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A glucose-triggered solubilizable polymer gel matrix for an insulin delivery system

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Summary

A poly(acrylamidophenylboronic acid-co-acrylamide) (poly(AAPBA-co-AAM)) gel was synthesized using diglucosylhexanediamine (DGHDA) as a crosslinking agent in order to prepare a glucose-responsive insulin delivery system. The system described here is a water-soluble polymer gel matrix whose erosion rate is glucose-dependent. At physiological pH, in the absence of glucose, anionic tetrahedral complexes between boronic acid moieties of the poly(AAPBA-co-AAM) and glucose moieties of DGHDA were maintained and the release rate of entrapped insulin was low. In the presence of glucose, however, the gel matrix was disrupted by replacement of DGHDA with glucose through competitive binding to boronic acid, resulting in the rapid solubilization of the matrix and a significant increase in the release rate of insulin.

Introduction

Various approaches have been investigated for the delivery of insulin in order to overcome problems associated with the conventional daily insulin injections, e.g., local discomfort and inadequate maintenance of euglycemia. An ideal insulin-delivery system for diabetic therapy would require a system capable of delivering insulin in response to changes in the physiological glucose levels.

There are three major approaches towards the development of glucose-responsive insulin-delivery systems: (i) a bioengineering approach which includes a computer system containing a glucose sensor and insulin injection pump (Rupp et al., 1982); (ii) a biological approach using cultured, living pancreatic β-cells encapsulated to constitute an insulin-delivery unit (Sun et al., 1985); (iii) the approach, which has been extensively investigated by many research groups, involving the development of a self-regulating insulin-delivery system based on biofeedback con-

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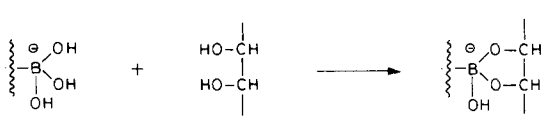
trol. Jeong et al. (1985) designed a system consisting of biologically active glycosylated insulin which was complementary to the binding sites of concanavalin A. Horbett et al. (1984) utilized a lightly crosslinked hydrogel copolymer membrane that released entrapped insulin according to the degree of swelling, which was modulated by the reaction between glucose oxidase and glucose. A similar protocol was investigated by Ishihara and co-workers (1984) employing hydrogels with different monomeric units. Siegel and Firestone (1988) developed a mechanochemical osmotic pump with altered water permeability in response to glucose levels which regulated the pump's output of insulin. Heller et al. (1987) have synthesized pH-sensitive, erodible poly(ortho esters) whose erosion rates increase sharply as the production of gluconic acid is increased. However, a glucose-responsive insulin-delivery system that delivers insulin over long periods of time and is biodegradable has not yet been developed.

In the present investigation, we propose a novel glucose-responsive insulin-delivery system based on a glucose-triggered solubilizable polymer matrix. A *m*-aminophenylboronic acid (*m*-APBA)-substituted polyacrylamide gel matrix was synthesized and crosslinked by a bispolyol. The gel may then be uncrosslinked by another polyol compound. This reversibly crosslinked polymer gel matrix is soluble in the presence of glucose, and the release of entrapped insulin occurs simultaneously with solubilization of the polymer matrix. Boronic acid, which is a key material in our system, is known to react with certain cyclic or acyclic diols and polyols to yield cyclic boronates as shown in Scheme 1. Thus, the molecule has been utilized for determination of the configuration of carbohydrates (Boeseken, 1949), as a protecting group in organic synthesis (Seymour et al., 1976), and as a ligand for affinity chromatographic separation of molecules with vicinal diol groups (Glad et al., 1980; Ducrocq et al., 1987).



Scheme 1.

In this report, the synthesis of poly(AAPBA-co-AAM) and its gelation by a reversible crosslinker, DGHDA are described. This study also examined the effect of glucose concentrations on the solubilization of copolymer matrix and the release rate of insulin.

Materials and Methods

Materials

m-Aminophenylboronic acid hemisulfate and bovine insulin (24.4 U/mg) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *N,N,N′,N′*-Tetramethylethylenediamine was obtained from Fluka AG (Buchs, Switzerland). Acrylamide, acryloyl chloride, hexanediamine, ammonium persulfate and α-D-glucose were supplied by Aldrich Chemical Co., Inc. (Milwaukee, WI, U.S.A.). Acrylamide was recrystallized twice from acetone and vacuum dried at 20°C. Triethylamine was obtained from Kanto Chemical Co. (Tokyo, Japan) and purified by distillation after drying for 1 day with calcium hydride. ^{125}I-insulin (porcine; lot no., AK81700; spec. act., 94.9 μCi/μg; unbound iodine, < 1%) was purchased from Du Pont NEN Research Products (Boston, MA, U.S.A.) and Coat-A-Count^{R} radioimmunoassay kit for insulin was obtained from Diagnostic Products Corp. (Los Angeles, CA, U.S.A). All other chemicals were reagent grade and were used without further purification.

