Novel Swelling/Shrinking Behaviors of Glucose-Binding Hydrogels and Their Potential Use in a Microfluidic Insulin Delivery System

Ronald A. Siegel,[1,2] Yuandong Gu,[1] Antonio Baldi,[3] Babak Ziaie[3]*

Departments of [1]Pharmaceutics, [2]Biomedical Engineering, and [3]Electrical and Computer Engineering, University of Minnesota, Minneapolis, MN 55455, USA
E-mail: siege017@umn.edu

Summary: Hydrogels based on lightly crosslinked poly(methacrylamidophenylboronic acid-co-acylamide) swell monototically at pH 7.4 with exposure to increasing concentrations of glucose. At pH 10 these hydrogels first shrink and then reswell with increasing glucose concentration. A mechanism for these disctinct behaviors is proposed and preliminary results validating this mechanism are presented. A simple microfabricated glucose-sensitive valve based on this hydrogel is described.

Introduction

In recent decades, the swelling/shrinking response of hydrogels in response to physical or chemical stimuli has caught the notice of researchers in drug delivery, because swelling changes can be converted to changes in either permeation rate of drugs, or changes in rate of delivery resulting from mechanical forces generated by the hydrogel. Hydrogels that swell or shrink in response to changes in temperature,[1,2] pH,[3,4] glucose concentration,[5-7] or in the presence of an electrical current,[8] have been considered for modulation of drug release rates.

Glucose-responsive hydrogel-based drug delivery systems have received considerable attention due to their potential application in the treatment of diabetes. Two fundamental mechanisms have been investigated. Early work focused on a mechanism in which acidic hydrogen ions (H^+), enzymatically extracted from glucose, binds to sidechains of a hydrogel.[9,10] For polybase gels, such binding increases the hydrogel's charge, causing the gel to swell. For polyacid gels, binding of H^+ has the opposite effects. Usually, a polybase gel is used since an increase in swelling, due ultimately to an increase in glucose concentration, leads to increased insulin release, which is the desired response.

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In the second, more recently studied mechanism, glucose binds reversibly to the hydrogel, causing changes in the hydrogel's permeability. For example, sol-gel transitions mediated by glucose have been used to switch permeation of insulin on and off.[5,6] Also, hydrogels containing derivatives of phenylboronic acid (PBA) have been shown to bind glucose and alter their charge, leading to changes in gel swelling and permeability to insulin.[7]

In this contribution, we report studies of a class of PBA-containing hydrogels which can either swell or shrink with increasing glucose level, and suggest a means by which such hydrogels can be incorporated into microfabricated valves that can rapidly gate the flow of insulin solutions in response to changes in blood glucose level.

Swelling and Shrinking of PBA-Containing Hydrogels

Background. As illustrated in Figure 1, PBA moieties form tetrahedral boronate$^-$ ions in the presence of free OH^- ions, and behave as Lewis acids. Molecules containing planar diols, such as glucose, reversibly condense with two of the hydroxyls on the boronate$^-$ and stabilize the charged form of the Lewis acid. This leads to an apparent shift in the Lewis acid's pKa in the acidic direction, according to

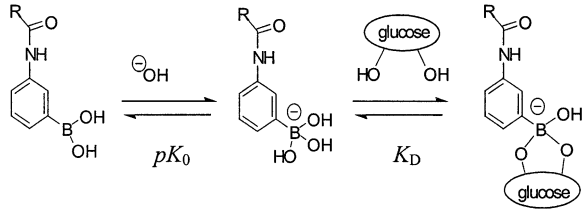

Figure 1. Scheme by which binding of glucose (or other planar diol) stabilizes the charged form of the MBPA moiety. After Aoki et al.[11]

$pK_{app} = pK_0 - \log(1 + [S]/K_D)$, where pK_0 is the Lewis acid constant of the PBA moiety, $[S]$ is the concentration of diol (glucose in Fig. 1), K_D is the dissociation constant of the diol-PBA complex (actually the equilibrium constant for the condensation of diol and tetrahedral boronate ion$^-$), and pK_{app} is the apparent acid constant of PBA moiety in the presence of diol.

