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Glucose Induced Permeation Control of Insulin through a Complex Membrane Consisting of Immobilized Glucose Oxidase and a Poly(amine)

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ABSTRACT: To control the release of insulin in response to the concentration of glucose, a glucose-responsive polymer membrane was designed by combining a glucose oxidase (GOD) immobilized membrane, a sensor for glucose, with a poly(amine) membrane which regulates the permeation rate of insulin. The permeability of insulin was increased by the addition of glucose. Gluconic acid produced by an enzymatic reaction between GOD and glucose induced a decrease in the pH value of the medium. This caused the protonation of tertiary amino groups in the membrane resulting in an increase in the water content of the poly(amine) membrane. The permeability of insulin through a complex membrane thus increases with glucose concentration.

KEY WORDS Glucose / Insulin / Glucose Oxidase / Poly(amine) / Membrane / Permeation / Swelling /

Recently, many investigations have been carried out on the controlled release systems of drugs utilizing polymeric materials and maintaining the concentration of drugs in plasma within a therapeutic range for extended periods of time.[1] Langer *et al.* demonstrated that ethylene–vinyl acetate copolymers impregnated with insulin maintained diabetic blood glucose levels near normal levels for one month.[2] In comparison with insulin infusion pumps, the advantage of these implantable polymer pellets is that large amounts of insulin can be concentrated in a small volume as opposed to a mechanized pumping which must be refilled daily.[3] However, the insulin release rate from these pellets dose not depend on variation in blood glucose levels and minor surgery is required for their implant and retrieval.

For an ideal insulin delivery system, insulin release should be controlled directly by the amount of blood glucose present at any particular time. This requires a continual feedback between the blood glucose level and insulin release rate.

Brownlee *et al.* proposed a self regulating insulin delivery system based on a combination of biological modulations and controlled release.[4] The design of this delivery system utilizes the concept of competitive and complementary binding behavior of concanavalin A with glucose and glycosilated insulin.

We reported in a previous paper that the permeation rate of insulin through an amphiphilic polymer membrane can be controlled by the water fraction of the membrane.[5] We also reported a preparation method for a polymer membrane capable of regulating the permeation of insulin in response to change in the concentration of glucose.[6] The membrane

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consists of a GOD immobilized membrane which functions as a sensor for glucose and forms hydrogen peroxide by an enzymatic reaction, and a redox polymer having a nicotinamide moiety to regulate the permeation rate of insulin by an oxidation reaction with the hydrogen peroxide.

It is well known that a polymer having tertiary amino groups is protonated by a decrease in the pH value of a medium and the hydrophilicity of the polymer increases.[7] Therefore, a glucose-responsive polymer membrane can be obtained by combining a poly(amine) membrane and GOD immobilized membrane since the pH value of the medium will decrease by the formation of gluconic acid produced from the reaction of GOD with glucose.

This article discusses the permeation control of insulin using a complex membrane of immobilized GOD and poly(amine) in response to the glucose concentration.

EXPERIMENTAL

Materials

N, *N*-Diethylaminoethyl methacrylate (DEA) and 2-hydroxypropyl methacrylate (HPMA) were distilled under reduced pressure in a nitrogen atmosphere and the fractions of bp 68°C/4 mmHg and bp 77°C/3.5 mmHg were used, respectively. 2,2′-Azobisisobutyronitrile (AIBN), *N*, *N*-dimethylformamide (DMF) were purified by conventional methods. Acrylamide (AAm) was purified by recrystallization from benzene. *N*, *N*′-Methylenebisacrylamide (BIS), *N*, *N*, *N*′, *N*′-tetramethylethylenediamine (TMEA), ammonium persulfate (APS) were an extra pure grade and used without further purification. GOD was purchased from Sigma Co., Ltd.