Preparation of m-APBA free form

The hemisulfate salt of *m*-APBA was converted into the free base form. Briefly, an aqueous solution (50 ml) containing 5 g of the salt was brought to pH 7.0 with 1 N sodium hydroxide solution and then concentrated to dryness in vacuo. The residue was extracted with dioxane, and the extracts were filtered and evaporated to dryness. The product was recrystallized from distilled water. Repeated recrystallization yielded 2.9–3.2 g (78–86%) of a white crystalline product (m.p. 165°C).

Synthesis of AAPBA

AAPBA was prepared by reacting *m*-APBA and acryloyl chloride as described previously

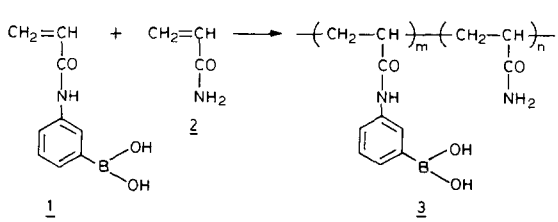
(Schott, 1972). *m*-APBA (0.685 g, 5 mmol) was dissolved in 50 ml of dried dioxane along with triethylamine (0.69 ml, 1 eq.) and acryloyl chloride (0.4 ml, 1 eq.). The reaction mixture was stirred at 20°C for 3 h, and then filtered to remove insoluble triethylammonium chloride. Upon mixing the filtrate with a large excess of methylene chloride, a yellowish precipitate was formed. Recrystallization of the isolated precipitate with distilled water afforded a yellowish crystalline solid (0.53 g, 55% yield); ^{1}H-NMR (DMSO-d_6) δ: 5.6, 5.4 (2H, CH_2), 6.2 (1H, CH), 7.1, 7.3, 7.4, 7.7 (4H, Ar-H), 7.9 (2H, $B(OH)_2$), 9.9 (1H, NH); IR ν^{KBr} cm^{-1}: 3380 (NH, OH), 1664 (CO), 1546 (NH), 1340 (BO).

Synthesis of DGHDA

α-D-Glucose (90 g, 0.5 mol) was suspended in 50 ml of anhydrous methanol containing hexanediamine (29.13 g, 0.5 eq.). The reaction mixture was refluxed at 65°C for 20 min (Pigman et al., 1951). After cooling, the mixture was combined with 300 ml of ethanol and maintained at 4°C until the formation of a pale yellowish precipitate. The product was collected by filtration and washed with cold methanol. The dried product (93.59 g, 85% yield, m.p. 104–106°C) was stored at −8°C. Analysis – Calcd. for $C_{18}H_{36}O_{10}N_2$: C, 49.1; H, 8.18; N, 6.36. Found: C, 47.5; H, 8.01; N, 6.12.

Synthesis of poly(AAPBA-co-AAM)

As shown in Scheme 2, poly(AAPBA-co-AAM) hydrogels were made of AAPBA and AAM at various molar ratios. Polymerizations (AAPBA : AAM molar ratios of 1 : 9 (P1), 2 : 8 (P2), 3 : 7 (P3), 5 : 5 (P4), and 7 : 3 (P5)) took place in methanol-water (1 : 1) solution at 20°C. Ammonium persulfate was used as an initiator and



Scheme 2.

N,N,N′,N′-tetramethylethylenediamine as an accelerator. The concentration of comonomers in the mixture was 2.22 mol and those of the initiator and accelerator were 0.5 and 4.0 mol% of the total comonomer content, respectively.

In the case of P1 and P2, reaction mixtures were solubilized in water and mixed with a large excess of methanol. The precipitated polymers were filtered and dried in vacuo at 40°C. The other copolymers (P3–P5) were solubilized in methanol and precipitated in excess diethyl ether. The resulting precipitates were filtered and dried in vacuo. Complete polymerization was suggested by the IR spectrum of each polymer sample exhibiting no residual C = C double bonds.