Methods. We prepared hydrogels consisting of 20 mol% 3-methacrylamidophenylboronic acid (MPBA: see Fig. 1), 80 mol% acrylamide (AAm), with methylene bisacrylamide as crosslinker, mixed together to a total monomer concentration of 200 mg/ml in 1N NaOH solution. Polymerization was initiated by the ammonium persulfate/TEMED couple and carried out at room temperature.
Equilibrium swelling measurements were performed at room temperature in degassed 0.15 M PBS set to particular pH values, and in the presence of glucose or fructose. Uniaxial static compression measurements were carried out on equilibrated hydrogels between the parallel plates of a rheometer.

Results. Figure 2 shows the joint dependence of swelling on pH and fructose concentration. With increasing fructose concentration, the swelling curve shifts in the acid direction. Comparing the midpoints of the swelling curves with the above equation relating apparent pK_A to concentration of diol, the observed shifts are well described by assuming that pK_0=8.86 and K_D=230 μM, which are the literature values for the acidity constant of free MPBA and for the binding constant of fructose to free benzene boronic acid (a close relative of MPBA), respectively.[11]

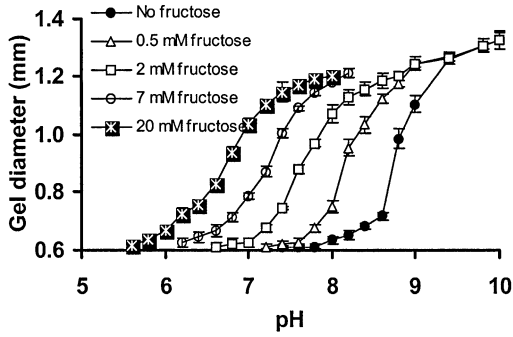

Figure 2. Swelling of p(MPBA-co-AAM) hydrogels as a joint function of pH and fructose concentration.

Figure 3 compares swelling of the poly(MPBA-co-AAm) hydrogels in glucose solutions at pH 7.4 and pH 10, and fructose solutions at pH 10. At pH 7.4, swelling increases monotonically with glucose concentration, as expected from the mechanism in Figure 1 in which glucose stabilizes the charge on the hydrogel. At pH 10, however, the hydrogel initial shrinks as glucose is added, and then reswells. The gel remains swollen

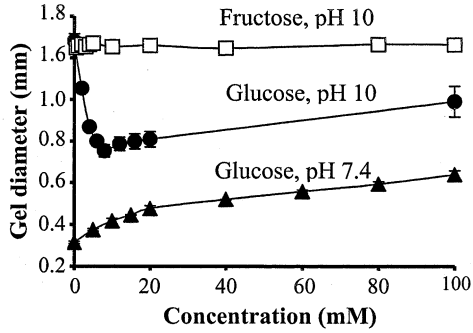

Figure 3. Swelling of p(MPBA-co-AAm) hydrogels in glucose and fructose solutions at pH 7.4 and 10.

at all tested concentrations of fructose, however.

At pH 10 the hydrogel is almost completely charged, so addition of glucose cannot increase charge and hence swelling. The initial shrinkage at low glucose concentrations at pH 10 is explained by noticing that the glucose molecule contains at least two pairs of planar diols. It therefore can bind simultaneously with two MPBA moieties attached to separate chains, thus forming new, albeit reversible crosslinks in the hydrogel. As glucose concentration increases further, however, free glucose molecules compete with the crosslinking glucose molecules for MPBA sites, causing the crosslinks to be broken. This mechanism is illustrated in Figure 4.

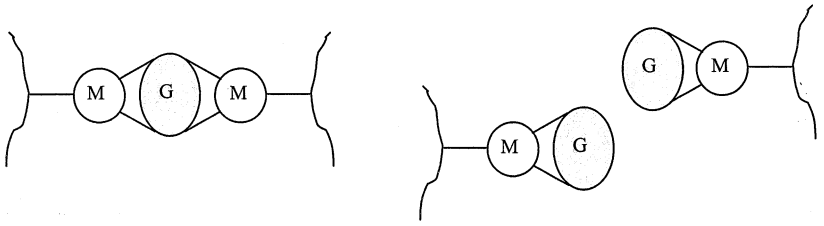

Figure 4. Left: formation of crosslinks between MPBA moieties (M) by glucose (G) at low concentrations. Right: breaking of these crosslinks by competition at higher glucose concentrations.

