Chapter 2

Glucose-Sensitive Phase-Reversible Hydrogels

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A novel hydrogel which undergoes sol-gel phase transformation by changes in glucose concentration of the surrounding medium was synthesized. The specific interactions between glucose and Concanavalin A (Con-A) was utilized to provide glucose sensitivity and reversible crosslinking. Glucose-containing copolymers were synthesized using allyl glucose and N-vinyl-2-pyrrolidinone (VP). Mixing of the copolymer and Con-A solutions resulted in the hydrogel formation. The efficiency of hydrogel formation increased as the relative concentration of Con-A to copolymer was increased. Upon addition of free glucose, the gel became a sol as a result of replacement of the polymer-attached glucose by free glucose molecules. The removal of the free glucose from the sol by dialysis caused the formation of hydrogel again. The sensitivity of the hydrogel to glucose can be adjusted by controlling the concentrations of copolymer and Con-A as well as the glucose concentration in the copolymer.

Hydrogels have been used in various applications ranging from controlled drug delivery to biotechnology. Many hydrogels have the ability to respond (i.e., swell, shrink, or degrade) to the changes in environmental stimuli, such as pH and temperature. Such a response has been limited to changes in the volume of hydrogels in the same gel phase. Currently, there are no hydrogels which undergo reversible sol-gel (i.e., liquid-solid) phase transformation in the presence of a specific molecule such as glucose. Recently, a few hydrogel systems which dissolve to the liquid state in the presence of excess glucose were developed (1, 2). Although they respond to changes in the environmental glucose concentration, they are not specific only to glucose and their sol-gel reversibility was not tested.

Here, we report the synthesis of hydrogels which can undergo phase transformation from sol to gel and vice versa depending on the presence of a specific biomolecule in the environment. In this particular study, we synthesized sol-gel phase-reversible hydrogels which are sensitive to glucose. The glucose sensitivity was chosen, since one of the goals of our study was to design self-regulating insulin delivery systems based on phase-reversible hydrogels.

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Sol-Gel Phase-Reversible Hydrogels Sensitive to Glucose

We used specific interactions between glucose and Concanavalin-A (Con-A) to form physical crosslinks between polymer chains. Con-A is a glucose-binding protein extracted from plants. Con-A consists of four polypeptide subunits, which is also called protomers. The molecular weight of each protomer is 26,000. We synthesized polymer chains containing glucose molecules. The glucose molecules attached to the polymer backbone react with Con-A. Since Con-A exists as a tetramer at physiological pH and each subunit has a glucose binding site, Con-A can function as a crosslinking agent for glucose-containing polymer chains. Because of the non-covalent interaction between glucose and Con-A, the formed crosslinks are reversible. Individual glucose molecules can compete with the polymer-attached glucose molecules. Thus, the maintenance of the crosslinks depends on the relative concentration of free glucose in the environment. This concept is described in Fig. 1.

The gel is formed by mixing a solution of glucose-containing polymers with a Con-A solution. Upon addition of free glucose molecules, the hydrogel becomes a sol (i.e., the hydrogel dissolves) due to the detachment of polymer chains from Con-A as a result of competitive binding of free glucose to Con-A. The sol can become a gel again upon removal of free glucose by dialysis. In Fig. 1, Con-A can also be attached to the polymer backbone. It is important to note that the sol-gel phase transition in our study is totally different from volume phase-transition, which is not really a phase transition, since the volume change occurs in the same gel state.

Materials and Methods

Synthesis of Allyl Glucose. Dry HCl (Aldrich) was dissolved in allyl alcohol (Aldrich). The allyl alcohol/HCl mixture was added to a-D-Glucose (MW 180.16; Aldrich) and reacted at 80°C for 4 h with reflux. After the reaction, unreacted allyl alcohol was evaporated under reduced pressure and heat. The prepared allyl glucose (MW 220.22) was then extracted with dry acetone. Allyl glucose was crystallized out from the concentrated extract.

Synthesis of Glucose-Containing Copolymers. Copolymers of allyl glucose and 1-vinyl-2-pyrrolidinone (VP; MW 111.14; Aldrich) were synthesized by free radical polymerization. The copolymers were synthesized from a solution of VP, allyl glucose, 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (Wako Pure Chemical Industries), and N,N,N',N'-tetramethylethylenediamine (Bio-Rad). Synthesized copolymers were dialyzed extensively against double distilled water. The molecular weight cutoff of the dialysis membrane was 6,000-8,000. Copolymers were then separated by precipitation with acetone and dried. A representative structure of the prepared copolymer is shown in Fig. 2.

Hydrogel Formation. Concanavalin-A (Con-A; MW 110,000; Sigma) was dissolved in 2X phosphate-buffered saline (PBS) solution containing 1mM $CaCl_2$, 1mM $MnCl_2$. The concentration of Con-A in the stock solution was 30% (w/v). The copolymer was also dissolved in PBS. The copolymer concentration was varied from 22.5 mg/ml to 180 mg/ml before the addition of Con-A solution. Equal volumes of Con-A and copolymer solutions were mixed to form hydrogels.

