Totally Synthetic Polymer Gels Responding to External Glucose Concentration: Their Preparation and Application to On−Off Regulation of Insulin Release

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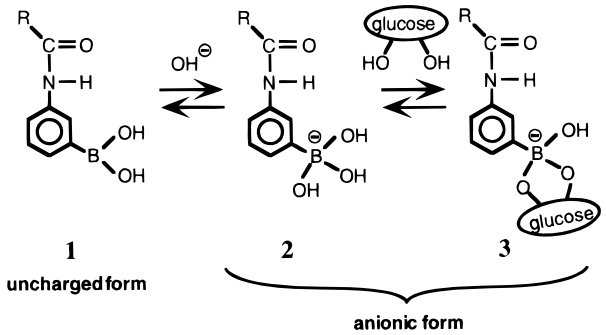
Received August 19, 1998

Recently, polymeric gels which change their physicochemical properties with external stimuli have been the subject of great interest because of their potential utility in diverse fields including controlled drug release, analytical and preparative separations, and sensor technologies[1]. Indeed, remarkable progress has been made in the development of gels responding to physical stimuli such as heat[2,3] and light.[4] Yet, few examples are known of totally synthetic gels responding to chemical stimuli, i.e., a concentration change in particular molecules in the milieu, regardless of their wide applicability in the fields described above. More specifically, development of sugar-responsive gels may give great impetus to construct a self-regulating insulin-delivery system for the treatment of diabetes,[5] a life-threatening disease showing an increased number of patients, especially in developed countries. We wish to present here the first successful preparation of a totally synthetic gel undergoing an abrupt change in the degree of swelling at a critical glucose concentration in aqueous medium. On−off regulation of insulin release from this glucose-responsive gel was also demonstrated in this study.

The major component of the matrix of our glucose-responsive gel is poly(*N*-isopropylacrylamide) (PNIPAAm) derivatized with a definite fraction of a phenylboronic acid group as the glucose-sensing moiety. A small amount of *N*,*N*′-methylene-bis-acrylamide (NMBA) was used as a cross-linker.

Phenylboronic acid and its derivatives are known to form covalent complexes with polyol compounds including glucose.[6] Because of this property, they have been utilized for such applications as sugar fractionation,[7,8] glyco-responsive polymer complexes,[9] glyco-sensors,[10−12] and synthetic mitogens for lymphocytes.[13] An important property of phenylboronic acid compounds in aqueous milieu is that they are in equilibrium between the undissociated (or uncharged) and the dissociated (or charged) form as shown in Scheme 1. Worth noticing is that one can shift the equilibrium in the direction of increasing charged phenylborates **(2 + 3)** through the complexation with glucose because only charged borates can form a stable complex with glucose in aqueous milieu. Direct complexation of the uncharged form **(1)** with glucose is known to be unstable in water because of its high susceptibility to hydrolysis, and thus its contribution to the equilibrium can be neglected in aqueous milieu.[14] Consequently, an increase in the concentration of glucose in the milieu increases the fraction of the total borate anions **(2 + 3)**, decreasing the fraction of the uncharged form **(1)**. Charged borates are certainly more hydrophilic than the uncharged form. Thus, a glucose-dependent change in the ratio between uncharged **(1)** and charged **(2 + 3)** borates in the polymer chain should crucially affect the polymer solubility, if the polymer chain has an amphiphilic character.[15] Indeed, in a gel system composed of PNIPAAm and 3-acrylamidophenylboronic acid (AAPBA), as reported here, a sharp transition in the swelling degree was achieved with glucose concentration.

