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High Ionic Strength Glucose-Sensing Photonic Crystal

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We demonstrate a colorimetric glucose recognition material consisting of a crystalline colloidal array embedded within a polyacrylamide−poly(ethylene glycol) (PEG) hydrogel, or a polyacrylamide−15-crown-5 hydrogel, with pendent phenylboronic acid groups. We utilize a new molecular recognition motif, in which boronic acid and PEG (or crown ether) functional groups are prepositioned in a photonic crystal hydrogel, such that glucose self-assembles these functional groups into a supramolecular complex. The formation of the complex results in an increase in the hydrogel cross-linking, which for physiologically relevant glucose concentration blue shifts the photonic crystal diffraction. The visually evident diffraction color shifts across the visible spectral region over physiologically important glucose concentration ranges. These materials respond to glucose at physiological ionic strengths and pH values and are selective in their mode of response for glucose over galactose, mannose, and fructose. Thus, we have developed a new recognition motif for glucose that shows promise for the fabrication of noninvasive or minimally invasive in vivo glucose sensing for patients with diabetes mellitus.

There is an ever increasing demand for continuous, noninvasive glucose monitoring due to the increasing number of people diagnosed with diabetes mellitus (both type I, insulin dependent, and type II, insulin independent).[1] The need for minimally invasive glucose monitoring has also increased due to the recognition that the long-term health outcome of patients with diabetes mellitus can be dramatically improved by careful glucose monitoring and control.[2] The development of accurate, reliable, continuous, and noninvasive glucose sensors would significantly improve the lives of patients with diabetes and decrease the long-term complications of persistent hyperglycemia and the immediate risks of hypoglycemia.

This need for glucose sensors has motivated the investigations of numerous approaches.[2] In the work here, we describe the fabrication of a new photonic crystal glucose-sensing hydrogel material that can determine physiologically relevant concentrations of glucose at the ionic strengths typical of bodily fluids. As shown in Figure 1, this glucose-sensing material (denoted as a PCCA) consists of a polyacrylamide hydrogel with an embedded crystalline colloidal array (CCA) that diffracts light to report on its lattice constant and, thus, on the hydrogel volume. We previously demonstrated the use of this photonic crystal sensor motif to sense metal cations, pH, ionic strength, and glucose.[3−5]

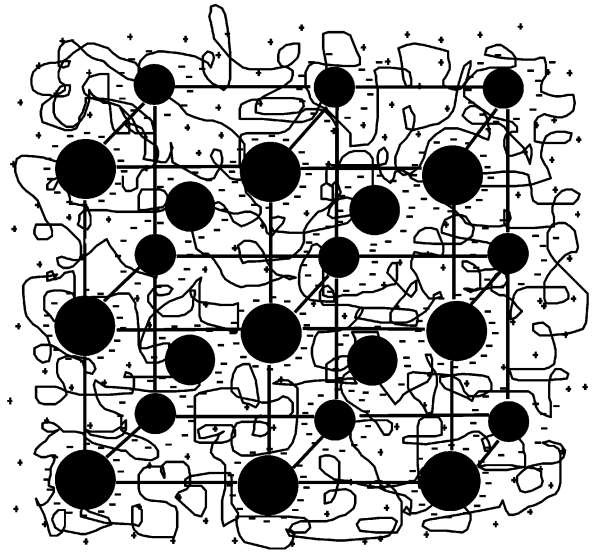

Figure 1. Polymerized crystalline colloidal array photonic crystal sensing material consisting of a crystalline colloidal array (generally fcc or bcc of ∼100-nm colloidal particles) embedded in a polymer hydrogel network containing a molecular recognition element. The embedded CCA of polystyrene colloidal particles efficiently diffracts light of a wavelength determined by the lattice constant. The diffracted wavelength is altered by hydrogel volume changes induced by interactions of the analyte with the molecular recognition agent.

Our first glucose sensor PCCA utilized glucose oxidase (GOx) as the molecular recognition element. The GOx conversion of glucose to gluconic acid reduced the GOx FAD prosthetic group, which became negatively charged. The formation of these covalently bound hydrogel anions resulted in a Donnan potential,

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which resulted in an osmotic pressure that caused the hydrogel to swell, which red shifted the Bragg diffraction.[3,4] Unfortunately, the utility of this motif for measuring glucose was limited to low ionic strength solutions. Thus, this motif could not be used to sense glucose at the ionic strengths typical of bodily fluids. Its response also depended upon the oxygen concentration (which reoxidized the FAD). In addition, the accompanying production of hydrogen peroxide was likely to be problematic for use of this sensor in some applications.

We recently reported the development of a new glucose-sensing polymerized crystalline colloidal array (PCCA), which utilizes phenylboronic acids as the glucose recognition element.[6] The ability of boronic acids to reversibly bind sugars has long been recognized,[7] and there is now a large body of literature on boronic acid-based carbohydrate sensors.[8−18] Boronic acid derivatives have been polymerized in the presence of sugars to create imprinted sugar-binding sites,[19,20] which have been utilized, for example, for the racemic resolution of sugars.[19] Polymers containing boronic acid groups have also been used for affinity chromatography.[21,22] In addition, boronic acid derivatives have been utilized to prepare carbohydrate-responsive hydrogels and polymers, for glucose sensing, and for insulin delivery purposes.[23−26] The use of boronic acids for carbohydrate sensing has been recently reviewed by James and Shinkai.[27]

This report is the second in a series of papers devoted to our use of boronic acids as a molecular recognition element in PCCA. Our objective is to utilize these photonic crystal glucose-sensing materials for in vivo glucose sensing. In our previous publication,[6] we demonstrated that a phenylboronic acid PCCA could sense glucose due to the decrease in the boronic acid pK_a upon glucose binding. The glucose binding induced the formation of boronate anions, which resulted in a Donnan potential, which gave rise to an osmotic pressure, which swelled the PCCA and red-shifted the diffraction. Unfortunately, this glucose-sensing material does not operate at the high ionic strengths of bodily fluids.

