dye and a positively charged boronic-acid-functional quencher.[4]

Boronic acids are known to bind to glucose reversibly under aqueous conditions.[5] When attached to a fluorophore, the acid molecules can modulate the fluorescence as a function of saccharide concentration.[6] In our approach, the boronic acid group is attached to a quencher instead of a dye, and the fluorescence is modulated upon binding of glucose to the boronic-acid-functional quencher. The signal transduction in this two-component system is based on the electrostatic attraction between the fluorophore and the quencher and is a function of the charge on the boron moiety.[4]

For a glucose sensor to be useful in a device, the sensing components must be immobilized to allow real-time monitoring. For in vivo use, the device must operate at physiological temperature, ionic strength, and pH values. In addition, it is preferable to use visible light for optical sensors to avoid complications associated with UV light and autofluorescence. To date, there has been no report of a boronic-acid-based glucose sensor that fulfils all these criteria.[7] We report the development of a two-component sensing system immobilized in a thin film hydrogel. The combination of a cationic boronic-acid-functional quencher **5** and an anionic dye **10** is shown to function as a CGM under physiological conditions.

Viologen **5** was prepared as indicated in Scheme 1. Commercially available 3,5-dimethylphenylboronic acid was first protected as a 1,3-propanediol ester in dichloroethane and then treated with NBS to give, after recrystallization from CH_3OH, the dibrominated species **3** in 52% yield. Reaction of **3** with vinylpyridine in a CH_2Cl_2/CH_3OH solution[8] gave



Scheme 1. Synthesis of positively charged quencher monomer **5**. a) 1,3-propanediol, $C_2H_4Cl_2$, CaH_2, reflux 3 h; b) *N*-bromosuccinimide (NBS), azobisisobutyronitrile, $C_2H_4Cl_2$, reflux, 3 h, 52%, 2 steps; c) vinylpyridine, CH_2Cl_2/CH_3OH (3:1), 40°C, 22 h, 52%; d) 4,4′-bipyridine, CH_3OH/dimethyl formamide (3:1), 40°C, 42 h, 39%.

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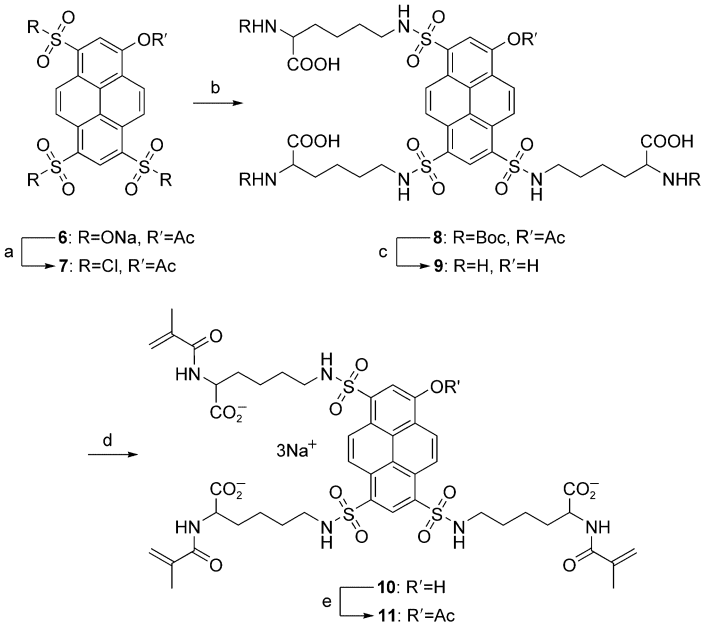
the monobromomethylphenylboronate **4**. Further quaternization with 4,4′-dipyridyl and chromatographic purification on C18-silica gel afforded tetracationic viologen **5** in 39%.

Monomeric dye **10** was synthesized in five steps from the commercially available sodium salt **6** (Scheme 2). Chlorination followed by sulfonamide formation gave the Boc-protected derivative **8** in 88%. Deprotection and reaction with methacryol chloride under standard conditions afforded the trifunctionalized derivative **10**, which was converted into the acetoxy-protected monomer **11** for use in polymerization.



Scheme 2. Synthesis of negatively charged dye monomer **11**. a) $SOCl_2$, reflux, 3 h, then H_2O, 81%; b) Boc-lysine, NaOH, terahydrofuran/CH_3CN/H_2O (2:7:1), RT, 24 h, then HCl, 94%; c) trifluroacetic acid, RT, 2 h, 65%; d) methacroyl chloride, H_2O, NaOH, RT, 1 h, 17%; e) acetic anhydride, NaOAc, 24 h, 70%. Boc = *tert*-butoxycarbonyl; Ac = acyl.

Pyranine derivatives functionalized with three sulfonamides have shifted excitation and emission peaks in comparison to unfunctionalized pyranine ($\lambda_{ex} = 454$ nm and $\lambda_{em} = 510$ nm)[9] and this shift is observed for **10**, for which $\lambda_{ex} = 491$ nm and $\lambda_{em} = 540$ nm.

