GLUCOSE STIMULATED INSULIN DELIVERY SYSTEMS

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Abstract - A self-regulating insulin delivery system is proposed based on
the competitive binding behavior of Con A with glucose. The results dem-
onstrated that glycosylated insulin concanavalin A complexes release
their glycosylated insulins, depending on glucose concentration present
and released glycosylated insulins permeate through the designed polymer
membranes.

INTRODUCTION

Brownlee and Cerami (Ref. 1) demonstrated that maltose-conjugated insulin bound to Concanava-
lin A (Con A) is released in the presence of a glucose solution. This glucose controlled in-
sulin delivery system is predicated on the concept of the competitive and complementary bind-
ing behavior of Con A with glucose and glycosylated insulin. Maltose insulin, however, may
not be used for the proposed system, because the binding constant to Con A is close to that
of glucose. The binding constant of glycosylated insulin to Con A should be relatively high
compared with that of glucose; otherwise, the displacement of glycosylated insulin by glu-
cose will take place at any glucose concentration even in a hypoglycemic condition. In the
course of synthesis of maltose insulin, the disaccharide opens one sugar ring in the coup-
ling reaction with insulin and the remaining sugar part attains a structure similar to glu-
cose. Recently, Jeong et al. (Ref. 2) synthesized and characterized eight glycosylated in-
sulins for the design of insulin delivery systems. The glycosylated insulin which is bound
to specific sites on Con A is displaced from Con A by glucose, thus insulin is released in
response to the concentration of glucose present. The schematic diagram of the self-regula-
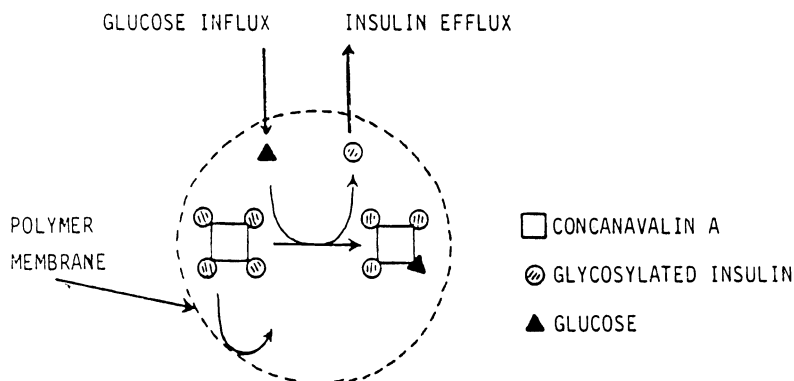ting insulin delivery system is illustrated in Fig. 1.

Fig. 1. Schematic diagram of self-regulating insulin delivery system.

Though the sugar binding site of Con A is most complementary to the α-D-mannopyranosyl resi-
due, Con A tolerates variation at the C-2 position (Ref. 3). Therefore, D-glucopyranosyl
unit also bind to Con A with less affinity than the D-mannopyranosyl unit. The C-3, C-4 and
C-6 hydroxyl groups of the D-arabino-configuration are the minimal requirement for interac-
tion with Con A. Since Con A has a wide range of affinities, by choosing proper sugar resi-
dues and possibly the appropriate spacer between the insulin molecule and the sugar, synthe-
sis of the best glycosylated insulin for the self-regulating insulin delivery system is
quite feasible.

Insulin diffusion through polymer membranes was studied to prepare the suitable membranes
for the self-regulating insulin delivery systems. Polymers used for the diffusion studies
include cellulose acetate (dense and porous type), regenerated cellulose (dense and porous

type), copolymer of lactic and glycolic acid (dense and porous type), poly(hydroxyethyl methacrylate) (p-HEMA) (dense and porous type), copolymer (hydroxyethyl methacrylate/methoxyethyl methacrylate) (p-HEMA/MEMA) (dense type) and copolymer (hydroxyethyl methacrylate/methoxyethoxyethyl methacrylate) (p-HEMA/MEEMA) (dense type) (Ref. 4).

