Characterization of Glucose Dependent Gel-Sol Phase Transition of the Polymeric Glucose-Concanavalin A Hydrogel System

Aiman A. Obaidat[1] and Kinam Park[1,2]

Received March 11, 1996; accepted April 19, 1996

Purpose. The main goal of this study was to synthesize and characterize hydrogels which undergo reversible gel-sol phase transformation in response to changes in glucose concentration in the surrounding environment.

Methods. The glucose-sensitive hydrogels were made by mixing the appropriate concentrations of acrylamide-allyl glucose copolymer and concanavalin A (Con A). To examine their phase reversibility, hydrogels in dialysis membranes were cycled between glucose-free and glucose-containing buffers. The binding affinity of allyl glucose (AG) to Con A was examined by using an equilibrium dialysis technique.

Results. The synthesized hydrogels underwent phase transition to sol in the presence of free glucose in the environment. The concentration of external free glucose (C_f) had to be at least 4 times that of polymer-bound glucose (C_p) to induce phase transition from gel to sol. The binding affinity study showed that binding of AG to Con A was four times stronger than that of free glucose. When C_p in the gel was 0.42 mg/ml or higher, C_f had to be much higher than 4 times C_p to induce phase transition.

Conclusions. The synthesized hydrogels underwent phase transition in the presence of free glucose in the environment, but the phase transition was not linearly dependent on the concentration of free glucose. This non-linear dependence was explained by the increased binding affinity of AG over native glucose to Con A, and the cooperative interactions between polymer-bound glucose and Con A.

KEY WORDS: hydrogels; gel-sol phase transition; glucose-sensitive; cooperative interaction; binding affinity.

INTRODUCTION

A self-regulated drug delivery system is capable of receiving information from the surrounding environment and adjusting drug release or output according to the received information. The feedback information either modulates the rate of drug release or triggers drug release at higher rates from a passive drug delivery device (1). Development of modulated drug delivery devices is especially important for the treatment of diabetes because simple replacement of insulin by periodic injections is unable to prevent the serious complications of the disease. Exogenous insulin needs to be released according to the fluctuating metabolic requirements of the body.

Several approaches have been utilized in the development of new insulin delivery systems. Brownlee and Cerami prepared a modulated insulin delivery device for the first time (2). Their system involved the preparation of glycosylated insulin. The glycosylated insulin molecules, which were bound to concanavalin A (Con A), could be displaced from Con A binding sites by free glucose. The displacement of glycosylated insulin was in direct proportion to the concentration of external glucose. A similar approach was developed by Kim and coworkers (3,4) with the emphasis of glycosylated insulin derivatives having variable binding constants to Con A. This made it possible to avoid easy displacement at low levels of external glucose. While this approach is highly elegant, modification of native insulin is not practical. Other studies utilized the pH-sensitive polymers (5,6) and liposomes (7). The reaction between glucose oxidase and glucose produced a pH change which caused pH-sensitive polymer to swell and release more insulin (5,6). Kim et al. developed pH-sensitive liposomes where glucose oxidase and insulin were coencapsulated (7). Enzymatic conversion of the permeated glucose to gluconic acid triggered destabilization of the liposomal membrane. Other studies utilized external stimuli such as electrical effect (8), magnetic effect (9), or ultrasound (10). In another approach, live islet cells were encapsulated in semi-permeable polymeric microspheres (11,12). Although this approach is promising and the cells survived the encapsulation process (13), the problems related to the long-term viability of cells have to be solved. In a recent clinical trial, simultaneous islet/kidney transplantation has been performed (14). Insulin independence (euglycaemia) was achieved in one case but with the extensive use of immunosuppression agents. Immunogenicity and cell viability problems constitute the major obstacles for this approach.

Previous reports from our laboratory described the synthesis and characterization of gel-sol phase-reversible hydrogels sensitive to glucose (15–18). A similar system was later described by Taylor et al. (19) who used polysucrose (Ficoll 400™) and Con A. Our gel-sol phase-reversible hydrogels utilize specific interactions between polymer-bound glucose and Con A. To synthesize polymer chains containing glucose as pendant groups, glucose was modified to allyl glucose (AG) and subsequently copolymerized with acrylamide (AAm). At physiological pH, Con A exists as a tetramer having four identical binding sites for glucose. Thus, glucose-containing polymer chains are physically crosslinked by Con A to form a gel. Since the interaction between Con A and glucose is not covalent, free glucose can compete with the polymer-bound glucose for the binding sites on Con A. Such competition results in loosening of the network structure and transformation of gel to sol. The gel-to-sol transformation, however, was not linearly dependent on the concentration of free glucose in the environment. To explain such a non-linear dependency, we characterized the binding affinity between Con A and AG.

MATERIALS AND METHODS

Synthesis of Allyl Glucose (AG)

AG was synthesized according to a published procedure (20). Briefly, 100 g of α-D-glucose (Aldrich, Milwaukee, WI) was reacted with 200 g of allyl alcohol (Aldrich, Milwaukee, WI) containing 3% (w/w) of dry HCl gas. The mixture was stirred at 70°C for 4.5 h under reflux. After cooling and neutralizing by ammonium hydroxide, unreacted allyl alcohol was removed by concentrating the solution under vacuum at 50°C

[1] Purdue University, School of Pharmacy, West Lafayette, Indiana 47907.