Preparation of polymer gel matrix containing insulin

Hydrogel matrices loaded with insulin were prepared as follows. To 4.5 ml of phosphate-buffered saline (PBS), pH 7.4, were added 10 mmol of a mixture of comonomers, 40 mg of insulin, and DGHDA (0.5 mol of AAPBA monomer content) and dissolved at 20°C. The initiator (ammonium persulfate) and accelerator (*N,N,N′,N′*-tetramethylethylenediamine) were then added. When the formation of a hydrogel matrix required iodine-labelled insulin, 5 μCi of radiolabelled insulin was mixed with 40 mg of unlabelled insulin. Immediately thereafter, the copolymer/insulin mixtures (approx. 5 ml) were cast into four Teflon molds (disk-shaped; diameter 16 mm, height 5 mm, volume 1 cm^3). Upon polymerization for 3 h at 20°C, disks were removed from the molds. These disks were kept in PBS buffer overnight in order to remove unreacted monomer and surface-adsorbed insulin.

In vitro insulin release test

An insulin-loaded disk was placed onto a wire pouch and immersed in the releasing medium with magnetic stirring. A volume of 200 ml of PBS, pH 7.4, with varying amounts of glucose, ranging from 0 to 100 mg/ml, was used as the release medium. Samples of 1 ml were taken at regular intervals and the amount of released insulin was determined quantitatively by radioim-

munoassay or radioactivity measurement of incorporated ^{125}I-insulin.

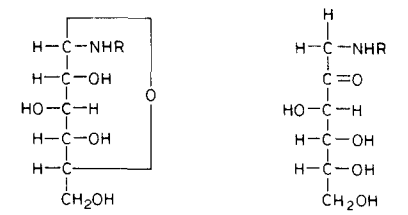
Measurements

^{1}H-NMR spectra of AAPBA and DGHDA in deuterated dimethyl sulfoxide were measured with a JEOL PMX 60SI NMR spectrometer. The ^{13}C-NMR spectrum of DGHDA in deuterated dimethyl sulfoxide was determined with a Varian Gemini 300 NMR spectrometer. The IR spectra of AAPBA, DGHDA and homo-, copolymer of AAPBA were recorded on an Alpha Centauri spectrophotometer. Elemental analyses of DGHDA and polymers were carried out with a Perkin Elmer 3030B atomic absorption spectrophotometer. The radioactivity of ^{125}I-insulin was determined on a Beckman DP 5500 γ-counter.

Results and Discussion

Synthesis of DGHDA

Structural analysis of the pyranose ring of DGHDA was performed, since the required configuration and conformation of the polyol of a glucopyranose are the most important factors in determining the affinities to boronic acid (Weith et al., 1970). The isolated DGHDA was *N*-glucoside (**I**), and isomerization of the molecule into isoglucosamine (**II**) by Amadori rearrangement was not observed (Scheme 3). The results obtained were based on structure determination by spectrophotometry, and were consistent with the report of Mitts et al. (1944). In the ^{13}C-NMR spectrum of DGHDA (Fig. 1), the resonances of the carbohydrate moiety were found downfield of tetramethylsilane at 90.7, 77.5, 77.4, 73.5, 70.5, and 61.4 ppm, in agreement with those observed for a glycoside of glucose (Walker et al., 1976). The lack of other resonances above 90 ppm indicates that the crosslinker is free from contaminating glucose (Rosevear et al., 1980). The resonances at 45.5, 29.9, and 26.8 ppm represent the three carbons in hexanediamine used as a spacer and those near 39 ppm correspond to dimethyl



Scheme 3.