Using three potential membranes, the efflux of glycosylated insulin by glucose substitution was examined. Two glycosylated insulins, that is, p-(succinylamido)-phenyl-α-D-mannopyranoside (SAPM)-insulin and p-(succinylamido)-phenyl-α-D-glucopyranoside (SAPG)-insulin, were selected based on optimum binding constant to concanavalin A (Con A). The obtained results support the feasibility of design for the self-regulating insulin delivery systems (Ref. 5).

MEASUREMENT OF GLYCOSYLATED INSULIN EFFLUX

The self-regulating insulin delivery system under development is predicated on the concept of the competitive and complementary binding behavior of Con A with glucose and glycosylated insulin. The derivatized insulin is bound to Con A which, in turn, is complementary to glucose. The glycosylated insulin is then displaced from Con A by glucose and insulin is released in response to, and proportional to, the amount of glucose present in the blood. The releases of two glycosylated insulins, SAPM-insulin and SAPG-insulin, from Con A complexes through three kinds of polymer membranes, porous type regenerated cellulose membrane, porous type membrane of the copolymer of 50% lactic acid and 50% glycolic acid and porous type p-HEMA membrane, were studied by the competitive substitution of glucose.

The apparatus used for release experiment of glycosylated insulins (Fig. 2) consists of a dialysis cell with an upper chamber, containing the complex of Con A and glycosylated insulin in tris buffer (pH 7.4) separated by a polymer membrane from a lower chamber, through which a given concentration of glucose in tris buffer (pH 7.4) is pumped at a constant flow rate (10 ml/h) and from which the effluent is sampled for measurement of radioactivity. Tris buffer (pH 7.4) contains Mn^{2+} and Ca^{2+} ions because Con A subunit contains one Ca^{2+} and one Mn^{2+} ion and both metals are needed for carbohydrate binding. The effluent was collected every hour for 24 hours, and ^{125}I-radioactivity was counted with a gamma-counter (Beckman Biogamma II, Beckman Instruments, Inc.) to determine the rate of release of glycosylated insulin.

0.5 mg of ^{125}I-labelled glycosylated insulin in 2.5 ml of tris buffer (pH 7.4) was mixed with 5 mg of Con A in 2.5 ml tris buffer and then incubated at room temperature to allow them to form complex for one hour just before the exchange diffusion experiment. The mixture was placed in the upper chamber of the cell and then tris buffer (pH 7.4) with no glucose was pumped into the lower cell for 6 hours to remove free glycosylated insulin which was not bound to Con A. This concentration process was monitored by counting the radioactivity of effluent. The radioactivity of effluent of the concentration process showed maximum at 1-2 hours and then gradually decreased, and about 10-15% of total glycosylated insulin was removed in this process. After the concentration process, given concentrations of glucose in tris buffer were pumped into the lower chamber of the cell for 24 hours. Four glucose concentrations were chosen, 50 mg/dl (hypoglycemic condition), 100 mg/dl (euglycemic condition), 300 mg/dl (hyperglycemic condition) and 500 mg/dl (hyperglycemic condition).
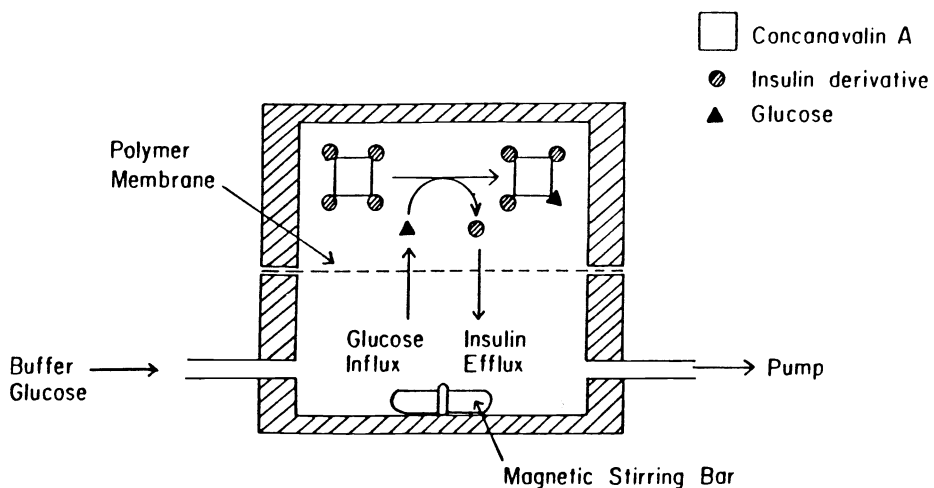

Fig. 2. Schematic diagram of the exchange diffusion cell.