[2] To whom correspondence should be addressed.

0724-8741/96/0700-989$09.50/0

to a thick syrup. The syrup was extracted vigorously with 1 liter of dry acetone (three to four times) at room temperature. The acetone extract was concentrated under vacuum at room temperature, seeded and placed in a refrigerator to crystallize AG. The produced AG was separated by filtration, air dried for a few hours, then dried under vacuum at room temperature for 24 h.

Synthesis of Acrylamide-allyl Glucose Copolymers

Copolymers of acrylamide (AAm) and allyl glucose (AG) were synthesized by free radical polymerization. AAm (BioRad, Richmond, CA) and AG were mixed in different molar ratios to produce copolymers with different concentrations of bound glucose. The concentration of AAm was fixed at 0.85 M, and the concentration of AG in the feed was varied. Azobisisobutyronitrile (AIBN, Eastman Kodak Company, Rochester, NY) was used as the initiator at the concentration of 1% (w/w) of monomers. Monomers and initiator were dissolved in dimethyl sulfoxide (DMSO) (21). Reaction mixtures were heated in sealed test tubes for 12 h at 70°C. Then the reaction mixtures were mixed with a large excess of methanol (~200 ml), and the precipitated copolymers were filtered. The copolymers were redispersed in methanol and stirred to ensure the removal of unreacted monomers and initiator. The washed copolymers were filtered and dried under vacuum for 24 h at room temperature. Selected copolymers were sent to Lark Enterprises (Webster, MA) for measuring the molecular weights by gel permeation chromatography.

For the determination of the reactivity ratios of the monomers, polymerization time was adjusted to obtain copolymers at low conversion, i.e., less than 10% of the total monomers. The other conditions were the same as those described above. These samples were analyzed by elemental microanalysis and the reactivity ratios were determined according to the method described by Fineman and Ross (22).

Formation of Hydrogels

Con A (Sigma Chemical Company, St. Louis, MO) and copolymers were dissolved in phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. Microturbidity measurements were carried out to find the optimal concentrations of Con A and copolymers to form the hydrogels. When hydrogel was formed, the turbidity of the sample increased. The experiments were performed by using Corning Disposable Non-Sterile Assay plates that contain 96 flat bottom wells with a capacity of 200 μl each. Eight serial dilutions from copolymer solution were made. To each well, 100 μl of copolymer solution and 100 μl of Con A solution were added in sequence and mixed. The copolymer concentration ranged from 15 mg/ml to 150 mg/ml. The concentration of Con A was either 50 mg/ml or 100 mg/ml. The absorbance of each well was measured at 630 nm using a microplate autoreader (EL 311 Microplate Autoreader, Bio-TeK Instruments).

Phase Reversibility Studies

A 3 ml mixture of the glucose-containing polymer and Con A was loaded to a dialysis cassette (Pierce Slide-A-Lyzer, Pierce Chemical Company, Rockford, IL) using a syringe needle. An accessory buoy was attached to make the cassette float on a solution. This assembly was transferred to a beaker containing PBS to which 1 mM $CaCl_2$ and 1mM $MnCl_2$ were added. The molecular weight cut-off of the dialysis membrane of the dialysis cassette was 10,000. Glucose was added at 0.2 g increments to the buffer solution every 25 min and the change in the absorbance of the gel at 630 nm was measured by placing the cassette in the light path of a Beckman DU-7 spectrophotometer. A dialysis cassette containing only PBS was used as a reference background.

Determination of AG Binding Affinity to Con A

Equilibrium dialysis between Con A and AG was run at room temperature at pH values of 5.0, 6.2, and 7.4 using a multi-sample microvolume dialyzer (EMD101B Equilibrium dialyzer, Hoefer Pharmacia Biotech Inc., San Francisco, CA). The dialyzer system consists of 8-well dialysis module and a dialysis membrane with a molecular weight cut-off of 6,000–8,000. The membrane divided each well into two chambers of equal volumes (0.5 ml). The module was attached to a dialysis mixer. Under gentle mixing, the ligand (e.g., AG, glucose, or mannose) diffused into the chamber containing Con A. The dialysis module was made of Teflon so that the protein would not aggregate on the surfaces of the dialysis module during the experiment. The buffer used in the dialysis experiments was citrate-phosphate buffer (0.1M citric acid and 0.2 M dibasic sodium phosphate). Con A was also dialyzed against glucose and mannose at pH 6.2. The concentration of ligands ranged from 50 μg/ml to 500 μg/ml. The exact concentration of Con A in the chamber was determined spectrophotometrically using an extinction coefficient of 11.4 at 280 nm (23). The concentration was 1% (0.89×10^{-4} M). After reaching equilibrium, aliquots were taken from both sides of the dialysis chambers, and the concentration of the sugar present was determined by phenol-sulfuric acid assay (24). Since the amounts of ligand present on both sides of the dialysis wells were determined for every well in each experiment, correction for the binding of ligand to the dialysis membrane was not necessary.