In this publication, we describe a more sophisticated boronic acid complexation motif designed for glucose sensing at physiological salinities. We carefully investigated the glucose-sensing mechanism and propose that it involves formation of a supramolecular bis-bidentate glucose−boronic acid complex, which is stabilized by PEG or crown ether-capped sodium cations. This bis-bidentate complex cross-links the hydrogel and shrinks the hydrogel volume, which blue shifts the photonic crystal diffraction from the embedded CCA. The visually evident diffraction color shifts across the visible spectral region, over the physiologically important glucose concentration ranges. Although all cis diol sugars bind to the boronates as mono-bidentate complexes, glucose is relatively unique within the physiologically important sugars in that it can bind at two cis diol sites to cross-link boronate sites.

In the final paper of this series,[28] we will discuss the chemistry involved in optimizing this supramolecular complex for detecting glucose in vivo. We will describe the optimization of the response of these sensors for detecting glucose at the high concentrations found in blood and in interstitial fluids and for determining glucose at the lower concentrations found in tear fluid.

EXPERIMENTAL SECTION

We fabricated our second-generation glucose-sensing PCCA by using a procedure essentially identical to that previously described[6] except that we also copolymerized 400-Da poly(ethylene glycol) monomethacrylate (PEG, Polysciences) or 4-acryloylamidobenzo-15-crown-5 (Acros Organics). The PEG was cleaned by exposing it to ion-exchange resin to remove ionic impurities. Typically, we used 80 mg of acrylamide (AA, Fluka), 20 mg of PEG, 2 g of CCA, 5 mg of cross-linker (methylenebisacrylamide, Fluka), and 3.8 μmol of photoinitiator (diethoxyacetophenone, Acros Organics).

The crown ether PCCA was prepared by copolymerization of a mixture of 100 mg of AA, 100 mg of 4-acryloylamidobenzo-15-crown-5, 3.0 g of CCA solution, 2 mg of methylenebisacrylamide, and 3.8 μmol of photoinitiator.

Chemical modification of the hydrogel backbone was accomplished as previously described[6] by hydrolysis to yield carboxylic groups, which were used to attach boronic acids to the acrylamide through carbodiimide coupling. We utilized either 3-aminophenylboronic acid (Acros Organics) or 4-amino-3-fluorophenylboronic acid synthesized in our laboratory.[28]

Diffraction from the sensing materials was measured at normal incidence by using a SI 400 (model 430/440) diode array spectrometer (Spectral Instruments).

RESULTS AND DISCUSSION

Response of the Sensor to Glucose. Figure 2A shows the glucose concentration dependence of diffraction of our boronic acid−polyacrylamide−PEG (BA−AA−PEG) photonic crystal sens-

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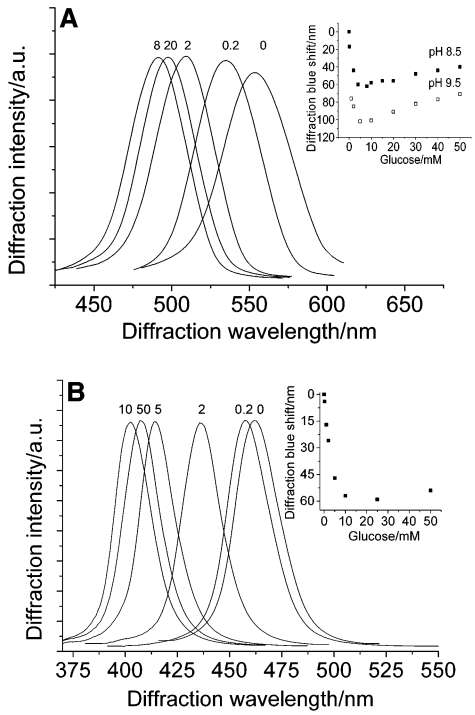

Figure 2. (A) Glucose concentration dependence of the diffraction spectrum of the BA−AA−PEG sensor in an aqueous solution containing 2 mM tris-HCl (pH 8.5) and 150 mM NaCl. Concentration of glucose (in mM) is shown above the diffraction peaks. Inset: Diffraction peak shift dependence on glucose concentration of the BA−AA−PEG sensor in 2 mM tris-HCl (pH 8.5) and 150 mM NaCl and in a solution containing 100 mM sodium carbonate−bicarbonate buffer (pH 9.5) and 150 mM NaCl. (B) Glucose concentration dependence of diffraction of the 4-amino-3-fluorophenylboronic acid BA−AA−PEG sensor in 2 mM tris-HCl (pH 7.4) and 150 mM NaCl. Inset: Dependence of diffraction peak shift on glucose concentration.

ing material in a pH 8.5, 2 mM tris-HCl buffer solution containing 150 mM NaCl. The spectral peaks result from diffraction by the 111 plane of the embedded face-centered cubic CCA. In the absence of glucose, the sensor diffracts 555-nm yellow-green light. The diffraction blue shifts for increasing glucose concentrations up until 8−10 mM. For example, the sensor diffracts 491-nm blue light at 8 mM. Further increases in glucose concentration cause diffraction red shifts. For example, at 20 mM glucose, the diffraction red shifts to 497 nm. The Figure 2A inset compares the sensor response for solutions at pH 8.5 and 9.5. The magnitude of the response is larger at pH 9.5 than at 8.5, presumably due to the increased population of the boronate form, which has a higher glucose affinity.[6] There is little or no response of these sensors to glucose at the physiological pH values of 7.4.