Determination of Glucose Concentration. The concentration of glucose molecules incorporated into the copolymer was measured by the phenol-sulfuric acid assay (3). A standard curve was constructed using various amounts of a-D-

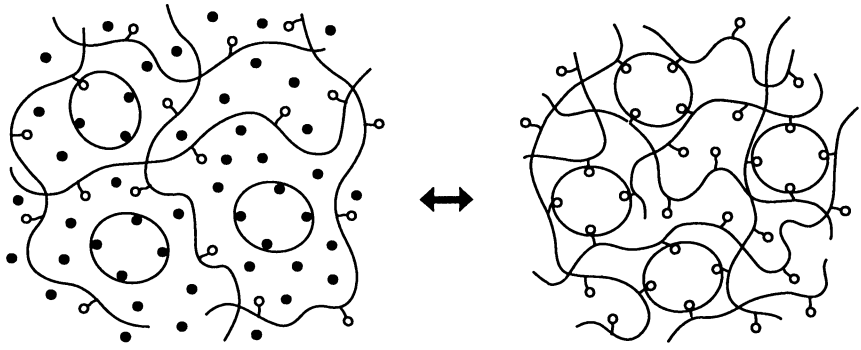

Figure 1. Pictorial representation of the sol-gel phase transition. Large circles represent Con-A molecules. Small open circles represent glucose attached to the polymer chain and small closed circles represent free glucose molecules.

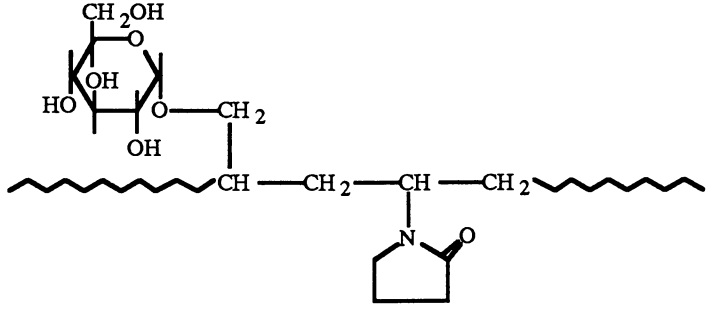

Figure 2. Representation of a copolymer made from allyl glucose and vinylpyrrolidinone monomers.

glucose. To glucose standard solutions and to copolymer solutions (2 ml each) was added 0.1 ml of phenol and 5 ml of concentrated sulfuric acid. After 30 min, the absorbance of the solutions at 490 nm was read on a Beckman DU-7 Spectrophotometer to determine the glucose concentrations.

Determination of Reactivity Ratios. For the measurement of the reactivity ratios, two monomers with various molar ratios in the initial feeds were copolymerized only for 10 min to produce copolymers at low conversion. Polymerization was stopped by pouring the reaction mixture into an excess amount of acetone, which resulted in dilution of more than 200 times by volume. From the measured concentrations of glucose and VP, the reactivity ratio was determined using the graphical method of Fineman and Ross (4).

Turbidity Measurements. Gelation of the mixture of Con-A and copolymers was determined by measuring the turbidity of the solution. To each well of a 96 well micro test plate were added 100 ml of copolymer solution and 100 ml of Con-A solution. The final concentration of copolymer solution was varied from 4.5 to 90.4 mg/ml, and that of Con-A solution was varied from 50 mg/ml to 150 mg/ml. The absorption was measured at 630 nm on EL311 Microplate Autoreader (Bio-Tek Instruments).

Results and Discussions

Polymerization of allyl glucose and VP produced copolymers containing various concentration of glucose on the backbone chain. Table I shows the compositions of the synthesized copolymers along with the molar ratios in the initial feed solution. The composition of the synthesized copolymer was determined by phenol-sulfuric assay. As shown in Table I, the incorporation of glucose into the copolymer increased with increase in initial feed composition of allyl glucose. The reactivity ratios obtained were 1.095 and -0.085 for r_1 (VP) and r_2 (allyl glucose), respectively. The reactivity ratio r_2 is essentially zero. The result indicates that the copolymer is composed of small blocks of VP with allyl glucose monomer placed in between the blocks.

Copolymer solutions were mixed with Con-A solution in test tubes at various concentrations to find out the condition for the gel formation. The final concentrations varied from 11 mg/ml to 90 mg/ml for copolymers and 50 mg/ml to 150 mg/ml for Con-A. The mixing resulted in either a viscous solution or a gel depending on the concentrations of the copolymer and Con-A. When the gel was formed, it formed immediately after the mixing. The sample became turbid and was not transparent any more. At that time, the gel could be taken out of the container as one solid piece. The results showed that the gel was formed more easily as the copolymer concentration became lower or the Con-A concentration became higher. The easier gel formation at low copolymer concentrations may be due to the less competition for Con-A among polymer chains. At higher Con-A concentrations, the competition among polymer chains is expected to be reduced and all the polymer chains can be crosslinked to form a gel.