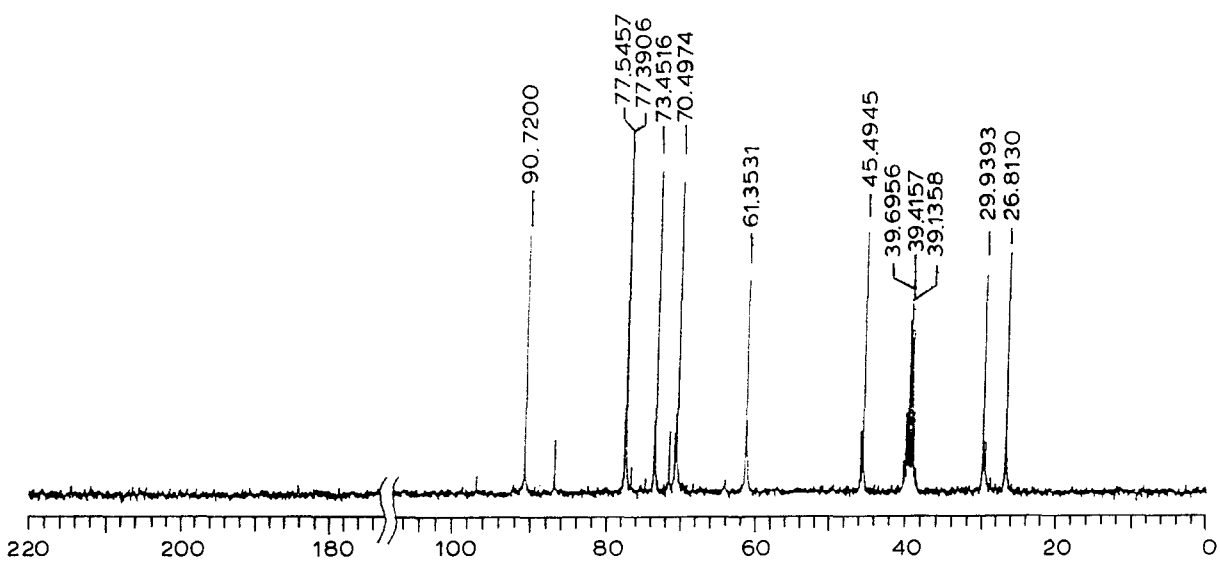


Fig. 1. The 75-MHz, proton-decoupled ^{13}C-NMR spectra of diglucosylhexanediamine.

sulfoxide as a solvent. The peak due to the ^{13}C-chemical shift of the carbonyl group (near 200 ppm), which provides the evidence that DGHDA is isoglucosamine, was not observed in that spectrum. These facts indicated that the synthesized DGHDA was *N*-glucoside having an intact pyranose ring structure. This was also supported by the IR spectrum where the N-H stretching band (1635 cm^{-1}) was distinctly resolved. Also, no absorption band in the C = O stretching region was observed. Although DGHDA was readily synthesized from glucose and hexanediamine in good yield, metachromatism was observed after 3–4 weeks at room temperature, which might be due to dissociation into glucose and amine. However, metachromatism was found to remain absent for longer than 1 year on storing the sample at −8°C.

Preparation of polymer and polymer matrix

Copolymer **3** in Scheme 2 was prepared by radical polymerization of AAPBA (**1**) and AAM (**2**). Table 1 lists the results of elemental analysis of poly(AAPBA-co-AAM). Poly(AAPBA-co-AAM) showed variation in solubility depending on the feed composition of the monomers. In general, poly (AAPBA-co-AAM) was soluble in methanol at high AAPBA monomer content and soluble in water at high AAM monomer content. Therefore, in preparing crosslinked copolymer gel matrix, it was necessary to determine the optimal composition of AAPBA and AAM in order to ensure sufficient crosslinking as well as water solubility of the uncrosslinked polymer. The copolymer was water-insoluble when the AAPBA monomer content was above 30%. The copolymer containing less than 3% of AAPBA was unable to form a gel matrix in the presence of the crosslinker (DGHDA) in PBS (pH 7.4). Above 30% of AAPBA, however, the resulting copolymer was soluble in sodium hydroxide or polyol solution, which might result from solubilization due to the full ionization of boronic acid or formation of water-soluble polyol complex, respectively. The crosslinked copolymer containing less than 3% of AAPBA formed a viscous solution with no mechanical integrity, and was likely due to the low degree of complex formation, i.e., low affinity between AAPBA and the glycosyl moiety of DGHDA. The complex formation constant (K) between benzeneboronic acid and glucose was reported to be relatively low (Lorand et al., 1959).

The degree of complex formation between boronic acid and polyol depends on (i) the availability in the compound containing the glycol group with the appropriate configuration and conformation, and (ii) the nature of neighboring charged groups of the boronic acid derivative (Weith et al., 1970). The pH of the solvent is also an important factor in complex formation, since the tetrahedral boronate anion is the active form in complex formation (Weith et al., 1970; Sienkiewicz et al., 1980). For example, benzeneboronic acids (pK_a 8.86) start to complex with D-glucose at about pH 6 and attain 100% formation of the complex at about pH 9. In the case of 3-nitrophenylboronic acids (pK_a 7.30) having electron-withdrawing groups in the aromatic ring, the pH value for 100% formation of the complex with D-glucose is close to pH 6.5 and