Figure 2B shows the response to glucose of a BA−AA−PEG sensor that contains 4-amino-3-fluorophenylboronic acid. This sensor responds to glucose under lower pH conditions and, thus, is able to sense glucose at physiological pH and salinity values (pH 7.4, 150 mM NaCl). This sensor responds in a manner similar to that of the phenylboronic acid BA−AA−PEG. For low concentrations of glucose, the diffraction blue shifts as the glucose concentration increases, while at higher glucose concentrations, the diffraction red shifts as the glucose concentration increases.

The response of these BA−AA−PEG sensors to glucose (Figure 2) differs dramatically from that of our previous BA−AA sensors.[6] Our previous BA−AA sensors (which do not contain PEG or crown ether) show no response to glucose at high ionic strength. At low ionic strengths (<5 mM NaCl), the BA−AA sensors red shift because glucose binding decreases the boronic acid pK_a. Glucose binding, thus, creates boronate anions covalently linked to the hydrogel, which gives rise to a Donnan potential that induces gel swelling. As discussed previously,[6] this ionically driven swelling does not operate at the high ionic strengths typical of bodily fluids.

The low glucose concentration BA−AA−PEG diffraction blue shifts must result from hydrogel shrinking. As discussed below, this shrinking appears to result from an increase in the hydrogel elastic constant.[29] The solution ionic strength is too high to permit Donnan potential shrinkage or swelling due to changes in the number of bound charged species.[5]

This elastic constant increase is likely to result from an increase in the hydrogel cross-link density due to the formation of glucose cross-links between two boronates in the hydrogel. This glucose−bis(boronate) complex is similar to the D-glucose and D-allose cross-linking of two boronates demonstrated by Shinkai et al.[30] for a bis(boronic acid) crown ether complex or for D-glucose and diphenylmethane-3,3′-diboronic acid.[31]

A less likely mechanism for the hydrogel shrinkage at low glucose concentrations is a decreased free energy of mixing of the hydrogel upon glucose binding. This seems unlikely because boronic acid glucose binding shifts the equilibrium toward the boronate form, which should have a larger free energy of mixing with water and, thus, should cause swelling.

The experimental work below examines the response of our sensor materials to different sugars and to changes in the PCCA composition in order to elucidate the underlying mechanism of the hydrogel glucose response. Also we theoretically modeled the observed gel volume changes in response to sugar binding using a refined hydrogel volume-phase transition model[5] in the framework of Flory's ionic polymer network model.[29]

Dependence of BA−AA−PEG PCCA Response on Sugar Structure. The pH dependence of the BA−AA−PEG sensor response (Figure 2) clearly indicates that binding of glucose to the tetrahedral *boronate* species is crucial to the sensor response mechanism. Boronates are known to strongly bind to species with appropriately situated vicinal alcohol functional groups such as sugars. Chart 1 shows the sugars we examined in our studies. These sugars can show numerous conformations both in solution and when bound to the boronates. For example, sugars can bind in their furanose or pyranose forms and in their chair or boat conformations.

For example, Shinkai et al. demonstrated high-affinity glucose binding to a series of bis(boronic acid) derivatives. For diphenylmethane-3,3′-diboronic acid,[31,32] the pyranose form of glucose forms a bis-bidentate complex with the two boronic acids that bind glucose at the 4-OH, 5-CH_2OH, and 1,2-diols. The work of Norrild

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Chart 1. Structures of Examined Monosaccharides