The effect of copolymer concentration on the formation of gel was examined by measuring the turbidity of the mixture of Con-A and copolymer. The microturbidity plate wells have a capacity of 0.2 ml. The formation of a gel resulted in higher opacity. Thus, the increase in absorbance value or the increase in turbidity can be followed for the progress of gel formation. Fig. 3 shows the effect of copolymer and Con-A concentration on the turbidity values. On the x-axis, the first number denotes the [AG]/[VP] ratio in the copolymer and the second number denotes the Con-A concentration (w/v). The figure clearly shows that the turbidity increases with decrease in copolymer concentration and/or increase in Con-A concentration.

Table 1. Copolymerization of AG and VP

Concentration of [VP] in feed	Molar ratio of [Ag]/[VP] in feed	Copolymer composition	
		[AG] mol%	[VP] mol%
8%	0.19	11.2	88.9
	0.36	19.2	80.8
	0.51	26.9	73.1
	0.72	35.6	63.4
12%	0.13	10.3	89.7
	0.16	15.3	84.7
	0.32	23.6	76.4
	0.48	31.5	68.5
16%	0.10	9.1	90.9
	0.15	15.4	84.6
	0.30	22.1	77.9
	0.45	25.2	74.8

In a study to examine the glucose sensitivity, we prepared the gel composed of 150 mg/ml of Con-A and 22.5 mg/ml of copolymer in dialysis tubes. The weight ratio of [AG]/[VP] was 0.61. From this information, it was calculated that the polymer-attached glucose concentration in dialysis tube was 8.6 mg/ml. The gel-containing dialysis tubes were placed in glucose solutions of various concentrations. The results showed that the gel remained in the gel state as long as the concentration of free glucose was less than 38 mg/ml. If the solution concentration of free glucose, however, was greater than 38 mg/ml, the gel was converted into a sol. The sol became a gel again when the free glucose was removed by placing the dialysis tube in PBS solution. The concentration of free glucose necessary to dissolve the gel was about 4 times that of the glucose concentration in copolymer. When copolymers containing different amount of glucose was used, the result was about the same, i.e., about 4 times larger free glucose concentration was necessary to dissolve the gel. This study has demonstrated that our hydrogels are able to undergo phase changes between the sol and the gel states, and such phase changes are sensitive to the glucose concentration in the environment.

The glucose-sensitive phase-reversible hydrogels are useful in the design of self-regulating insulin delivery systems as well as glucose sensors. The phase transition and the resulting changes in permeability of insulin through the gel layer can be utilized to control the delivery of insulin. The sol state resulting from high free glucose levels is expected to allow faster insulin diffusion, and thus more insulin release. The gel state present at low free glucose levels, however, is expected to slow down or inhibit the release of insulin. The ability to sense glucose levels using our hydrogel instead of enzymes such as glucose oxidase (5) is that the Con-A system is independent of the oxygen level which often affect the result of the enzymatic reactions.

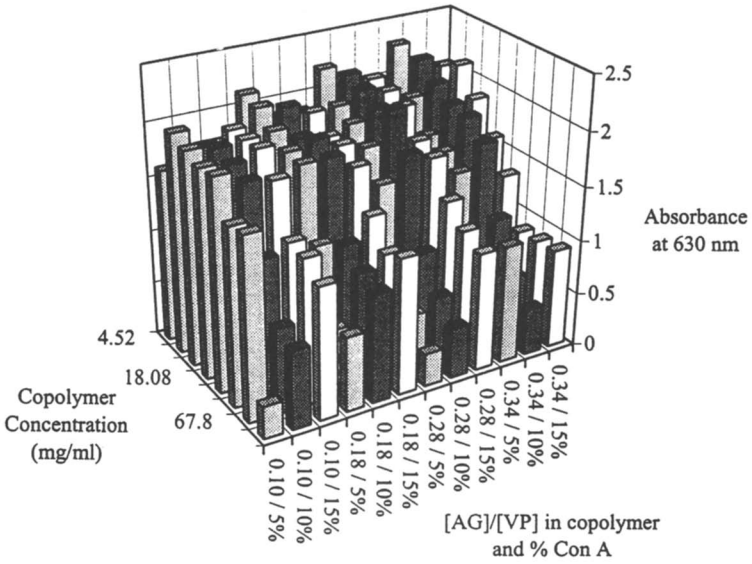

Figure 3. Turbidity of the mixtures of Con-A and copolymer solutions as a function of the copolymer concentration. Absorbance was measured at 630 nm. The left numbers (0.10, 0.18, and 0.28) in the x-axis indicate the molar ratios of [AG]/[VP] in the copolymer. The right numbers (5%, 10%, 15%) indicate the final concentrations of Con-A.

In this study we have focused on the synthesis of glucose-sensitive phase-reversible systems. The optimization of our hydrogel system can certainly lead to the development of better self-regulating insulin delivery systems and better glucose sensors. The existence of many specific interactions in nature provides opportunity for other new sol-gel phase-reversible hydrogels. The presence of antibody to almost any molecule makes it possible to synthesize hydrogels sensitive to any biomolecule.

Acknowledgments

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