To evaluate the ability of **5** to quench the fluorescence of **10** in the absence and presence of different saccharides, fluorescence spectroscopic measurements were carried out in aqueous buffer solutions at pH 7.4. Upon titrating **10** with **5**, significant fluorescence quenching was observed (Figure 1), with an apparent Stern–Volmer quenching constant of $K_{sv} = 1.3 \times 10^4\,\text{M}^{-1}$. Titration of **10** with **5** in the presence of 5 mM glucose decreased the quenching efficiency of **5** ($K_{sv} = 7.2 \times 10^3\,\text{M}^{-1}$).

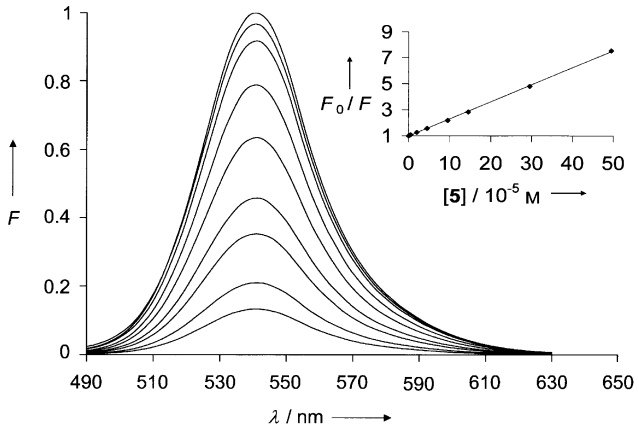
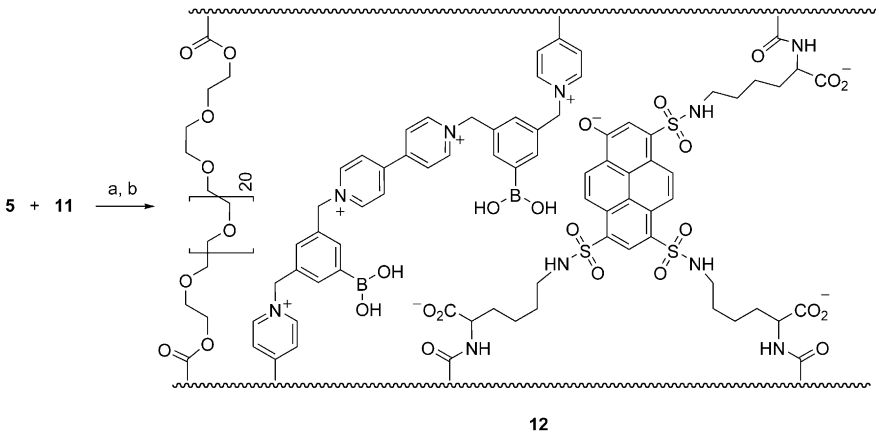


Figure 1. Fluorescence emission spectra of **10** (4×10^{-6} M) with increasing concentrations of **5**. Inset: Stern–Volmer plot, where F is the fluorescence intensity of **10** in the presence of **5** and F_0 is the fluorescence intensity of **10** in the absence of **5**. $\lambda_{ex} = 470$ nm.

The relative affinity of **5** towards different saccharides was then determined. Titration of solutions containing **10** (4×10^{-6} M) and **5** (1.2×10^{-4} M) were carried out with fructose, glucose, and galactose. Binding constants for each sugar were calculated from the binding isotherms.[10] The measured affinities are ranked in the order: fructose ($2063\,\text{M}^{-1}$) > glucose ($275\,\text{M}^{-1}$) > galactose ($210\,\text{M}^{-1}$). Phenyl boronic acids normally show a 3:1 greater affinity for galactose over that for glucose[6] but **5** binds glucose more strongly than galactose.

With the quenching and sugar-sensing ability of the dye/quencher combination established, the sensing elements were immobilized in a thin film (Scheme 3). A 50% aqueous solution containing **5, 11**, 2-hydroxyethyl methacrylate, polyethyleneglycol dimethacrylate, and an initiator was injected between two glass plates separated by a 25.4-μm teflon spacer. Free-radical polymerization was carried out at 40 °C for 15 h. The resultant film was leached in pH 9 buffer for one day and equilibrated in pH 7.4 buffer for one day. The swollen



Scheme 3. Synthesis of a glucose-sensing polymer **12** with its sensing elements in proximity to one another. a) 2-Hydroxyethyl methacrylate, polyethylene glycol dimethacrylate ($M_W = 1000$), VA-044 (initiator), 40 °C, 15 h; b) NaOH solution, pH 9, 24 h.

hydrogel was found to contain 40% water (by weight). The deprotected film was subsequently tested for its sugar-sensing ability.