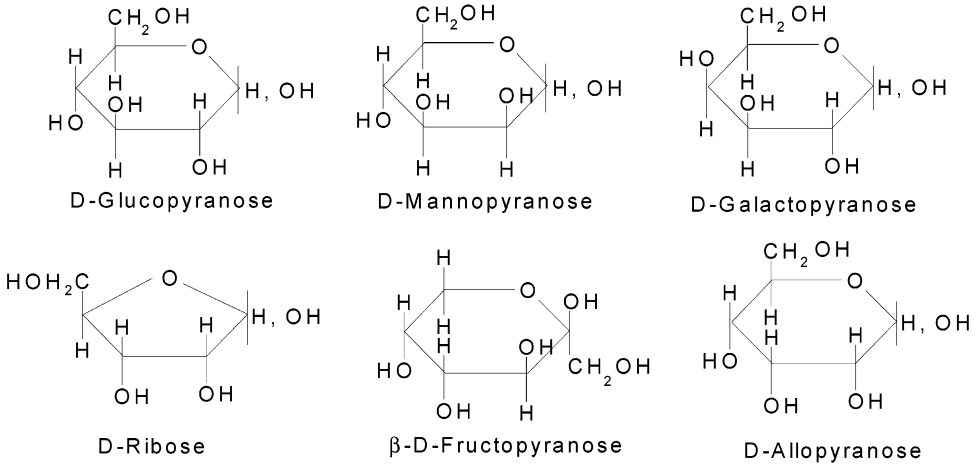
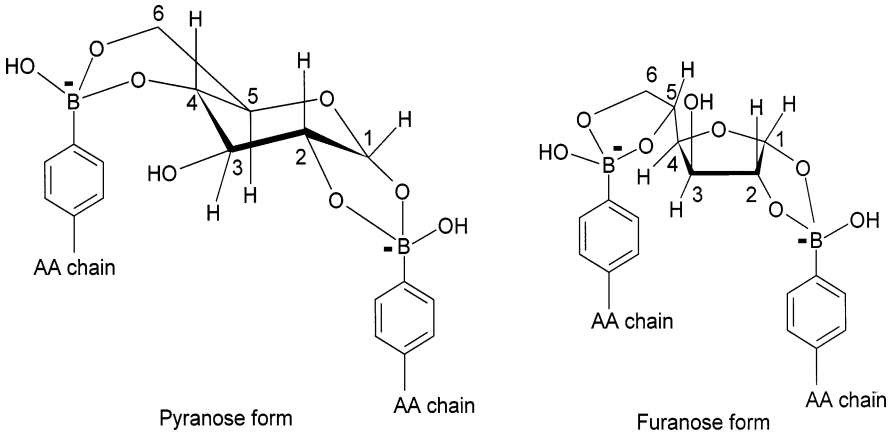


Chart 2. Proposed Glucose Bis(boronate) Complex



et al.[13,33,34] also shows that glucose can form a bis-bidentate complex with boronic acid derivatives; however, Norrild found that glucose binds in its furanose form through 1,2- and 3,5-diols in neutral aqueous solutions or even through 1,2- and 3,5,6-diols in aqueous alkaline solutions.[34] Although controversies still remain concerning the glucose conformations that bind to boronic acid derivatives,[35] we conclude that D-glucose forms cross-links within the BA−AA−PEG hydrogel with a geometry similar to that shown in Chart 2.

Shinkai et al. also observed glucose−bis(boronate) complexes in a bis(boronic acid)−ferrocene derivative.[36] They found that D-galactose and D-mannose did not form bis-bidentate complexes. This presumably results from the fact that in D-glucose the 2-hydroxy and 4-hydroxy groups adopt the same configuration that easily binds to the boronate, while in D-mannose and D-galactose, these hydroxyl groups adopt opposing configurations. Shinkai et al.[31] concluded that this leads to steric distortions that prevent D-galactose and D-mannose from forming bis-bidentate complexes.

Our proposed glucose−bis(boronate) complex is also similar to those found in the D-glucose and D-allose complexes of a crown ether bis(boronic acid) derivative.[37] This crown ether bis(boronic acid) derivative does not form a bis-bidentate complex with D-fructose. As shown in Figure 3A, these results parallel those found for our BA−AA−PEG, where identical concentrations of D-mannose, D-galactose, and D-fructose give rise to increasing large red shifts, while Figure 3B shows that D-ribose and D-allose give rise to blue shifts smaller than that induced by 4 mM glucose. We interpret the diffraction blue shifts to result from glucose−, ribose−, and allose−bis(boronate) complexes, which cross-link the hydrogel, while red shifts result from mono-bidentate sugar−boronic acid binding.

Although it is not yet clear in detail how sugar hydroxyl conformational differences impact the affinity and stoichiometry of boronic acid binding, the magnitudes of our observed red shifts closely parallel the affinities Lorand et al.[7] observed for 1:1 sugar−boronate complexes of mannose, galactose, and fructose (Table 1). The configurations of D-allose and D-ribose at the 1,2 positions are identical to glucose, as is the 4,6 diol configuration of D-allose and the 3,5-diol configuration of D-ribose. We conclude that these are the sites of binding of these sugars to two boronic acids.

We are aware of Norrild et al.'s[38] NMR study of a tolylboronate species, which demonstrates bis-bidentate- and mono-tridentate fructose binding. However, this behavior was only observed at

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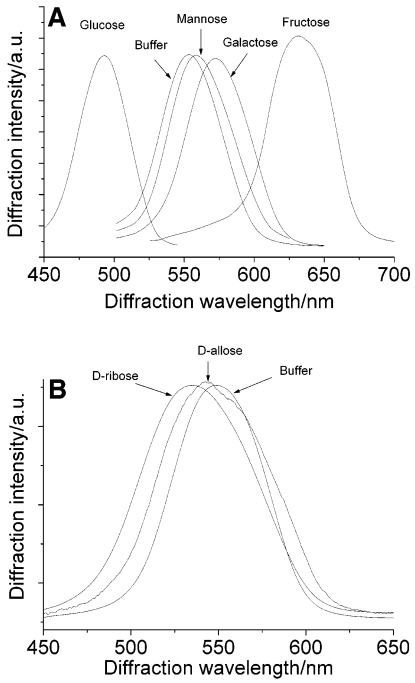


Figure 3. Response of the BA−AA−PEG sensor to (A) 4 mM concentrations of D-glucose, D-mannose, D-galactose, and D-fructose; (B) 4 mM concentrations of D-allose and D-ribose in 2 mM tris-HCl, pH 8.5, 150 mM NaCl solution.