RESULTS

The synthesized allyl glucose (AG) was identified by the melting point and optical rotation measurements. The melting point ranged between 98–100°C and the polarimeter reading was $[\alpha]_D = +150°$ in water. These values are similar to the values of 100.5–101.5°C and $[\alpha]_D = +151.1°$ reported in literature (20). The yield of the synthesized AG was 18–20%.

Several copolymers containing different concentrations of glucose were synthesized. A representative structure of copolymers of AG and acrylamide (AAm) is shown in Figure 1. The molar ratio of AG to AAm in the feed was varied from 0.21 to 0.75. The concentration of glucose on the copolymer chains was measured by elemental microanalysis and phenol-sulfuric acid assay. The compositions of the synthesized copolymers based on the results of elemental microanalysis are shown in Figure 2. As shown in Figure 2, the molar ratio of AG in the synthesized copolymers did not increase more than 0.25 even though its molar ratio in the feed was increased to 0.75. This can be attributed to the low reactivity ratio of AG compared to that of AAm.

To determine the reactivity ratios of the monomers, a series of copolymerization reactions with varying ratios of the two

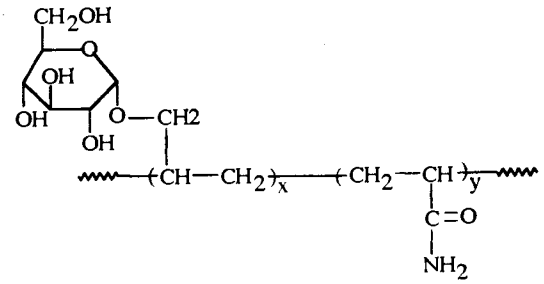

Fig. 1. A representative structure of glucose-containing copolymers made of allyl glucose and acrylamide.

monomers were conducted. The copolymers were isolated at the stage of 8.4–9.7% monomer conversion, and their compositions were calculated using elemental microanalysis. The reactivity ratios of r_1 and r_2 for AAm and AG were found to be 0.70 and 0.22, respectively. These values suggest that the copolymer may have a tendency to an alternating structure.

Hydrogels were formed in PBS containing $MnCl_2$ and $CaCl_2$. The presence of the divalent metals is required for the binding of polymer-bound glucose to Con A (25). The optimal concentrations of the copolymers to form gels were determined by microturbidity measurements. Formation of hydrogels at different concentrations of the copolymers was examined by tracking the change in turbidity at 630 nm. Figure 3 shows the change in turbidity by hydrogel formation as a function of the copolymer concentration. The concentration of Con A was fixed at 100 mg/ml. When the copolymer concentration was increased more than 70 mg/ml, gel was not formed probably due to the lack of enough Con A to crosslink all the available copolymer chains. At lower concentrations of copolymers, gel was formed more readily as the polymer-bound glucose concentration decreased. In addition, it is expected that at high copolymer concentration (more than 70 mg/ml) and a constant concentration of Con A, the four pendant glucose molecules which Con

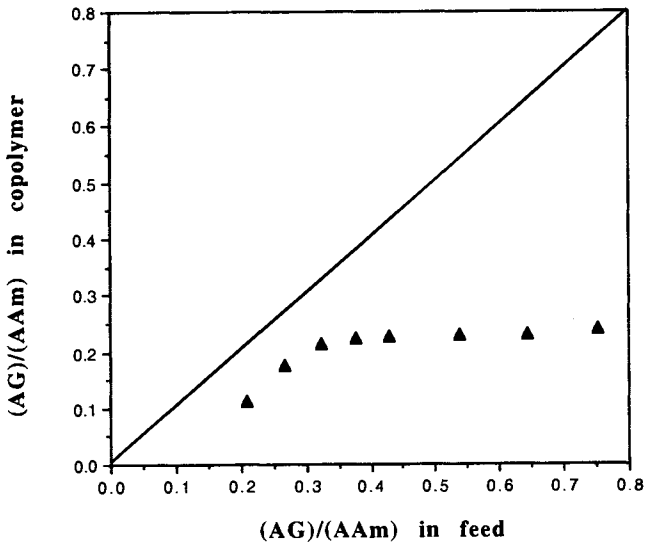

Fig. 2. The molar ratio of allyl glucose (AG) to acrylamide (AAm) in copolymers as a function of (AG)/(AAm) in the reaction feed. The linear line indicates the 1:1 ratio. The error bars were smaller than the size of the symbols (n = 3).