Copolymerization of DEA with HPMA and Preparation of the DEA–HPMA Copolymer Membrane

In 101.2 ml of DMF, 10.9 ml of DEA, 68.0 ml of HPMA, and 0.148 g of AIBN were dissolved and the solution was placed in a polymerization tube. The tube was degassed and sealed by the conventional method. It was shaken for 24 min at 60°C. The reaction mixture was then cooled and poured into an excess of acetone–hexane mixture (1 : 5). The precipitate was dissolved in DMF and the solution was poured again into an excess of acetone–hexane mixture. The precipitated copolymer was filtered and dried *in vacuo*. The mole fraction of DEA in the copolymer was 0.12, as determined by elemental analysis. The structure of the copolymer is shown in Figure 1.

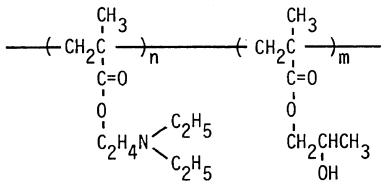

Figure 1. Structure of the DEA–HPMA copolymer.

The DEA–HPMA copolymer obtained was dissolved in DMF and the solution was cast on a Teflon plate. The solvent was slowly evaporated in an oven for 3 days at 40°C to prepare a DEA–HPMA copolymer membrane and it was dried *in vacuo*. The thickness of the DEA–HPMA copolymer membrane was 0.10 mm.

Preparation of the GOD Immobilized Membrane

A solution of 1.31 g of AAm, 65.3 mg of BIS and 10 mg of GOD dissolved in 7 ml of water was poured into a glass dish. To this was added 0.09 mg of TMEA and 0.5 ml of aqueous solution containing 25 mg of APS. The polymerization was initiated immediately and the GOD immobilized membrane, in a transparent, gel state, was obtained. The thickness of the membrane was about 1.0 mm. The membrane was washed with water to remove the unreacted substances and applied in the following experiment. The degree of immobilization exceeded 90%.[6]

Measurement of the Water Content of the DEA–HPMA Copolymer Membrane

The DEA–HPMA copolymer membrane was immersed in a 0.1 M sodium barbiturate buffer solution of various pH and swollen in a vessel thermostated at 30°C. The swollen membrane was taken out at regular intervals and the excess solution was removed with a filter paper. The membrane was then weighed. The water content of the membrane was calculated by the following equation,

$$\text{Water content } (\%) = \frac{(\text{Wt of swollen membrane}) - (\text{Wt of dry membrane})}{(\text{Wt of swollen membrane})} \times 100$$

Measurement of the Amount of Insulin Permeated through the DEA–HPMA Copolymer Membrane

The following permeation experiment was carried out. The DEA–HPMA copolymer membrane was swollen in a buffer solution of given pH at room temperature. After the water content of the copolymer membrane reached equilibrium, the copolymer membrane was interposed between two glass cells. The permeation area of the cell was 7.07 cm^2. A buffer solution 50 ml, containing 8.35 mg of insulin was placed in one cell and 50 ml of the buffer solution in the other. Both solutions were stirred slowly. Aliquots of the buffer solution were taken out after given periods of time and the absorbance at 274 nm based on insulin in the solution was measured to determine the amount of insulin which had permeated the membrane. The permeation coefficient was calculated from the slope of the permeation profile of insulin through the membrane.[5,6]

Measurement of the Amount of Insulin Permeated through a Complex Membrane

The insulin permeation experment using a complex membrane was carried out as follows. The GOD immobilized membrane and preswollen DEA–HPMA copolymer membrane were combined and interposed between two glass cells. The apparatus is shown in Figure 2. In the insulin permeation experiment without glucose, 50 ml of 1 mM sodium phosphate buffer solution (pH 7.10) containing 0.95 g of Na_2SO_4 and 8.35 mg of insulin were placed in the cell facing the DEA-HPMA copolymer membrane and 50 ml of the buffer solution were added to the cell facing the GOD immobilized membrane. In the experiment with glucose, 50 ml of the buffer solution containing a given amount of glucose were placed in the GOD immobilized membrane side. The amount of permeated insulin was then determined spectrophotometrically with a Shimadzu UV-240 Spectrophotometer