Table 1. Formation Constants for Polyol−Phenylboronic Acid Complexes *K* (According to Lorand et al.[7])

polyol	*K*
poly(vinyl alcohol)	1.9
ethylene glycol	2.76
D-glucose	110
D-mannose	172
D-galactose	276
D-fructose	4370

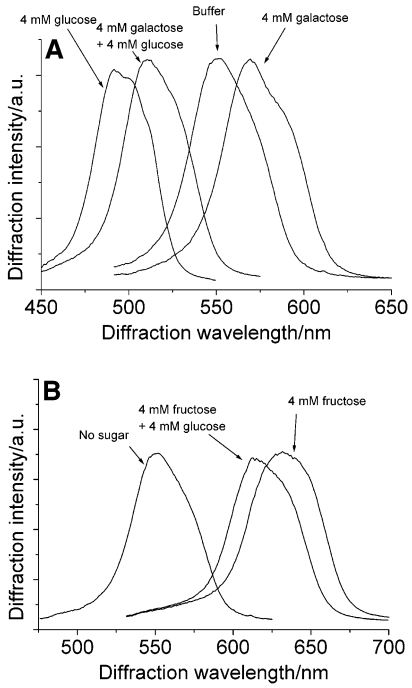


Figure 4. Response of BA−AA−PEG sensor to (A) 4 mM D-glucose, 4 mM D-galactose and to a mixture of 4 mM D-glucose and 4 mM D-galactose; (B) 4 mM D-fructose and to a solution containing 4 mM concentrations of both D-fructose and D-glucose. The solutions also contained 2 mM tris-HCl, pH 8.5, and 150 mM NaCl.

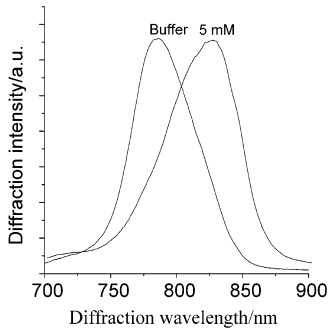


Figure 5. Response of the BA−AA−PEG sensor to a 5 mM concentration of glucose in a pH 8.5 solution containing 2 mM tris-HCl in the absence of NaCl.

high pH and at high tolylboronate concentrations; fructose cross-linking is unlikely to occur under our experimental conditions.

Our proposal is also supported by the observation (Figure 4) that 4 mM of glucose competitively binds in the presence of 4 mM galactose or fructose. This is shown by the observation that a blue shift occurs upon glucose addition. We presume that the red shifts observed at higher glucose concentrations result from saturation of the boronate-binding sites, where the equilibrium is shifted toward a 1:1 BA−glucose complex. This is supported by the observation that addition of free BA to a pH 8.5 solution containing 5 mM glucose and 150 mM NaCl causes the BA−AA−PEG sensor to red shift 10 nm. Our hypothesis is that this red shift results from competition of the free BA for the hydrogel-bound glucose.

Dependence of Response on PEG and Cations. There is no response of the BA−AA PCCA to glucose in a 150 mM NaCl solution at pH 8.5 in the absence of PEG, indicating that glucose cross-linking of BA does not occur without PEG. However, in the absence of NaCl, the BA−AA−PEG diffraction red shifts upon addition of 5 mM glucose (pH 8.5 buffered solution) due to formation of boronate anions (Figure 5). Thus, in the absence of NaCl, glucose appears to complex to single boronic acid groups; we conclude that the putative glucose cross-linking requires both NaCl and PEG.

We studied the dependence of the BA−AA−PEG and BA−AA PCCA diffraction on different salts (Figure 6). The Figure 6 inset shows the NaCl concentration dependence of the diffraction of a simple polyacrylamide−bis(acrylamide) PCCA (AA), a hydrolyzed AA, and a BA−AA PCCA. The AA PCCA shows little diffraction dependence on NaCl concentration except at the highest NaCl concentrations, where there is a small diffraction red shift. This presumably results from an increased AA hydrogel free energy of mixing at >1 M NaCl. In contrast, a hydrolyzed AA PCCA contains numerous free carboxylate groups, which are ionized at neutral pH and cause the PCCA to swell in pure water and the diffraction to red shift. Addition of even 1 mM NaCl causes a ∼90-nm blue shift as the Donnan potential becomes swamped.[5] The BA-AA PCCA as synthesized also contains a few carboxylates; it blue shifts ∼20 nm, a much smaller amount than the hydrolyzed AA PCCA.

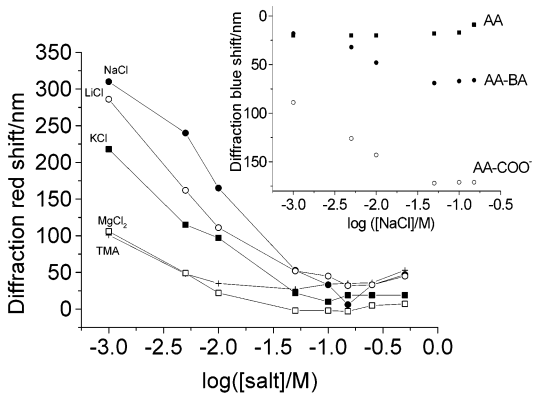


Figure 6. Salt concentration dependence of diffraction of the BA−AA−PEG PCCA. (TMA, tetramethylammonium chloride). Inset: Dependence of diffraction of an AA PCCA, a hydrolyzed AA PCCA (AA-COO^-), and a BA-AA PCCA on the NaCl concentration. The diffraction shift is relative to that in pure water.