Confirming evidence for this mechanism is obtained from shear moduli measured by compression of the hydrogels after exposure to varying glucose concentrations at pH 10. As shown in Figure 5, the modulus first rises sharply, and then falls with increasing glucose concentration. The peak modulus is observed at the same concentration as the

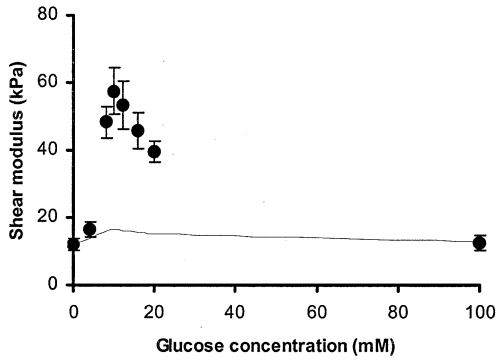

Figure 5. Symbols: Shear moduli of hydrogels determined by uniaxial compression measurements. Solid line: Values of shear moduli predicted based on change in hydrogel volume alone.

minimum swelling observed in Fig. 3. The solid line in Fig. 5 shows the trend in the modulus that would be expected from the change in volume alone, based on the $\phi^{1/3}$ scaling law,[12] where ϕ is the volume fraction of polymer. Since this trend greatly underpredicts the data, extra crosslinks must be formed as the gel contracts.

Fructose, unlike glucose, does not cause the gel to shrink. This is consistent with literature in which glucose is shown to form 1:2 complexes with free PBA molecules, while fructose only forms 1:1 complexes.[13]

Microfluidic Valve for Glucose-Sensitive Insulin Gating

Swelling and shrinking of the poly(MPBA-co-AAm) hydrogel, when the gel is inside a valve, can be used to switch on and off the flow of insulin solution when glucose

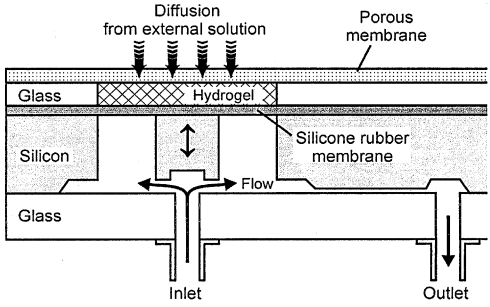

Figure 6. Schematic of hydrogel-based microvalve.[14] (© 2003 IEEE).

concentration changes. A schematic for a microfabricated valve is shown in Figure 6. The hydrogel is sandwiched between a rigid, porous membrane and a flexible, silcone rubber diaphragm, and the latter is connected to an embossment, which can open and shut a microchannel. Glucose diffuses through the porous membrane and affects hydrogel swelling as described above. When the hydrogel swells, it pushes down on the membrane, causing the embossment to close the inlet to a microchannel and stopping fluid flow through the channel. Flow is re-established when the gel shrinks due to elastic retraction of the diaphragm. Figure 7a shows the inside of the microvalve looking from the bottom, while Figure 7b is the bottom view of the microvalve with the glass plate and flow channel inlet and outlet attached.

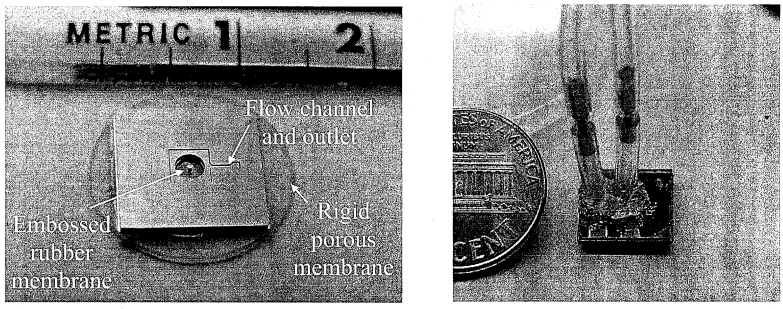

Figure 7. a) Microvalve as seen from below, with interior chamber, flexible rubber membrane and embossment exposed. b) Microvalve as seen from below with glass underplate, inlet and outlet attached.[14] (© 2003 IEEE).