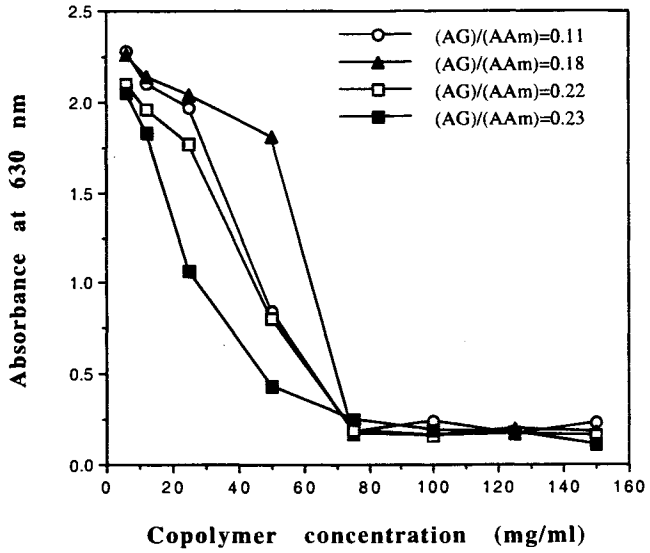

Fig. 3. Absorbance at 630 nm as a function of the copolymer concentration. The absorbance at 630 nm increases as hydrogel is formed from a clear solution. The concentration of Con A was maintained constant at 100 mg/ml. The inset shows the molar ratios of allyl glucose (AG) to acrylamide (AAm) in the copolymers used (n = 3).

A can combine to are mainly in the same copolymer chain. Therefore, gel can not be formed because the other available copolymer chains are not crosslinked. On the other hand, when the concentration of the copolymer is low, Con A molecule can interact with four glucose molecules contributed from different copolymer chains. This will result in gel formation due to the crosslinking of a large number of copolymer chains. Our previous studies with copolymers of N-vinyl-2-pyrrolidone and AG also showed a similar trend (15–18). Of the many glucose-containing copolymers, we used the copolymer with the molar ratio of AG to AAm of 0.11 throughout subsequent studies. The molecular weight of the copolymer with (AG)/(AAm) of 0.11 was determined by gel permeation chromatography. The weight average molecular weight (M_w), number average molecular weight (M_n), and M_w/M_n were 69,835, 13,518, and 5.2, respectively.

Phase reversibility studies showed that gels formed by poly(AAm-co-AG)-Con A complex were sensitive to free glucose. Cycling of the hydrogels between glucose-containing buffers and glucose-free buffers showed repeated phase transitions. Figure 4 shows a transition of a gel containing 0.41 mg/ml of polymer-bound glucose to a sol in the presence of external free glucose. The transition of a gel to sol was determined by measuring the decrease in absorbance at 630 nm. The gel remained as one solid piece until the absorbance value decreased to less than 1.5. The gel and the sol coexisted when the absorbance value was in between 0.5 and 1.0. The decrease in absorbance reached a point where no further significant decrease in absorbance was observed as shown in Figure 4. The concentration of free glucose required to induce the complete gel-to-sol transformation was measured from the intersection point. In Figure 4, the intersection point was found to be 1.64 mg/ml as indicated by an arrow. Table I shows the concentrations of free glucose required to induce phase transitions of hydrogels containing different amounts of polymer-

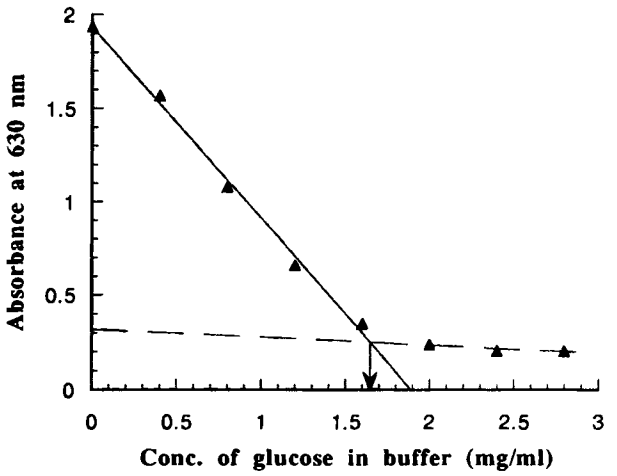

Fig. 4. Absorbance at 630 nm as a function of the glucose concentration in the external buffer solution. The copolymer with (AG)/(AAm) of 0.11 was used to form the gel. As the hydrogel starts phase transition to sol, the absorbance at 630 nm decreases. The concentrations of polymer-bound glucose and Con A were 0.41 mg/ml and 100 mg/ml, respectively. The arrow indicates the concentration of free glucose necessary for complete gel-to-sol transition.

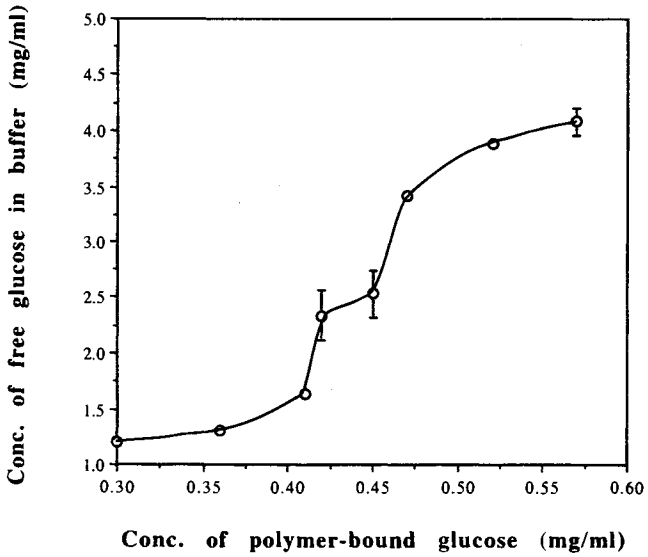

Fig. 5. The concentration of free glucose in buffer necessary to induce phase transition as a function of the concentration of polymer-bound glucose. The concentration of Con A was 100 mg/ml. The copolymer with (AG)/(AAm) of 0.11 was used to form the gel (n = 3).