Scheme 1. Equilibria of (Alkylamido)phenyl Boronic Acid (**1**)



The gel with an AAPBA content of 10 mol % was prepared in this study as follows: Under argon atmosphere, *N*-isopropylacrylamide (3.37 g), 3-acrylamidophenylboronic acid (0.63 g), and *N*,*N*′-methylene-bis-acrylamide (0.05 g) as a cross-linker were dissolved in 20 mL of DMSO with 2,2′-azobis(2,4-dimethylvaleronitrile) (1 mg/mL) as an initiator of radical polymerization. The prepared solution was then injected between two Teflon sheets (10 cm × 10 cm) separated by a Teflon gasket (1.0-mm thickness) and backed by glass plates. The solution was polymerized at 60 °C for 16 h. The formed gel slab was immersed successively into a series of DMSO/water mixtures graded in the order of 100/0, 75/25, 50/50, 25/75, and 0/100 to remove unreacted compounds. The slab was kept at least 1 day in each mixed solution. Finally, disks of 15-mm diameter were punched out from the gel slab using a cork borer and dried under ambient conditions for 1 day, followed by overnight drying in vacuo. To explore the glucose-induced change in swelling, disks were immersed in a pH 9 buffered solution adjusted to physiological ionic strength (0.1 M CHES buffer containing 0.12 M of NaCl ($I = 0.15$)) with varying concentrations of glucose at different temperatures and allowed to reach equilibrium swelling. Results are shown in Figure 1 as a function of temperature. Obviously, a remarkable change in the temperature-swelling curve was observed with glucose concentra-

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10.1021/ja982975d CCC: $15.00
Published on Web 11/19/1998

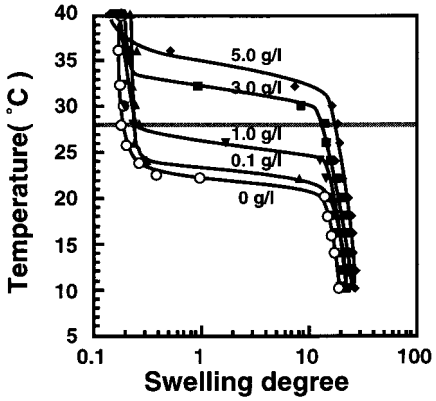

Figure 1. Temperature vs swelling curves for NB gel at different glucose concentrations: (○) 0 g/L, (▲) 0.1 g/L, (▼) 1.0 g/L, (■) 3.0 g/L, (◆) 5.0 g/L. The swelling degree was defined as the water content per mass of dried gel [(Wwet − Wdry/Wdry)].

tion in the milieu. An increase in glucose concentration from 0 to 5 g/L led to a rise in the critical swelling temperature from 22 °C to 36 °C. Estimated from the pK_a value of phenylboronic acid moieties in the linear copolymer of NIPAAm with AAPBA, the fraction of borate anions **(2 + 3)** in the NB gel should increase from 0.56 to 0.93 on the addition of 5 g/L glucose in the milieu at pH 9.0. This makes the gel more hydrophilic, and with a concomitant increase in ion osmotic pressure due to the counterions, the phase-transition temperature of the gel shifts to higher value in the presence of glucose compared to the condition without glucose.

It is also obvious from Figure 1 that the NB gel undergoes a remarkable change in the swelling, responding to the glucose concentration under isothermal conditions in the range of 25 to 30 °C. In this context, the NB gel may be applicable as a chemical valve to regulate solute permeation responding to glucose. Insulin should be a good candidate as the solute, considering the future application of this type of glucose-responsive gel for diabetes treatment. The on−off regulation of insulin release from the NB gel responding to a change in glucose concentration is demonstrated in Figure 2. Fluoresceinisothyocyanate (FITC)-labeled insulin (Sigma, MO) was used in this study to monitor the insulin release from the gel by a change in fluorescence intensity of the external milieu. As shown in Figure 2, almost no insulin release from the gel was observed with a glucose concentration below 1 g/L. On the other hand, a remarkable release of insulin took place for the gel immersed in the buffer with 3 g/L of glucose. This result clearly indicates the presence of a threshold concentration of glucose to trigger insulin release from the gel through hydration of the gel network with an increase in the fraction of the phenylborate anion. Further, Figure 3 shows the results of repeated on−off release of insulin from the gel on changing the concentration of external glucose. Rapid release of insulin from the NB gel with increased glucose concentration was effectively shut off by decreasing glucose concentration below the critical value. This on−off regulation of insulin release was successfully repeated in a synchronizing manner with a change in the concentration of external glucose.