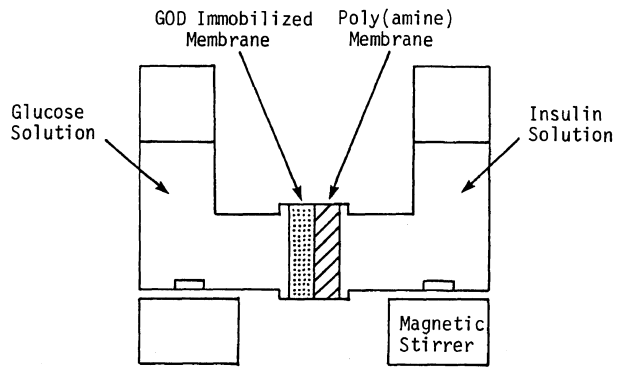

Figure 2. Apparatus for the insulin permeation experiments.

as mentioned above.

RESULTS AND DISCUSSION

Swelling Behavior of the DEA–HPMA Copolymer Membrane

Figure 3 shows the pH dependence of the equilibrium water content of the DEA–HPMA copolymer membrane. The water content increased with decreasing the pH of the medium, there was a particularly large increase in the water content below pH 6.80. It was confirmed that the water content of the poly(HPMA) membrane was independent of the pH of the medium and thus the change in water content of the DEA–HPMA copolymer membrane resulted from the introduction of the DEA moiety. The DEA moiety was considered to be protonated by a decrease in the pH of the medium followed by an increase in the hydrophilicity of the DEA moiety. This explains the pH dependence of the water content.

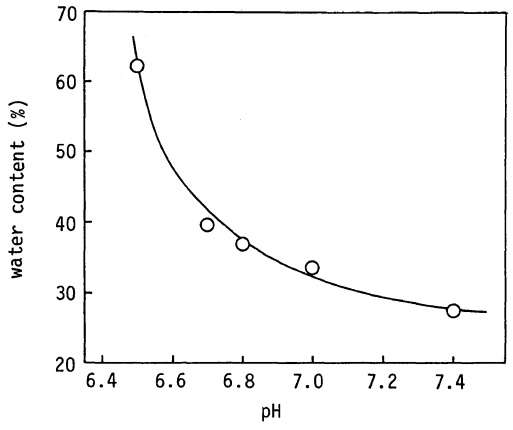

Figure 3. pH dependence of water content of the DEA–HPMA copolymer membrane at 30°C.

To investigate the reversibility of the change in the water content of the DEA–HPMA copolymer membrane induced by pH change, its time dependence was measured, when the pH of the medium was changed reversibly. The results are shown in Figure 4. When the preswollen membrane in a medium of a low pH (pH 6.50 or 6.65) was put in one of high pH (pH 7.40), the water content decreased. With a subsequent decrease in pH, the water content recovered its original value. Thus, the water content of the DEA–HPMA copolymer membrane changes reversibly by change in the pH of the medium.

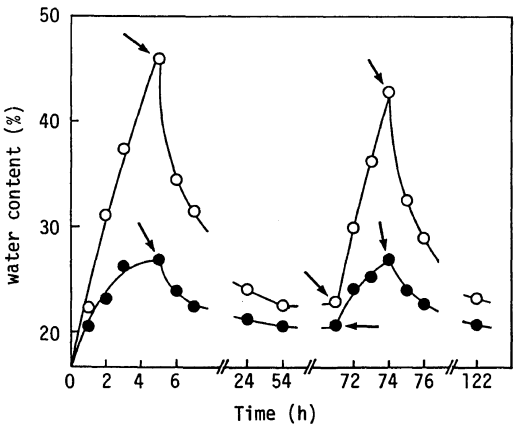

Figure 4. Reversible change in water content of the DEA–HPMA copolymer membrane induced by the pH change at 30°C: (A) pH 6.50; (B) pH 7.40; (C) pH 6.65.