bound glucose. The concentration of free glucose (C_f) to induce phase transition was about 4 times the concentration of polymer-bound glucose (C_p) for the first three gels in Table I. When C_p was 0.42 mg/ml, C_f started to increase to more than 5 times C_p. For the last three gels in Table I, C_f increased to more than 7 times C_p. The data in Table I were replotted in Figure 5 to make it easier to see such a trend. A small change in C_p from 0.41 to 0.42 mg/ml resulted in increase in C_f more than 4 times C_p. There was another quantum jump in C_f when C_p increased from 0.45 to 0.47 mg/ml.

To understand the trend in Table I (or Figure 5), we examined the binding affinity between AG and Con A using an equilibrium dialysis technique. Con A was dialyzed against AG at room temperature at three pH values. Equilibrium was reached after 12 h. Time to reach equilibrium was determined by dialyzing the ligands (e.g., AG, glucose, and mannose) against buffer free of Con A. It took 12 h for the concentrations of the ligands to be equal in both sides of the dialysis chambers.

Table I. Concentrations of Free External Glucose Required to Induce Phase Transformation of the Gels. The Gels Were Formed by Using Varying Concentrations of the Copolymer with (AG)/(AAm) of 0.11

Concentration (mg/ml) of Glucose Attached to the Copolymer (C_p)	Concentration (mg/ml) of Free Glucose to Induce Phase Transition (C_f)	C_f/C_p
0.30	1.20	4.0
0.36	1.31 ± 0.02	3.6
0.41	1.64 ± 0.01	4.0
0.42	2.34 ± 0.23	5.5
0.45	2.53 ± 0.21	5.6
0.47	3.42 ± 0.03	7.2
0.52	3.89 ± 0.02	7.4
0.57	4.08 ± 0.13	7.1

Calculation of the mass balance revealed that no ligands were bound to the dialysis membranes at equilibrium. The data of equilibrium dialysis were analyzed according to the Scatchard plot of r/c = Kn − Kr (26). In the equation, r is the ratio of the molar concentration of bound AG to that of Con A, c is the molar concentration of free AG, n is the number of binding sites on the protein, and K is the association constant in L/mole. The Scatchard plot of AG binding to Con A at pH 6.2 is shown in Figure 6. From the intercept on the r/c axis, an association constant of 2.71×10^3 L/mole for AG was obtained. Calculations were based on a molecular weight of 112,000 daltons for Con A. Linear plots were obtained at the pH values studied with n equal to 4 which is the number of glucose binding

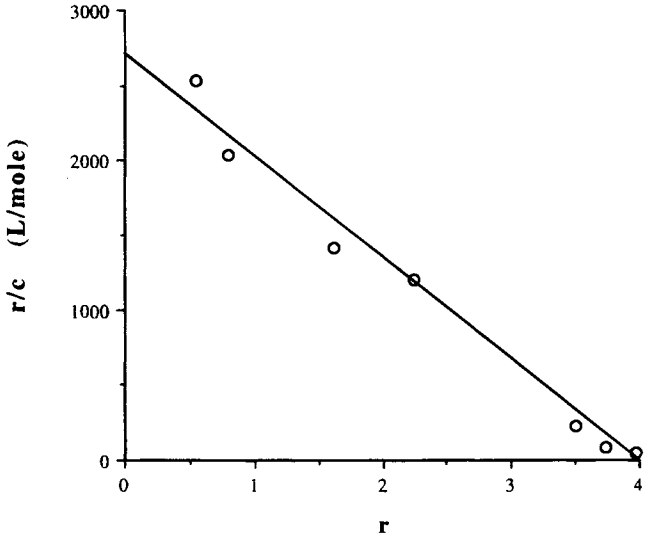

Fig. 6. Scatchard plot for the binding of allyl glucose (AG) to Con A at pH 6.2. r is the ratio of the molar concentration of bound AG to that of Con A, and c is the molar concentration of free AG.

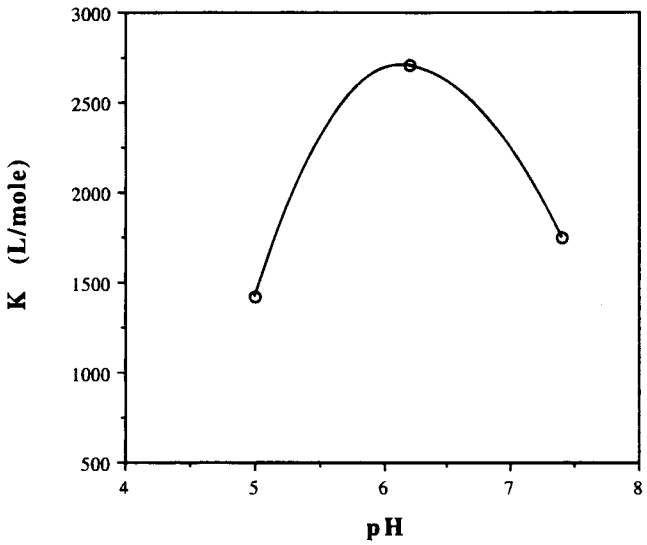

Fig. 7. pH dependence of the binding constant (K) of allyl glucose to Con A.