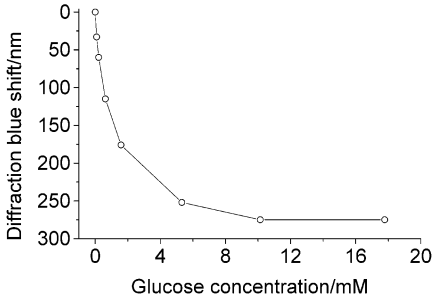
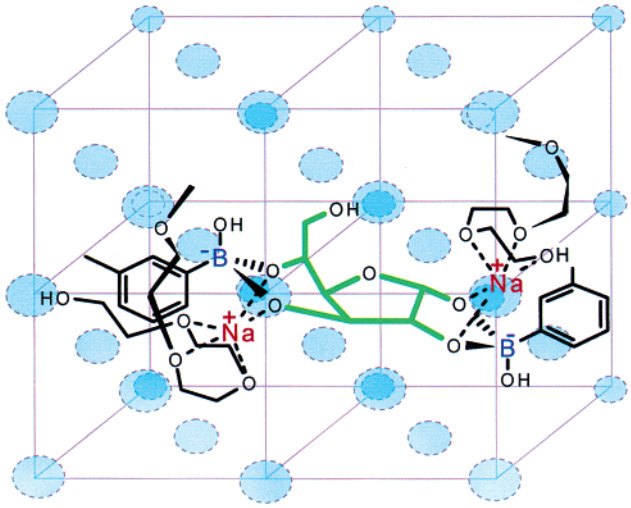


Figure 7. Glucose concentration dependence of the diffraction of the BA-AA PCCA with covalently attached 4-acryloylamidobenzo-15-crown-5 in aqueous solution of 2 mM tris-HCl, pH 8.5, 150 mM NaCl.

Chart 3. Bis-Bidentate Complex Formation between Glucose (in Furanose Form) and Two Boronates Stabilized by PEG−Na^+ Complex



In marked contrast, the BA−AA−PEG sensor red shifts dramatically upon addition of even 1 mM concentrations of NaCl, LiCl, KCl, $MgCl_2$, and TMA. For example, 1 mM NaCl red shifts the diffraction by 310 nm, while addition of 1 mM TMA red shifts it by only ∼100 nm. The other salts give intermediate red shifts. All red shifts decrease as the salt concentrations increase to 1 M. The red shifts at 1 mM salt must derive from complexation of cations by the PEG that serves to localize charge on the hydrogel. Na^+ appears to be most strongly bound, while TMA is least strongly bound among the cations tested herein.

Previous studies have shown that PEG binds cations in organic solvents;[39] these complexes have been exploited as phase-transfer agents. PEG will also bind cations in aqueous solution. Indeed, Na^+ binding is evident in the NMR data of Cabane,[40] which shows increased relaxation rates for ^{23}Na ions in poly(ethylene oxide) (PEO)−dodecyl sulfate (SDS) micelle solutions due to binding of Na^+ to the PEO−SDS aggregates; these relaxation rates are proportional to PEO concentration. Dubin et al.[41] indicated that sodium binds directly to the PEG oxygen atoms. This favors interaction of cationic Na−PEG with anionic SDS micelles.

The binding of Na^+ by PEG in our BA−AA−PEG PCCA is evident from the accumulation of Na^+ within the hydrogel; atomic emission determination of the sodium content of the BA−AA−PEG PCCA (Desert Analytics Inc.) shows that BA−AA−PEG hydrogel has ∼4 times higher sodium content as compared with the surrounding solution. Atomic emission measurements show no sodium accumulation for the AA or the BA-AA PCCA. These data clearly indicate that the PEG localizes Na^+ within the hydrogel.

The importance of Na^+ localization to the glucose response is evident from the glucose response of a BA−AA hydrogel in which we copolymerized 4-acryloylamidobenzo-15-crown-5 (Acros Organics), instead of PEG, 400 monomethacrylate. This crown ether should show a modest association constant for Na^+ (we estimate[42] $K \sim 0.6$). Figure 7 shows that in 150 mM NaCl the glucose-induced blue shift of this crown ether BA−AA PCCA is larger than that of the BA−AA−PEG PCCA (Figure 2).

Mechanism of Glucose Sensing. The fact that both PEG (or crown ether) *and* cations are required for the glucose-induced blue shift suggests the mechanism noted in Chart 3 for the glucose response of the BA−AA−PEG. At low glucose concentrations, the glucose cross-links two boronates while the PEG (or crown ether) moieties localize cations close to the two boronates to form ion pairs to electrostatically stabilize the dianion complex by minimizing the electrostatic repulsion between the two anionic boronates.

These glucose-induced hydrogel cross-links increase the elastic restoring force and shrink the hydrogel volume, which blue shifts the diffraction. Higher glucose concentrations saturate the boronate sites to break the glucose cross-links and red shift the diffraction. Cross-linking of the boronate anion complex is evident because no response to glucose occurs for the phenylboronic acid at pH values below 7.5 where only small amounts of the boronate species exist. PEG or crown ethers increase the local cation concentrations to stabilize the glucose−bis(boronate) dianion complex.

This mechanism for boronate binding is reminiscent of Shinkai's recently demonstrated allosteric crown ether bis(boronate) glucose-binding derivatives.[37] In Shinkai's complexes, the

(39) See, for instance: *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications;* Harris, J. M., Ed.; Plenum Press: New York, 1992; and references herein.