RATE OF RELEASE OF GLYCOSYLATED INSULIN

The binding constant of glycosylated insulin to Con A should be much higher than that of

glucose, otherwise the displacement of glycosylated insulin by glucose will take place at any glucose concentration even in a hypoglycemic condition if the binding constant of glycosylated insulin to Con A is close to that of glucose. Table 1 summarizes the estimated glycosylated insulin binding to Con A which was normalized as the binding constant of glucose to Con A is unity. As seen from Table 1, p-(succinylamido)-phenyl-α-D-mannopyranoside (SAPM)-insulin and p-(succinylamido)-phenyl-α-D-glucopyranoside (SAPG)-insulin have fairly high binding constants to Con A, therefore, these two glycosylated insulins were chosen and used for exchange diffusion experiments.

TABLE 1. Estimated glycosylated insulin binding to concanavalin A (normalized values to glucose) (Ref. 6).

Glycosylated insulin	Relative binding constant
Maltose-insulin	1
Succinyl and glutaryl glucosamine-insulin	<0.5
Succinyl and glutaryl amido-phenyl-glucopyranoside-insulin	45
Succinyl and glutaryl amido-phenyl-mannopyranoside-insulin	200
Glucopyranosyloxy-phenyl-thiocarbamoyl-insulin	45

The membranes used in the exchange diffusion experiments were porous regenerated cellulose, porous copolymer of 50% (w/w) lactic and 50% (w/w) glycolic acid, and porous p-HEMA. Released glycosylated insulins caused by glucose competitive substitution versus the time they were measured after introduction of the glucose solution into the exchange diffusion cell. It was shown that glycosylated insulins were released through polymer membranes depending on the glucose concentration, indicating the feasibility of the self-regulating insulin delivery system (Ref. 5).

As might be expected from the difference in binding constants of SAPM-insulin and SAPG-insulin (the estimated binding constants of SAPG-insulin and SAPM-insulin to Con A are 45 and 200, respectively, when normalized to glucose), SAPG-insulin was released at a higher rate than SAPM-insulin.

Among the three polymer membranes, the porous copolymer of lactic/glycolic acid allowed the highest rate of release of glycosylated insulins, and porous regenerated cellulose showed the lowest rate of release while porous p-HEMA exhibited intermediate release rates.

When the porous copolymer of lactic/glycolic acid was used as a membrane, the rates of release of glycosylated insulins reached maxima in four hours and then decreased rapidly, indicating depletion of glycosylated insulin from the system.

SAPG-insulin may not be useful with the porous copolymer of lactic/glycolic acid because SAPG-insulin has a low binding constant to Con A compared with SAPM-insulin and subsequently the SAPG-insulin is depleted very rapidly if the porous copolymer of lactic/glycolic acid is used as a membrane in the self-regulating insulin delivery system.

RESPONSE OF GLYCOSYLATED INSULIN RELEASE TO ALTERATION IN GLUCOSE CONCENTRATION

Insulin release response to the alteration in glucose concentration was studied. The glucose concentration was changed alternately between 50 and 500 mg/dl at four hour intervals. The results are shown in Figs. 3-8.

The system with the combination of porous regenerated cellulose and SAPM-insulin did not show a clear response because this combination consisted of a tight membrane and a high binding constant glycosylated insulin (Fig. 3).