sites of Con A. The linearity of the plots indicates that the binding sites are identical and independent. Con A was also dialyzed against α-D-glucose and mannose to determine their association constants. K values were found to be 6.61×10^2 L/mole and 2.56×10^4 L/mole for glucose and mannose, respectively. These values are in agreement with the values reported in literature (27). These measurements show that AG binds to Con A 4 times stronger than glucose, but still 9 times weaker than mannose. In Figure 7, the association constant (K) for AG is plotted as a function of the three pH values studied. It can be observed that K reached a maximum at pH 6.2.

DISCUSSION

Glucose-sensitive phase-reversible hydrogels were synthesized before by Kitano et al. (28). Their system employed phenyl boronic acid attached to N-vinyl-2-pyrrolidone polymer as a glucose sensing moiety. The system was not really glucose specific since phenyl boronic acid is not glucose-specific. We were able to make our system glucose-specific by using Con A, a glucose binding protein. In our previous study, glucose-sensitive phase-reversible hydrogels were prepared by using glucose-containing N-vinyl-2-pyrrolidone polymer and Con A (17). In this study we have synthesized glucose-containing acrylamide copolymers. Hydrogels made from this copolymer and Con A were also phase-reversible in response to glucose. The specific interaction between glucose and Con A was used previously by other investigators (29,30). In their reports, however, only precipitates were formed instead of hydrogels. In our study we were able to form hydrogels using glucose-containing polymers and Con A by adjusting the relative concentrations of the copolymer and Con A.

Con A exists as a tetramer of identical subunits at physiological pH. Each subunit contains 237 amino acids residues, one calcium ion, one manganese ion, and one binding site for glucose, mannose, or fructose like-saccharide (31–33). Binding to Con A requires a hexose saccharide structure with unmodified hydroxyl groups on C3, C4, and C6 positions. Configurational factor at C2 hydroxyl group is highly important, since mannose with the axial positioned C2 hydroxyl group has 40 times higher binding affinity than mannose with the equatorial positioned C2 hydroxyl group (33). In the etherification reaction of α-D-glucose with allyl alcohol, a vinyl functionality was attached to C1 of glucose through the formation of an ether bond. Since the reaction left C3, C4, and C6 hydroxyl groups of glucose unmodified, AG was expected to bind to Con A. Copolymerizing AG with AAm was successful in obtaining polymer chains containing glucose as pendant groups. However, the reactivity ratio of AG in this system of comonomers was low, and the content of glucose in the synthesized copolymers was less than 25 mol%.

The gel formed more readily as copolymer concentrations decreased at the fixed Con A concentration. This may be due to less competition for Con A binding sites among polymer chains when the polymer concentration is low. At higher concentrations of Con A, however, it is expected that the competition among polymer chains for the binding sites is reduced and the available polymer chains can be crosslinked to form a gel. In this study, we were able to prepare gels that showed phase transition from gel to sol at various concentrations of free glucose ranging from 1.2 mg/ml to more than 4.0 mg/ml.

As shown in Table I and Figure 5, the ratio of the concentration of free glucose required for phase-transition to that of polymer-bound glucose in the gel started to increase from 4 to 5.5 and to more than 7. When the concentration of polymer bound-glucose (C_p) was 0.41 mg/ml, the concentration of free glucose required to induce phase-transition (C_f) was 1.64 mg/ml. This is expected from the binding affinity of AG to Con A which is 4 times stronger than glucose. When C_p was changed to 0.42 mg/ml, however, C_f was increased to 2.34 mg/ml. The ratio of C_f/C_p was 5.5. The ratio larger than 4 could be explained by the cooperative interaction between polymeric glucose and Con A. At a concentration of 0.42 mg/ml, the polymer-bound glucose molecules start to have cooperative interactions with Con A, and the cooperative interaction becomes maximal when C_p was increased to 0.47 mg/ml. The cooperative interactions play a variety of biological roles ranging from modulating the formation of biological mediators to directing DNA recognition (34). Cooperative interaction exerted by polymeric glucose is simply due to its multiple binding ability at C_p higher than 0.42 mg/ml.

As discussed earlier, C_f was found to be at least 4 times larger than C_p due to the 4 times higher binding affinity to Con A by AG. If the binding constant of AG is similar to that of free glucose, the displacement of AG from the binding sites of Con A by free glucose should take place at any glucose concentration. This is not desirable since the system should not respond to glucose concentration in the hypoglycemic state. Thus, the difference in binding affinity between glucose and AG to Con A is an advantage. Con A is known to have a higher degree of binding to saccharides which have conformation at the anomeric carbon. Studies on Con A binding to carbohydrates showed that it has a higher degree of binding to p-nitrophenyl-α-D-mannopyranoside and p-nitrophenyl-α-D-glucopyranoside more than the native carbohydrates (35,36). These results support our finding that AG has higher binding affinity to Con A than the native glucose does. Our study, however, showed that mannose binds to Con A 9 times stronger than AG. Mannose is known to have the highest binding affinity to Con A (37). Con A recognizes and interacts with the hydroxyl groups at C3, C4, and C6 of the saccharide, but the orientation of the C2 hydroxyl

group, which is different in glucose and mannose, is very important for the binding affinity (36,38).