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two phenylboronic acids are localized next to one another through their linkage to a crown ether. Glucose is proposed to form a bis(boronate) cross-link in this crown ether complex. The affinity of the neutral boronic acid for glucose was dramatically increased by the Lewis acid interactions of the boronic acid groups with amines within the crown ether. Cation binding by the crown ether removed the amine−boronic acid interactions and changed the bis(boronic acid) complex geometry to one with a significantly decreased glucose affinity.

Shinkai's glucose−bis(boronic acid) complexes are quite distinct from our BA−AA−PEG glucose complex, since the geometry adopted by our phenylboronic acid groups is relatively unconstrained by the soft polymer hydrogel framework, as is the geometry that can be adopted by the PEG−cation complexes.

Modeling of Sugar Response of BA−AA−PEG PCCA. We modeled the BA−AA−PEG PCCA response to sugars by improving our previous models,[5,6] which were based on Flory's ionic network model.[29] These models require that the total osmotic pressure at equilibrium must equal zero:

$$\Pi_T = \Pi_M + \Pi_E + \Pi_{Ion} = 0$$

where Π_M and Π_E are the osmotic pressures associated with the free energy of mixing and with the network elasticity and Π_{Ion} is the osmotic pressure due to the difference in mobile ion concentration inside and outside the gel (Donnan potential):

$$\Pi_M = -\frac{\partial \Delta G_M}{\partial V} = -\frac{RT}{V_s}\left[\ln\left(1-\frac{V_0}{V}\right)+\frac{V_0}{V}+\chi\left(\frac{V_0}{V}\right)^2\right]$$

$$\Pi_E = -\frac{\partial \Delta G_E}{\partial V} = -\frac{RTn_{cr}}{V_m}\left[\left(\frac{V_m}{V}\right)^{1/3}-\frac{1}{2}\frac{V_m}{V}\right]$$

$$\Pi_{Ion} = RT(c_+ + c_- - c_+^* - c_-^*) \qquad (1)$$

where R is the universal gas constant, T is the temperature, χ is the Flory−Huggins interaction parameter for the polymer network and the solution, V_s is the molar volume of the solvent, n_{cr} is the effective number of cross-linked chains in the network, V is the existing volume of the gel, V_m is the volume of the relaxed network (i.e.. the volume at which cross-links were introduced into the gel[5,43,44]), V_0 is the volume of dry polymer network, c_+ and c_- are the concentrations of cations and anions inside the gel, and c_+^* and c_-^* are the concentrations outside the gel.

For high ionic strength solutions, the Donnan potential is negligible and

$$\Pi_M + \Pi_E = 0 \qquad (2)$$

We ignore changes in χ upon glucose binding, since the small free energy of mixing change is likely to be negligible compared to cross-linking changes. Thus, our main assumption is that the change in hydrogel volume results solely from formation or breakage of glucose cross-links across two boronic acid sites attached to the hydrogel network.

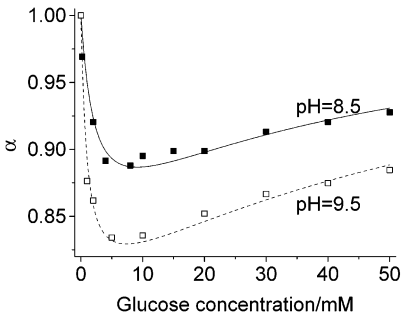

Figure 8. D-Glucose concentration dependence of the linear deformation factor, α, for BA−AA−PEG PCCA in 2 mM tris-HCl, pH 8.5, 150 mM NaCl and in 100 mM sodium carbonate buffer, pH 9.5, 150 mM NaCl. The lines through the data points are the theoretical fits as discussed in the text.

To model the volume-phase transition, we need to separately treat the two types of hydrogel cross-links. The total number of cross-linked chains is $n_{cr} = n_{cr}^0 + 2n_{B_2G}$, where n_{cr}^0 is the effective number of permanently cross-linked chains formed during the original polymerization of the network and n_{B_2G} is the number of additional cross-links due to glucose complexation to two boronic acid sites.

The original cross-links formed during PCCA polymerization allowed the chains to adopt their most probable chain configurations at the original PCCA volume. However, the glucose cross-links form at varying hydrogel volumes. Nevertheless, if these additional cross-links show reversible glucose binding, we can assume that they can relax to their most probable configurations at any hydrogel volume.

If we explicitly recognize this difference in the nature of the cross-links, the osmotic pressure arising from network elasticity is

$$\Pi_E = -\frac{\partial \Delta G_E}{\partial V} = -\frac{RTn_{cr}^0}{V_m}\left[\left(\frac{V_m}{V}\right)^{1/3}-\frac{1}{2}\frac{V_m}{V}\right] - RTc_{B_2G}$$

where c_{B_2G} is the concentration of the additional cross-links ($c_{B_2G} = n_{B_2G}/V$).

We can calculate the volume of the hydrogel (and the diffracted wavelength) at each sugar concentration by calculating the number of sugar bis(boronic acid) (boronate) cross-links formed as indicated in the Appendix.

We utilized the previously[5] determined value $n_{cr}^0/V_m = 1.46 \times 10^{-3}$ M for the hydrogel cross-link density and a $pK_a = 8.5$ for 3-acetamidoboronic acid.[6,45] We used elemental analysis to determine the amount of boronic acid in the PCCA ($n_T = 0.323$ mol/L of dry polymer). We estimated a Flory−Huggins interaction parameter $\chi = 0.495$ from the gel swelling in the absence of glucose ($\chi = 0.48-0.49$ for polyacrylamide[46,47]).