The system with the combination of porous regenerated cellulose and SAPG-insulin showed a response to the change of glucose concentration, but the lag time of response was the same as the intervals of the change of glucose concentration and, consequently, the system showed the release curve which fluctuated out of phase from the alteration in glucose concentration (Fig. 4). The curve of the release rate of this system showed a gradual upward trend
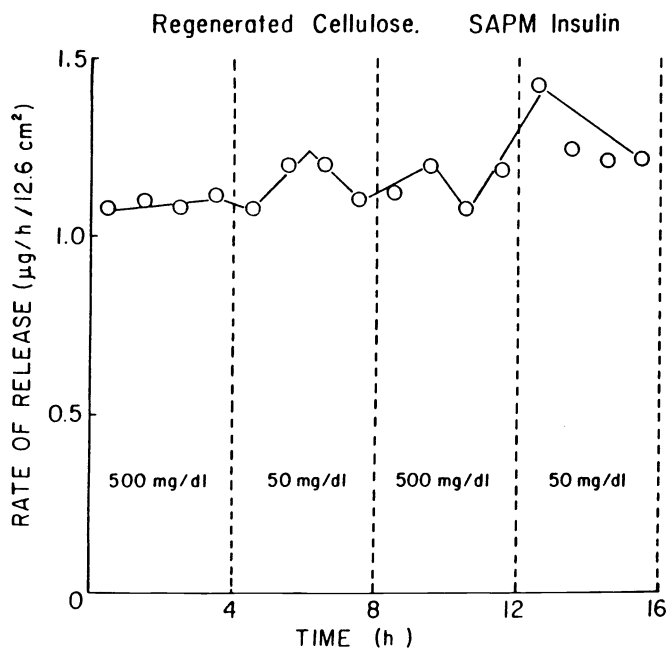
Regenerated Cellulose.   SAPM Insulin

Fig. 3. Response of SAPM-insulin to alteration in glucose concentration
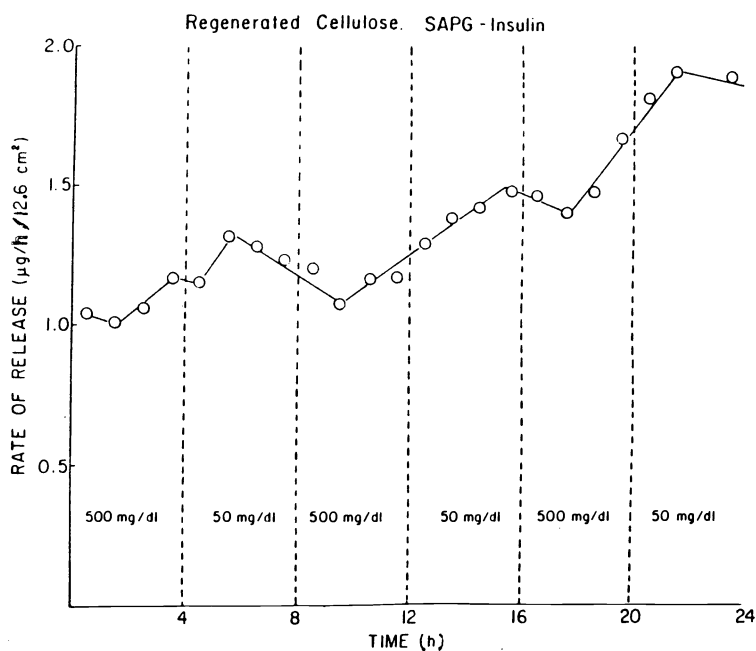through regenerated cellulose porous membrane.

Fig. 4. Response of SAPG-insulin to alteration in glucose concentration
through regenerated cellulose porous membrane.

as a whole, indicating the cumulation of replaced glycosylated insulin inside the upper cham-
ber of the cell because of the low diffusivity of porous regenerated cellulose and the low
binding constant of SAPG-insulin.

The system with porous copolymers of lactic acid and glycolic acid and SAPM-insulin showed a
fairly good response to the change of glucose concentration and the lag time of response was
about 1.5 hours (Fig. 5). The response curve of this system gradually went down wholly as
responding to the glucose concentration. This may be due to the depletion of glycosylated
insulin from the system.

Though the combination of porous copolymer of lactic acid and glycolic acid and SAPG-insulin