Permeation of Insulin through the DEA–HPMA Copolymer Membrane

Figure 5 shows the relation between the permeation coefficient of insulin through the DEA–HPMA copolymer membrane and the pH of the medium. The permeation coefficient increased with decreasing pH, and there was a particularly large increase in the permeation coefficient below pH 6.80. This tendency was

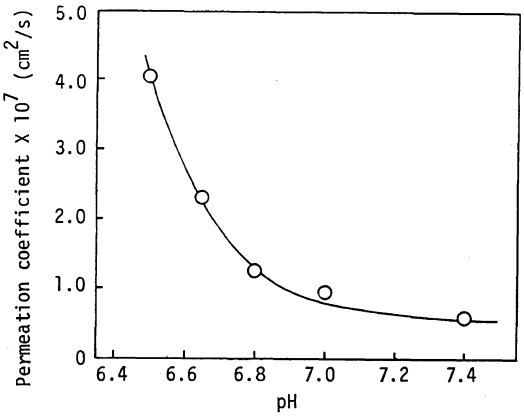

Figure 5. pH dependence of the permeation coefficient of insulin through the DEA–HPMA copolymer membrane at 30°C.

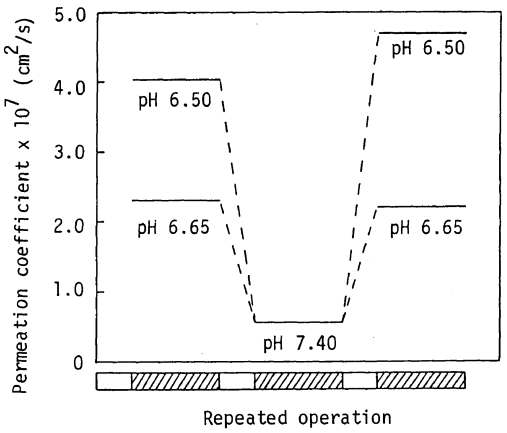

Figure 6. Various permeation coefficients of insulin through the DEA–HPMA copolymer membrane: (□)soaking, (▨) permeation.

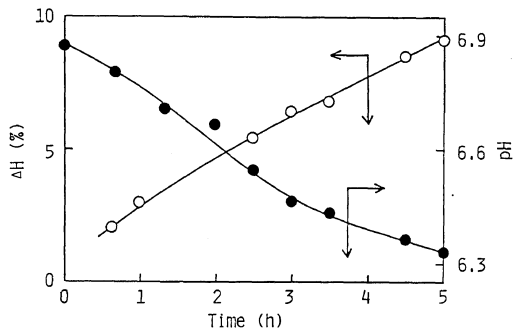

Figure 7. Changes in water content of the DEA–HPMA copolymer membrane and pH of the medium in 1 mM phosphate buffer solution containing 0.1 M glucose with the GOD immobilized membrane at 30°C: ΔH represents the change in membrane water content.

also observed for the water content of the membrane (see Figure 3). It is generally known that the permeability of a substance through a swollen polymer membrane depends on its water content.[8] In such a system, it is considered that an increase in the permeation coefficient at a low pH value is induced by an increase in the water content of the DEA–HPMA copolymer membrane.

Figure 6 shows the results of permeation experiments of insulin through the DEA–HPMA copolymer membrane with continuous change in the pH of the medium. It is clear that the permeation coefficient of insulin through the membrane changes reversibly with pH change. This corresponds to the swelling behavior of the DEA–HPMA copolymer membrane shown in Figure 5.

Thus, if the pH is changed through an appropriate chemical reaction, the permeability of insulin through the DEA–HPMA copolymer membrane should also change in response to the reaction.

Permeation of Insulin through a Complex Membrane Composed of the GOD Immobilized and DEA–HPMA Copolymer Membranes

The change in the water content of the DEA–HPMA copolymer membrane induced by an enzymatic reaction GOD and glucose was investigated. The experiment was carried out in 1 mM phosphate buffer solution containing 1 mM of glucose. The time dependence of change in water content and pH of the medium is shown in Figure 7. The pH of the medium decreased and the water content of the DEA–HPMA copolymer membrane increased at the same time with an increase in the reaction time. A decrease in pH was considered to result from the enzymatic reaction between GOD and glucose to form gluconic acid. The increase in water content of the DEA–HPMA copolymer membrane was attributed to protonation of the tertiary amino groups of the copolymer in response to a decrease in the pH.