Figure 8 shows that our model can fit the BA−AA−PEG PCCA experimental data (Figure 2) at both pH 8.5 and 9.5. We plot the

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linear deformation factor $\alpha = \lambda/\lambda_o = (V/V_o)^{1/3}$, where λ and λ_o (V and V_o) are the diffracting wavelengths (volumes) at the glucose concentration of interest and in the absence of glucose, respectively. The model predicts both the initial low glucose concentration blue shift and the higher glucose concentration red shift.

We allowed the model to find the best-fit boronic acid and boronate equilibrium binding constants. This best fit was obtained with $K_1^* = 110\ M^{-1}$ and $K_2^* = 4500\ M^{-2}$ for pH 8.5 and $K_1^* = 135\ M^{-1}$ and $K_2^* = 12\,000\ M^{-2}$ for pH 9.5 (see the Appendix for association constant definitions).

These best-fit association constant values are in the range of experimentally determined association constants.[6,7] However, our modeling, and the precision and accuracy of our experimental measurements, are not sufficiently robust to accurately calculate the numerous individual association constants (K_1^1, K_1^2, K_2^1, K_2^2, K_2^3); K_1^* and K_2^* are the apparent association constants. Our model best fit can utilize families of values for the association constants. One set consistent with previous measurements is $K_1^1 = 80$, $K_1^2 = 140$, $K_2^1 = 0$, $K_2^2 = 14\,000$, and $K_2^3 = 4000$. These modeling results indicate that we have developed a good understanding of the glucose-sensing response mechanism of our BA−AA−PEG PCCA.

As shown in Figure 2B, we have recently synthesized a fluorinated phenylboronic acid derivative that can be used to fabricate a BA−AA−PEG PCCA that can determine glucose at the pH values and salinities of bodily fluids. Our next publication will discuss the synthesis of this derivative and the optimization of this sensor material for use in determining glucose in bodily fluids such as blood, interstitial fluid, and tear fluid.

CONCLUSIONS

We demonstrate a new molecular recognition motif, in which boronic acid and PEG (or crown ether) functional groups are prepositioned in a photonic crystal hydrogel, such that glucose self-assembles these functional groups into a supramolecular complex. The formation of the complex is associated with an increase in the hydrogel cross-linking, which blue shifts the photonic crystal diffraction. The visually evident diffraction color shifts across the visible spectral region, from red to blue over physiologically important glucose concentration ranges. These materials respond to glucose at physiological ionic strengths and are selective in their mode of response for glucose over galactose, mannose, and fructose. Thus, we developed a new recognition motif for glucose that shows promise for the fabrication of noninvasive or minimally invasive in vivo glucose sensing for patients with diabetes mellitus.

The next paper in this series will discuss the optimization of this glucose-sensing material. This optimization will extend its range such that the diffraction shift spans the visible spectral region over the physiologically relevant glucose concentrations, such that these sensing materials can be used to determine glucose in blood, interstitial fluid, and tear fluids.

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APPENDIX

As discussed below, glucose binding involves a complex equilibrium due to the ionization of the boronic acid boronate derivatives and because glucose will form complexes of both 1:1 and 1:2 stoichiometries.

We consider the following equilibria: $B + OH^- \leftrightarrow BOH^-$ with association constant K_1. Since $[BOH^-] = K_1[B][OH^-]$, the total concentration of boronic acid and boronates [B*] uncomplexed to glucose is

$$[B^*] = (1 + K_1[OH^-])[B]$$

The following two equilibria give rise to 1:1 boronic acid (boronate)−glucose complexes: (a) $B + G \leftrightarrow BG$, with association constant K_1^1 and (b) $BOH^- + G \leftrightarrow (BOH)G^-$, with association constant K_1^2.

At constant pH, we can define the equilibrium, $B^* + G \leftrightarrow BG^*$, with equilibrium constant

$$K_1^* = \frac{K_1^1 + K_1^2 K_1[OH^-]}{1 + K_1[OH^-]} \tag{3}$$

The following three equilibria involve the 2:1 boronic acid (boronate)−glucose complexes: (a) $2B + G \leftrightarrow B_2G$, with equilibrium constant K_2^1, (b) $2BOH^- + G \leftrightarrow (BOH)_2G^{-2}$, with equilibrium constant K_2^2, and (c) $B + BOH^- + G \leftrightarrow B(BOH)G^-$, with equilibrium constant K_2^3.

We can define the following equilibrium, $2B^* + G \leftrightarrow B_2G^*$, with equilibrium constant

$$K_2^* = \frac{K_2^1 + K_2^2(K_1[OH^-])^2 + K_2^3 K_1[OH^-]}{(1 + K_1[OH^-])^2} \tag{4}$$

It should be noted that K_1^* and K_2^* depend on the solution pH.

The concentration of boronic acid sites in the PCCA is

$$C_T = [B^*] + K_1^*[B^*][G] + 2K_2^*([B^*])^2[G] = \frac{n_T}{V}$$

and the concentration of glucose−bis(boronic acid) (boronate) cross-links is

$$c_{B_2G} = [B_2G^*] = K_2^*([B^*])^2[G]$$

where n_T is the total number of boronic acid sites in gel and [G] is the glucose concentration in solution.

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