responding very sharply to the first fluctuation of glucose concentration, the system became unresponsive quite rapidly due to the depletion of glycosylated insulin from the system (Fig. 6).
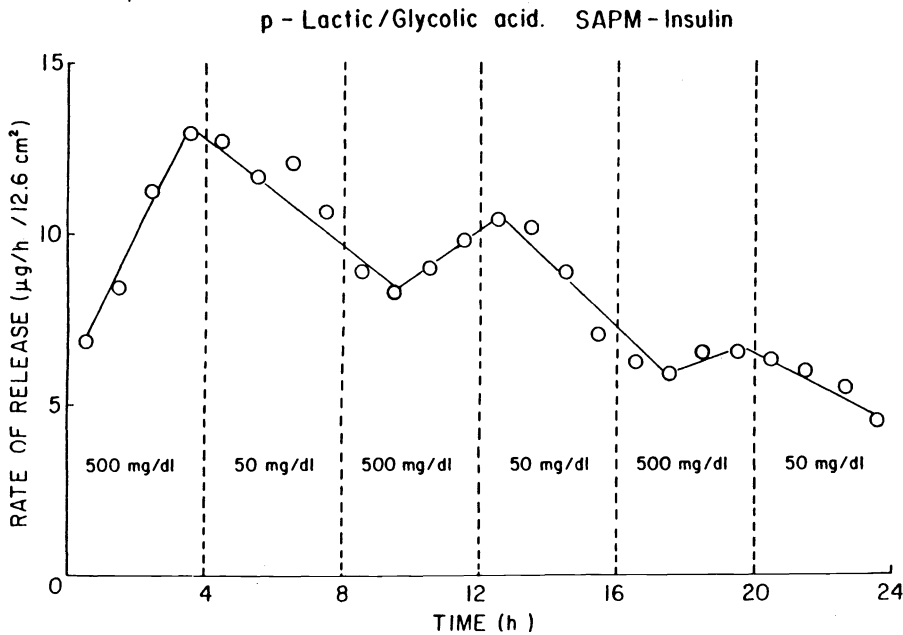

Fig. 5. Response of SAPM-insulin to alteration in glucose concentration through polylactic/glycolic acid porous membrane.
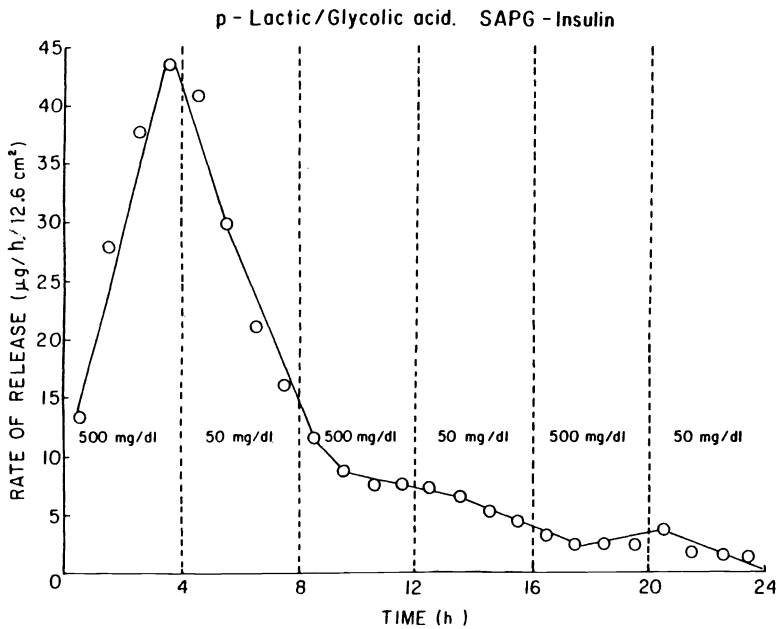

Fig. 6. Response of SAPG-insulin to alteration in glucose concentration through polylactic/glycolic acid porous membrane.

The systems with porous p-HEMA membranes (Figs. 7 & 8) demonstrated the feasibility of the self-regulating insulin delivery system. These systems showed a moderate but steady response to the change of glucose concentration. The lag time was shorter than one hour. Lag time is one of the most important factors of the design of the self-regulating insulin delivery system and can be related to the thickness of membrane.