Therefore, since the water content of the DEA–HPMA copolymer membrane can be changed by the enzymatic reaction between GOD and glucose, the permeation of insulin through the polymer membrane may change in response to the glucose concentration. A schematic representation of the glucose-induced permeation control of insulin using a complex membrane composed of the GOD immobilized membrane and a polyelectrolyte membrane having tertiary amino groups such as the DEA–HPMA copolymer membrane is shown

in Figure 8.

Figure 9 shows the results of insulin permeation through the complex membrane at various concentrations of glucose. It can be seen that the permeation rate of insulin in the presence of glucose was greater than that without glucose. The permeability of insulin through the GOD immobilized membrane alone was not changed by the addition of glucose and was about 10 times greater than that through the DEA–HPMA copolymer membrane.[6]. Moreover, the permeation rate of insulin through the complex membrane composed of the crosslinked poly(AAm) membrane instead of the GOD immobilized membrane, and the DEA–HPMA copolymer membrane in the medium with 0.1 M glucose was almost the same as that without glucose. In this case, the enzymatic reaction between GOD and glucose did not occur. Therefore, this increase in the permeation rate was attributable to the enzymatic reaction between GOD and glucose. In our previous article, the permeability of insulin through an amphiphilic polymer membrane was found to be influenced by the water content of the membrane.[5] Thus, increase in the permeation rate of insulin through the complex membrane was considered due to the change in the water content of the DEA–HPMA copolymer membrane induced by the enzymatic reaction between GOD and glucose.

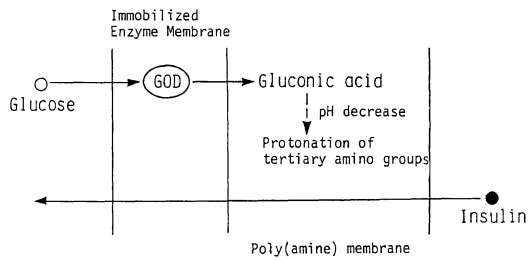

Figure 8. Schematic representation* of the glucose-responsive polymer membrane consisting of a poly(amine) and GOD immobilized membranes.

In the initial period of the permeation profile, the permeation rate of insulin in the medium with 0.2 M glucose was greater than that with 0.1 M glucose, but later both rates became nearly equal. The difference in the initial permeation rates was possibly due to the different initial rates of the enzymatic reaction owing to the different glucose concentration.

Thus, it may be concluded that the permeation control of insulin in response to glucose concentration is possible by using a complex membrane consisting of the GOD immobilized and DEA–HPMA copolymer membranes. Since the change in the permeation of

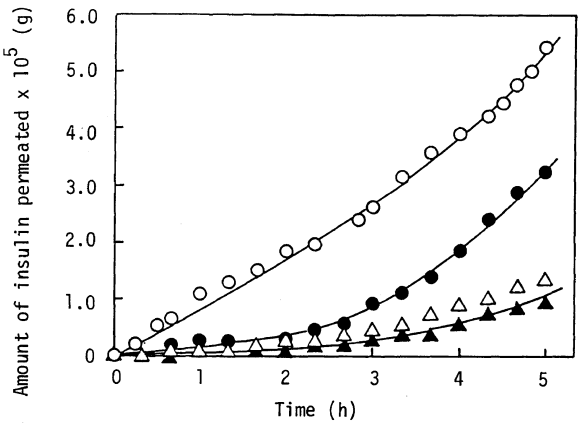

Figure 9. Permeation profile of insulin through a complex membrane consisting of the GOD immobilized and DEA–HPMA copolymer membranes at 30°C. Glucose concentration: (○) 0.2 M; (●) 0.1 M; (▲) 0 M; (△) 0.1 M using the crosslinked poly(AAm) membrane instead of the GOD immobilized membrane.

insulin through the DEA–HPMA copolymer membrane is reversible in response to the pH of the medium, the reversible permeation control of insulin is possible through the change in the glucose concentration by the complex membrane.

Glucose–responsive polymer membranes such as this complex membrane should be applicable to the controlled release of insulin as a responsive function of glucose concentration.

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