The hydrogel was mounted into a flow cell and phosphate buffer of ionic strength 0.1M was circulated through the cell. The film was excited at 470 nm by front-face illumination and the emission at 540 nm was monitored over time. The temperature was kept constant at 37°C. After a stable baseline had been obtained, the buffer solution was replaced with saccharide solution and the change in fluorescence intensity was measured. As indicated in Figure 2, the infusion

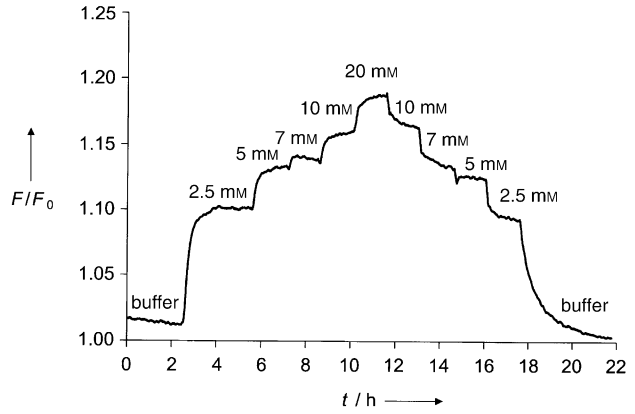


Figure 2. Relative fluorescence intensity change of emmission from **12** over time in the presence of a range of concentrations of glucose. F is the fluorescence intensity of **12** in the presence of glucose and F_0 is the fluorescence intensity of **12** in the absence of glucose. $\lambda_{ex} = 470$ nm and $\lambda_{em} = 540$ nm.

of glucose into the hydrogel resulted in a stepwise change in fluorescence intensity that was dependent on sugar concentration. Importantly, the sensor detected glucose in the physiological range of 2.5–20 mM. Moreover, the changes in fluorescence were completely reversible. Similar profiles were obtained for fructose and galactose and the apparent binding constants for each sugar were determined. The selectivity for each saccharide changed once the components were immobilized in a polymer. The immobilized system is more selective for glucose and less selective for fructose and galactose than the free components; the relative affinities are: fructose ($666\,M^{-1}$) > glucose ($333\,M^{-1}$) > galactose ($111\,M^{-1}$).

Hydrogels are known to mimic the behavior of polymer chains in solution in that a high degree of segmental mobility is possible even though long-range diffusion cannot occur. Functional groups attached to the polymer chains are free to move about and interact at least locally. This movement appears to occur in **12**. Since the quenching mechanism appears to be predominately static,[11] the dye and quencher units in the polymer chain must have the freedom to associate and dissociate within the polymer matrix depending on the local saccharide concentration.

The saccharide-sensitive hydrogel reported herein shows promise as the basis for a continuous glucose monitoring system. Through a reversible electrostatic interaction within its polymer matrix, the chemosensor **12** is able to 1) detect glucose in the physiologically important range of 2.5–20 mM and 2) operate under physiological conditions (37°C, 0.1 μM ionic strength, pH 7.4). Moreover, the fluorescence signal obtained is completely reversible, which allows real-time monitoring of glucose levels. Further studies are underway to determine the scope of this approach in continuous glucose monitoring.

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[1] The Diabetes Control and Complications Trial Research Group, *N. Engl. J. Med.* **1993**, *329*, 977.
[2] G. Freckmann, B. Kalatz, B. Pfeiffer, U. Hoss, C. Haug, *Exp. Clin. Endocrinol. Diabetes* **2001**, *109*, S347.
[3] a) T. Koschinsky, L. Heinemann, *Diabetes/Metab. Res. Rev.* **2001**, *17*, 113; b) R. J. Russell, M. V. Pishko, C. C. Gefrides, M. J. McShane, G. L. Cote, *Anal. Chem.* **1999**, *71*, 3126; c) R. Ballerstadt, J. S. Schultz, *Anal. Chem.* **2000**, *72*, 4185.
[4] a) J. T. Suri, D. B. Cordes, F. E. Cappuccio, R. A. Wessling, B. Singaram, *Langmuir* **2003**, *19*, 5145; b) J. N. Camara, J. T. Suri, F. E. Cappuccio, R. A. Wessling, B. Singaram, *Tetrahedron Lett.* **2002**, *43*, 1139.
[5] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769.
[6] T. D. James, S. Shinkai, *Top. Curr. Chem.* **2002**, *218*, 159.
[7] Boronic acid based sensors have been prepared that use UV light as the excitation source, see A. E. Colvin, U.S. Pat. Appl. Publ. 2002039793, **2002**.
[8] Vinylpyridinium salts are very unstable and are known to spontaneously polymerize, see a) T. Kanbara, M. G. Mikhael, A. B. Padias, H. K. Hall, *J. Polym. Sci. Part A* **1997**, *35*, 2787; b) S. A. Ross, M. Pitie, B. J. Meunier, *J. Chem. Soc. Perkin Trans. 1* **2000**, 571; c) W. K. Fife, P. Ranganathan, M. A. Zeldin, *J. Org. Chem.* **1990**, *55*, 5610. We found that the use of methanol as a cosolvent inhibits polymerization.
[9] a) E. Koller, R. Moser, *Appl. Fluoresc. Technol.* **1989**, *1*, 15; b) G. J. Mohr, T. Werner, O. S. Wolfbeis, *J. Fluoresc.* **1995**, *5*, 135.
[10] K. A. Connors, *Binding Constants—The measurement of Molecular Complex Stability*, Wiley, New York, **1987**.
[11] UV/Vis measurements in solution studies confirmed the formation of a ground-state complex between **5** and **10**.