TABLE 1

Elemental analysis data of poly(AAPBA-co-AAM)

Sample no.	Feed molar ratio (AAPBA : AAM)	Elemental analysis data (wt%)					
		Feed monomer (calculated)			Polymer (found)		
		C	H	N	C	H	N
P1	1:9	52.1	6.6	16.9	48.1	6.5	15.3
P2	2:8	53.1	6.3	14.7	48.6	6.8	13.2
P3	3:7	53.9	6.1	13.1	52.5	7.0	11.9
P4	5:5	55.0	5.7	10.7	51.8	5.9	9.7
P5	7:3	55.8	5.5	9.0	53.3	5.7	8.3

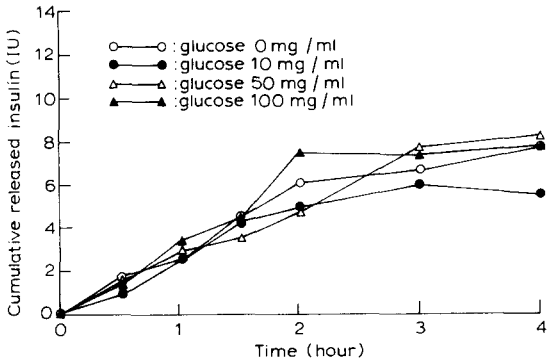


Fig. 2. Cumulative released amount of insulin entrapped in poly(AAPBA-co-AAM) (AAPBA feed monomer 5%) gel matrix in response to various glucose concentrations.

the molecules form complexes with D-fructose even at pH 3.5 (Barker et al., 1973; Myohanen et al., 1981). As a rule, alkaline pH favors complex formation.

In the present investigation, copolymers of 5–10% AAPBA monomer content were chosen. The crosslinked copolymer gel matrices described above were soluble only in the polyol solution (e.g. glucose or sorbitol) and under acidic conditions (< pH 5.0). The rate of solubilization of the polymer matrix under acidic conditions was much slower than that in polyol solution due to the poor water solubility of unionized benzeneboronic acids at acidic pH values. It is interesting to note that the prepared polymer matrix maintained its integrity in buffer solution (pH 7.4) for several weeks except for slight swelling.

In vitro insulin release studies

Insulin release experiments were performed with polymer matrices prepared using 5% (M1), 8% (M2), and 10% AAPBA monomer content (M3). Figs 2 and 3 show the results of experiments on M1 and M2, respectively. In these experiments (Ml and M2), the amounts of released insulin were determined by the RIA method. Lag times were not observed. Obviously, the release of insulin began immediately upon placing the hydrogel matrices in the releasing medium. Both M1 and M2 were completely solubilized within 4 h in PBS containing glucose. The differences in insulin release rates in response to the various glucose concentrations were not significant in the case of M1 (Fig. 2). The low content of AAPBA may have resulted in insufficient crosslinking with DGHDA and the polymer networks were not bound tightly enough to exclude the diffusional pathway of the insulin. This effect may be related to the glucose-independent insulin-release characteristics of M1. In the case of the hydrogel matrix of M2 (Fig. 3), the insulin-release pattern was similar to that of M1 at 100 mg/ml of glucose solution. In contrast, the insulin-release rate in PBS (pH 7.4) was lower than that of M1, and insulin release was not observed in the latter phase. However, it should be noted that the total amounts of insulin in Figs 2 and 3, determined by RIA, were considerably lower than the expected value (24.4 IU × 8 mg = 195.2 IU). The reason for this discrepancy is not yet fully understood but might be related to the method of sample preparation which involves the direct polymerization of a copolymer/insulin mixture. The possible formation of degradation products or the denaturation of insulin due to direct polymerization has not yet been investigated. It is quite possible, however, that this problem might be overcome by loading insulin and crosslinker after the polymerization of comonomers has reached completion, since the uncrosslinked poly(AAPBA-co-AAM) was viscous enough to mix with the insulin solution.