The ultimate application of this glucose-sensitive hydrogels is in the development of self-regulating insulin delivery systems. The hydrogels currently have the ability to sense the changes in glucose concentration in the environment and the ability to respond to such changes by undergoing reversible phase transition from gel to sol. This particular property makes the hydrogel useful as a glucose-sensor, since the phase transition of the hydrogels can be quantitated. The phase transition is also expected to control the release of insulin through the hydrogels. While it was indeed shown that the glucose-sensitive hydrogels could control the release of insulin (19), the time it took to begin and shut off the insulin release was not fast enough to be practical. This particular problem may be overcome by designing the hydrogel membrane into a very thin membrane or filling the small pores of the membrane with the hydrogels. Further characterization of the glucose-sensitive phase-reversible hydrogels will be useful in the development of modulated insulin delivery systems.

CONCLUSIONS

Mixing two solutions of Con A and glucose-containing copolymer at appropriate concentrations resulted in the formation of a hydrogel. The non-covalent interaction between polymer-bound glucose and Con A provides gel-sol phase reversibility. The concentration of external free glucose (C_f) had to be at least 4 times higher than that of the polymer-bound glucose (C_p) to induce phase transition. When C_p was 0.42 mg/ml and above, polymer-bound glucose started to have cooperative interaction with Con A and as a result, C_f necessary for phase transition became much larger than 4 times C_p.

ACKNOWLEDGMENTS

This study was supported partly from the American Diabetes Association, Indiana Affiliates. Aiman A. Obaidat is supported by a fellowship from Jordan University of Science and Technology, Irbid-Jordan.