In order to test the function of the microvalve, the assembly was placed in alternating baths containing PBS or PBS with 20 mM glucose. In both cases pH was set to 7.4 and the ionic strength was 0.155 mM, and the test was performed at room temperature. The inlet to the microflow channel was connected to a gravity-fed fluid line with pressure 6 kPa, and the outlet was connected to a fluid line. Fluid velocity was monitored by observing the movement of bubbles injected into the outflow line.

The results of this test are shown in Figure 8. In the absence of glucose, the hydrogel is relatively unswollen, and the microflow channel is open and fluid flow is seen. When glucose is added, however, the hydrogel expands, causing the embossment to close off the inlet to the channel, and shutting off fluid flow. The response time is close to 20 min.[14]

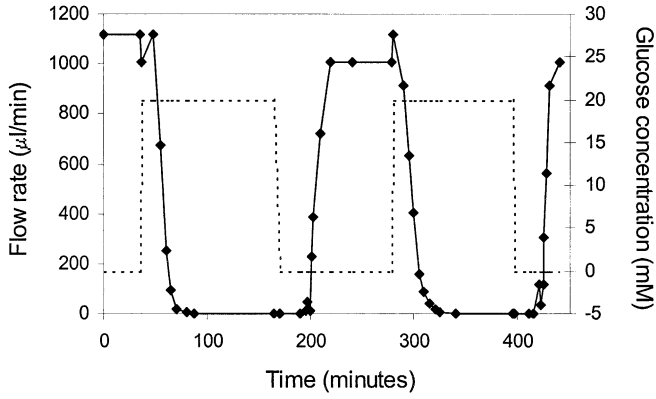

Figure 8. Response of microvalve to changes in glucose concentration between 0 mM and 20 mM PBS solutions at pH 7.4, ionic strength 0.155 mM, and at room temperature. Fluid pressure at inlet is 6 kPa relative to outlet.[14] (© 2003 IEEE).

Discussion

The microvalve prototype just discussed is suggestive of a system for automatic control of insulin delivery based on changes in blood glucose concentration. Such a valve might be placed at the tip of a catheter from a pressurized insulin reservoir, for example. The reservoir could be a small capsule worn outside the body, and the catheter would then cross the skin, leading to the microvalve, which sits in interstitial fluids whose glucose concentrations reflect, to an adequate approximation, the concentration of glucose in blood. With proper design, the reservoir could be replaced every few days, while the microvalve would be implanted indefinitely. Obviously, several improvements are needed for this scenario to be realized.

While the response time of the microvalve (20 min) is much faster than those seen for other polymer-based glucose-responsive systems, this time needs to be shortened to about 5 minutes in order for the microvalve to be therapeutically useful. Generally , the simplest means to speed up response is to miniaturize even further, for example by making the hydrogel, the rigid porous membrane, and the gap between the boss and the microflow channel inlet thinner. Thus far, the limits of miniaturization have not been reached, and work is being pursued in that direction.

A more fundamental problem with the microvalve lies in its polarity. Notice that the present microvalve design permits flow at low glucose concentrations, but shuts off flow at high glucose concentrations. When insulin is involved, the opposite response is

desired. One might change the mechanical design of the microvalve. However, polarity can also be reversed using a hydrogel that shrinks with increasing glucose concentration. As shown in Figure 3, when pH is above the pK_0 of the pendant PBA moieties, such shrinkage is observed due to crosslinking of the hydrogel by glucose. While pK_0 for MPBA is 8.86, this characteristic can be lowered by incorporating electron withdrawing groups into the phenyl ring[15] or by placing positively charged groups proximal to the boronate ion.[16,17] Both the mechanical and chemical approaches for reversing polarity are presently under investigation.

Acknowledgments

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