Figs 4 and 5 show the release profiles of ^{125}I-insulin from the M3 matrix in response to a

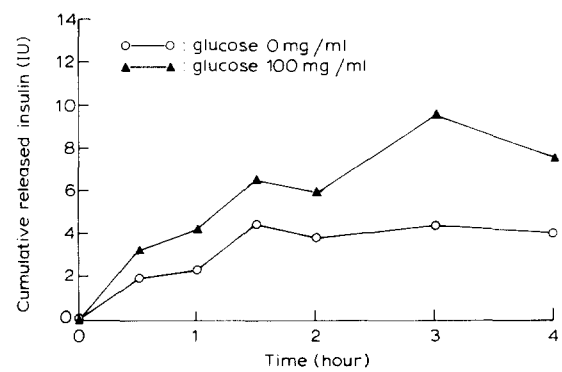


Fig. 3. Cumulative released amount of insulin entrapped in poly(AAPBA-co-AAM) (AAPBA feed monomer 8%) gel matrix in response to various glucose concentrations.

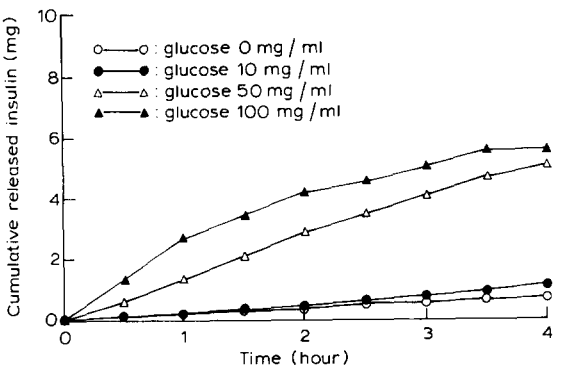


Fig. 4. Cumulative released amount of insulin entrapped in poly(AAPBA-co-AAM) (AAPBA feed monomer 10%) gel matrix in response to various glucose concentrations.

continuous and a stepwise glucose challenge, respectively. In Fig. 4, the release rate of insulin was highest at 100 mg/ml of glucose and decreased with decreasing glucose concentration. At steady state, insulin-release rates from hydrogel matrix were 0.16, 0.32, 1.35, and 2.0 mg/h at 0, 10, 50, 100 mg/ml glucose concentration, respectively. It was also observed that at high glucose concentrations (50 and 100 mg/ml), the polymer gel matrix was completely solubilized within 5 h but the gels in PBS alone maintained their integrity, except at a low degree of swelling (approx. 20%). These data indicated that the solubilization of gels resulted from the replacement of crosslinker (DGHDA) by glucose and

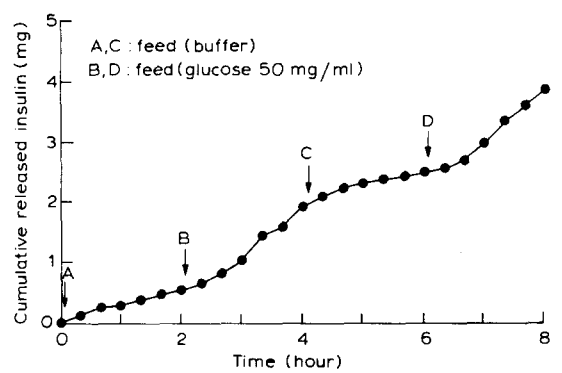


Fig. 5. In vitro insulin-release profile of poly(AAPBA-AAM) gel matrix in response to the stepwise glucose challenge.

that the degree of replacement is dependent upon the concentration of glucose. As shown in Fig. 5, the response of insulin to the step function challenge was moderate and steady, however, insulin release did take place during the buffer phase. The absence of a complete 'off' phase might be due to a spontaneous passive diffusion of insulin through the water channels in the matrix. However, it should be borne in mind that the glucose concentrations (50 to 100 mg/ml) for solubilizing the gel matrix are much higher than the physiological blood glucose level (> 100 mg/dl).

Conclusion

A novel glucose-soluble hydrogel was synthesized as a glucose-responsive insulin-delivery system. The hydrogel consisted of a water-soluble copolymer containing a boronic acid moiety and a crosslinker comprising polyol groups. Upon introduction into a glucose solution, the boronic acid-polyol complex between the copolymer and crosslinker was broken by excess glucose, resulting in an uncrosslinked, water-soluble polymer. The degree of sensitivity to glucose and gel integrity were influenced by the boronic acid content, such as the AAPBA molar ratio, and by the binding constant of crosslinker to boronic acid. The evidence indicated that various copolymer hydrogel systems could be prepared with different degrees of sensitivity to glucose. This possibility could be of interest for the design of a glucose-responsive insulin-delivery system.

Future studies will be focused mainly on determination of the biochemical characteristics of released insulin, the development of a suitable and safe insulin-loading technique and the optimization of the delivery system in order to be able to respond to changes in physiological blood glucose levels.

Acknowledgment

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