REFERENCES

1. J. Heller. Chemically self-regulated drug delivery systems, *J. Controlled Release* **8**:111–125 (1988).
2. M. Brownlee and A. Cerami. A glucose-controlled insulin delivery system: semisynthetic insulin bound to lectin. *Science* **206**:1190–1191 (1979).
3. S. Y. Jeong, S. W. Kim, M. J. D. Eenink, and J. Feijin. Self-regulating insulin delivery systems I. Synthesis and characterization of glycosylated insulin. *J. Controlled Release* **1**:57–66 (1984).
4. S. Sato, S. Y. Jeong, J. C. McRea, and S. W. Kim. Self-regulating insulin delivery systems II. In vitro studies. *J. Controlled Release* **1**:67–77 (1984).
5. J. Kost, T. A. Horbett, B. D. Ratner, and M. Singh. Glucose sensitive membranes containing glucose oxidase: activity, swelling, and permeability studies. *J. Biomed. Mater. Res.* **19**:1117–1133 (1985).
6. R. A. Siegel. pH-sensitive gels: swelling, equilibria, kinetics, and application for drug delivery. In J. Kost (ed.), *Pulsed and self-regulated drug delivery,* CRC Press, Boca Raton, FL, 1990, pp. 129–157.
7. C. K. Kim, E. B. Im, S. J. Lim, Y. K. Oh, and S. K. Han. Development of glucose-triggered pH-sensitive liposomes for a potential insulin delivery. *Int. J. Pharm.* **101**:191–197 (1994).
8. K. Sawahata, M. Hara, H. Yasunaga, and Y. Osada. Electrically controlled drug delivery systems using polyelectrolyte gels. *J. Controlled Release* **14**:253–262 (1990).
9. D. S. T. Hsieh, R. Langer, and J. Folkman. Magnetic modulation of release of macromolecules from polymers. *Proc. Natl. Acad. Sci. USA* **78**:1863–1867 (1981).
10. S. Miyazaki, C. Yokouchi, and M. Tanaka. External control of drug release: controlled release of insulin from a hydrophilic polymer implant by ultrasound irradiation in diabetic rats. *J. Pharm. Pharmacol.* **40**:716–717 (1988).
11. M. V. Sefton, R. L. Broughton, M. E. Sugamori, and C. L. Mallabone. Hydrophilic polyelectrolytes for the microencapsulation of fibroblasts or pancreatic islets. *J. Controlled Release* **6**:177–187 (1987).
12. A. M. Sun and G. M. O'Shea. Microencapsulation of living cells-a long-term delivery system. *J. Controlled Release* **2**:137–141 (1985).
13. H. Uludag, J. E. Babensee, T. Roberts, L. Kharlip, V. Horvath, and M. V. Sefton. Controlled release of dopamine, insulin and other agents from microencapsulated cells. *J. Controlled Release* **24**:3–12 (1993).
14. P. F. Gores, J. S. Najarian, E. Stephanian, J. J. Lloveras, S. L. Kelley, and D. E. R. Sutherland. Insulin independence in type I diabetes after transplantation of unpurified islets from single donor with 15-deoxyspergualin. *Lancet* **341**:19–21 (1993).
15. S. J. Lee and K. Park. Synthesis of sol-gel phase-revrsible hydrogels sensitive to glucose. *Proc. Intern. Symp. Control. Rel. Bioact. Mater.* **21**:93–94 (1994).
16. S. J. Lee and K. Park. Synthesis and characterization of sol-gel phase-reversible hydrogels sensitive to glucose. *Polymer Preprints* **35**(2):391–392 (1994).
17. S. J. Lee and K. Park. Glucose-sensitive phase-reversible hydrogels. *ACS Symposium Series* **627**:11–16 (1996).
18. S. J. Lee and K. Park. Synthesis and characterization of sol-gel phase-reversible hydrogels sensitive to glucose. *J. Molecular Recognition,* in press.
19. M. J. Taylor, S. Tanna, and G. G. Adams. Insulin delivery system using a novel glucose sensitive formulation. *Proc. Intern. Symp. Control. Rel. Bioact. Mater.* **22**:746–747 (1995).
20. E. A. Talley, M. D. Vale, and E. Yanovsky. Allyl ethers of carbohydrates III. Ethers of glucose and galactose. *J. Am. Chem. Soc.* **67**:2037–2039 (1945).
21. W. S. Kim, S. H. Lee, I. K. Kang, and N. K. Park. Release of 8-hydroxyquinoline from copolymers of 8-quinolinyl acrylate and acrylamide. *J. Controlled Release* **9**:281–285 (1989).
22. M. Fineman and S. D. Ross. Linear method for determining monomer reactivity ratios in copolymerization. *J. Polym. Sci.* **2**:259–262 (1950).
23. B. B. L. Agrawal and I. J. Goldstein. Protein-carbohydrate interaction VII. Physical and chemical studies on concanavalin A, the hemagglutinin of the jack bean. *Arch. Biochem. Biophys.* **124**:218–229 (1968).
24. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**:350–356 (1956).
25. A. J. Kalb and A. Levitski. Metal binding sites of concanavalin A and their role in binding of α-D-glucopyranoside. *Biochem. J.* **109**:669–672 (1968).
26. G. Scatchard. The attractions of proteins for small molecules and ions. *Annal. N. Y. Acad. Sci.* **51**:660–672 (1949).
27. L. L. So and I. J. Goldstein. Protein-carbohydrate interaction XX. On the number of binding sites of concanvalin A, the phytohemagglutinin of the jack bean. *Biochim. Biophys. Acta* **165**:398–404 (1968).
28. S. Kitano, Y. Koyoma, K. Kataoka, T. Okano, and Y. Sakurai. A novel drug delivery system utilizing a glucose responsive polymer complex between poly(vinyl alcohol) and poly(N-vinyl-2-pyrrolidone) with a phenyl boronic acid moiety. *J. Controlled Release* **19**:162–170 (1992).
29. J. E. Morris, A. S. Hoffman, and R. R. Fisher. Affinity precipitation of proteins by polyligands. *Biotech. Bioeng.* **41**:991–997 (1993).
30. K. Nakamae, T. Miyata, A. Jikihara, and A. S. Hoffman. Formation of poly(glycosyloxyethyl methacrylate)-concanavalin A complex and its glucose sensitivity. *J. Biomater. Sci. Polym. Edn.* **6**:79–90 (1994).

31. J. B. Sumner and S. F. Howell. The identification of the hemagglutinin of the jack bean with concanavalin A. *J. Bacteriol.* **32**:227–237 (1936).
32. J. L. wang, B. A. Cunningham, and G. M. Edelman. Unusual fragments in the subunit structure of concanavalin A. *Proc. Natl. Acad. Sci. USA* **68**:1124–1130 (1971).
33. E. Smith and I. J. Goldstein. Protein-carbohydrate interaction V. Further inhibition studies directed toward defining the steriochemical requirements of the reactive sites of concanavalin A. *Arch. Biochem. Biophys.* **121**:88–95 (1967).
34. P. F. Predki and B. Sarkar. Cooperative interaction of estrogen receptor 'zinc finger' domain polypeptides on DNA binding. *Biochem. J.* **305**:805–810 (1995).
35. S. D. Lewis, J. A. Shafer, and I. J. Goldstein. Kinetic parameters for the binding of p-nitrophenyl-α-D-mannopyranoside to concanavalin A. *Arch. Biochem. Biophys.* **172**:689–695 (1976).
36. R. D. Farina and R. G. Wilkins. Kinetics of interaction of α- and β-D-monosaccharides with concanavalin A. *Biochim. Biophys. Acta* **631**:428–438 (1980).
37. V. Horejsi, M. Ticha, and J. Kocourek. Studies on lectins XXXI. Determination of dissociation constants of lectin sugar complexes by means of affinity electrophoresis. *Biochim. Biophys. Acta* **499**:290–300 (1977).
38. R. D. Poretz and I. J. Goldstein. An examination of the topograghy of the saccharide binding sites of concanavalin A and of the forces involved in complexation. *Biochemistry* **9**:2890–2896 (1970).