Chick et al. (Ref. 7) measured insulin release response to the alteration in glucose concentration using beta cells cultured in artificial hollow fibers. The interval of the altera-

tion in glucose concentration was two days in their experiments and the rate of insulin re-
lease was represented as mU/48 hours. Compared with Chick's results, the results obtained
here support the feasibility of the self-regulating insulin delivery system.
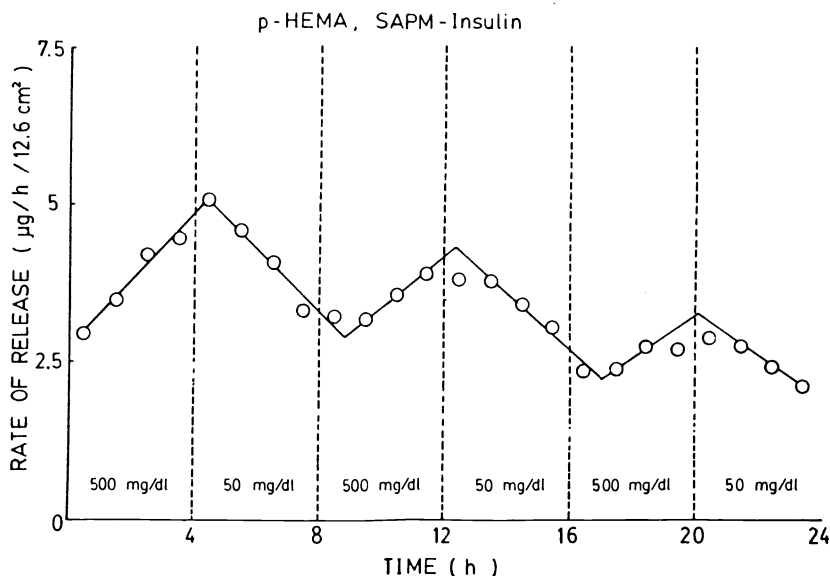

Fig. 7. Response of SAPM-insulin to alteration in glucose concentration
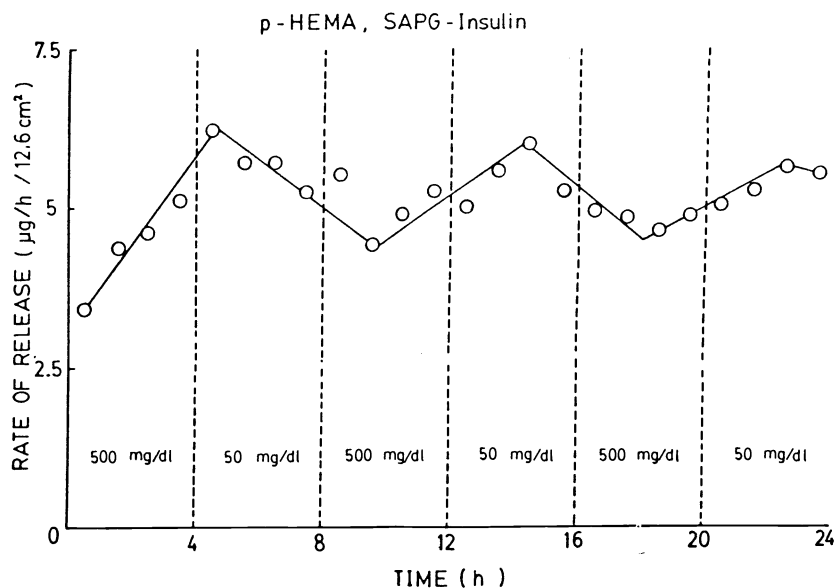through poly-HEMA porous membrane.

Fig. 8. Response of SAPG-insulin to alteration in glucose concentration
through poly-HEMA porous membrane.

REFERENCES

1. M. Brownlee and A. Cerami, Science 206, 1190-1191 (1979).
2. S.Y. Jeong, M.J.D. Eenick, J. Feijen and S.W. Kim, J. Controlled Release (in press).
3. R.D. Poretz and I.J. Goldstein, Biochemistry 9, 2890-2896 (1970).
4. S. Sato and S.W. Kim, submitted to Int. J. Pharmaceutics.
5. S. Sato, S.Y. Jeong, J.C. McRea and S.W. Kim, J. Controlled Release (in press).
6. I.J. Goldstein and C.E. Heyes, Adv. Carbohyd. Chem. Biochem. 38, 127-205 (1978).
7. W.L. Chick, A.A. Like, V. Lauris, P.M. Galletti, P.D. Richardson, G. Panol, T.W. Mix and
   C.K. Colton, Trans. Am. Soc. Art. Int. Org. 21, 8-15 (1975).