Many research studies have so far been devoted to the development of self-regulated releasing systems of insulin, in which a biochemical reaction system for glucose plays a key role. Namely, lectin (concanavalin A)[5,17] and an enzyme (glucose oxidase)[18,19] are used in these studies as the essential component

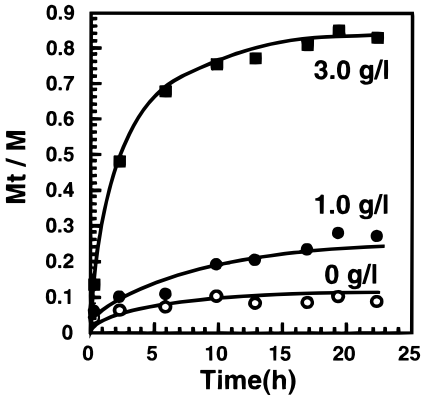

Figure 2. Release profiles of FITC-insulin from NB gel at 28 °C, pH 9.0 under varying concentrations of glucose. Glucose concentration: (○) 0 g/L, (●) 1.0 g/L, (■) 3.0 g/L. FITC-insulin was loaded into the NB gel by immersing the gel disk in 50 mL of 0.1 M CHES buffered saline (I = 0.15) at 5 °C, pH 9.0, containing 130 mg/L of FITC-insulin for 24 h. The gel was then promptly transferred into the same buffer adjusted to 28 °C, pH 6.0, for 60 min to form a surface skin layer for entrapping FITC-insulin inside of the gel. Release experiments of insulin from the insulin-entrapped gel were conducted in 100 mL of 0.1 M CHES buffered saline (I = 0.15) at 28 °C, pH 9.0, with varying concentrations of glucose. The fluorescence intensity of the solution at 520 nm (excitation wavelength: 495 nm) was monitored at given time intervals to determine the released amount of FITC-insulin (Mt) from the gel based on the calibration curve. The total amount of entrapped insulin (M) in the gel was defined as the cumulative released amount of FITC-insulin from the NB gel for 2 days at pH 9.0.

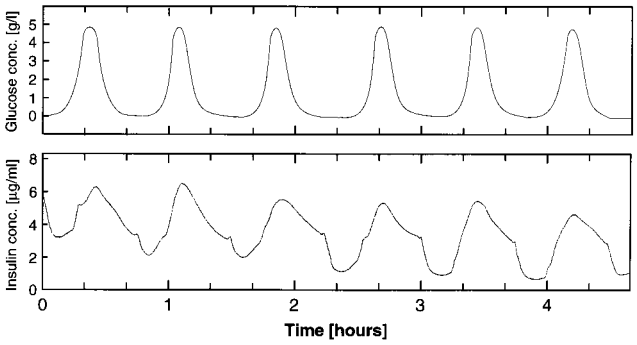

Figure 3. Repeated on−off release of FITC-insulin from the NB gel at 28 °C, pH 9.0, in response to external glucose concentration. FITC-insulin loaded gel was settled in a thermostated flow chamber connected with an inflow circuit equipped with a valve alternately switching the flow with high and low glucose concentrations.[16] Computer-controlled pumps were used to adjust the flow rate of the solutions. Glucose and FITC-insulin in the outflow of the chamber were monitored by reflective index and fluorescence detectors, respectively.

of the system to trigger the property change in the insulin-loaded matrix, allowing regulation of the release profile of insulin. Nevertheless, the use of biological components may involve such issues as stability, toxicity, and immunogenicity. Totally synthetic devices utilizing glucose-responsive polymer gels may have the potential to overcome these issues.

In conclusion, a remarkable change in the swelling induced by glucose was demonstrated for the gel composed of PNIPAAm with phenylboronic acid moieties. On−off regulation of insulin release from the gel was achieved through a drastic change in the solute transport property as a result of the formation and disruption of the surface barrier layer of the gel. This novel type of glyco-sensitive gel may have potential utilities in self-regulated drug-releasing systems as well as in other applications such as actuators, regulators, and separation systems with glyco-sensitivity.

JA982975D

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