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SELF-REGULATING INSULIN DELIVERY SYSTEMS

I. SYNTHESIS AND CHARACTERIZATION OF GLYCOSYLATED INSULIN

Seo Young Jeong, Sung Wan Kim*
Department of Pharmaceutics, University of Utah, Salt Lake City, UT 84112 (U.S.A.)
Marinus J.D. Eenink and Jan Feijen
Department of Chemical Technology, Twente University of Technology, Enschede (The Netherlands)

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A design for a self-regulating insulin delivery system based on the competitive binding of glucose and glycosylated insulin to the lectin Concanavalin A is proposed. A different approach to diabetes therapy is the attempt to effect a permanent cure of the disease by supplementing the patient's defective pancreas with a normally functioning transplant. However, pancreatic transplantation in humans is still in its early stage, and the major problems including rejection of the transplants still remain unsolved. In phase one, eight glycosylated insulin derivatives were synthesized. Maltose was directly coupled to bovine insulin by reductive amination. Succinyl- and glutaryl-glucosamine derivatized insulins were synthesized by a mixed anhydride method using the appropriate substituted glucosamines. Monosaccharide derivatives p-aminophenyl-α-D-glucopyranoside and p-aminophenyl-α-D-mannopyranoside were also coupled to insulin via succinate and glutarate spacers. p-(α-D-glucopyranosyloxy)-phenyl-thiocarbamoyl insulin was obtained by reacting insulin with p-isothiocyanatophenyl-α-D-glucopyranoside, which was obtained through conversion of p-aminophenyl-α-D-glucopyranoside with thiophosgene. Unreacted maltose and other carbohydrate derivatives were removed by gel permeation chromatography or dialysis; unmodified insulin was removed by affinity chromatography. The yield and purity of the carbohydrate derivatives were determined by IR, NMR and MS/GC.

INTRODUCTION

The development of an insulin delivery system based on a biological or bioengineering approach has been attempted for the past decade, because blood glucose levels cannot be controlled in an optimal way with conventional administration of insulin.

The biological approach has been the transplantation of islet cells, but a major obstacle is formed by the recipient's immune rejection of the transplant. Because of the limited availability of human pancreatic tissue, there is considerable interest in finding ways to transplant tissue from animals into humans without immunological rejection. The strategy in this approach is to develop a protective encapsulation that will allow the access of genetically dissimilar islet cells to a diabetic patient's blood stream, but which

*To whom request for reprints and correspondence should be addressed.

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prevents the activation of the patient's immune defense system.

Chick et al. [1] cultured pancreatic islet cells on hollow fibers, which were sealed into plastic tubes, and surgically implanted in the upper leg of diabetic rats. It was found that the beta cells continued to secrete insulin, in approximate response to alterations in the glucose concentration of the perfusate. There was no sign of immunological rejection, and the blood glucose levels of the diabetic rats were normalized by the devices. Although this approach appears to hold promise, it requires surgery and a device needing a continuous intravenous connection. Moreover, the risk of infection and clotting may be serious for application to a human, in addition to the immunogenic problems.

Recently, Sun et al. [2] reported an alternative approach to the problem of islet transplantation. Pancreatic islet cells of healthy rats were microencapsulated and injected in diabetic rats. The polymer membrane, composed of polylysine and polyethyleneimine, had pores which allowed the permeation of glucose and insulin, but prevented the transport of immunoglobulins. The promising results obtained for a period of more than 90 days justify further evaluation of encapsulated islet cells as a bio-artificial pancreas for human application.

The bioengineering approach is directed towards the design of insulin infusion pumps. Several hundred diabetics in the U.S. presently use external battery-operated pumps. The pump injects insulin continuously through a needle attached to a catheter inserted into a vein or into subcutaneous tissue. The flow can be adjusted manually when a different delivery rate of insulin is required. The units are usually worn on a belt or strapped to a leg.

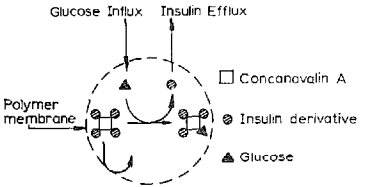
Still in an experimental stage are pumps delivering an amount of insulin which is controlled by a sensor which measures blood glucose levels. Though successful progress has been made in this area, these pumps are still too heavy to be portable. Another difficulty is that the system needs an apparatus for the continuous sampling of blood, an analyzer to determine the blood glucose level rapidly and continuously, a computer to analyze the results and to determine the appropriate insulin dose, and an infusion pump to deliver insulin intravenously in a manner approximating the delivery by the beta cells of the pancreas. Efforts are underway to reduce the size of the system and prolong its sensor's life. The "vest pocket" model, a system the size of a cigarette pack containing glucose sensor, power source, computer, insulin reservoir and pump, has been devised [3]. Another obstacle at present is the lack of an accurate implantable electrode to sense the concentration of blood glucose. Again, a through-the-skin connection to the patient's blood stream for long periods presents risks of infection and clotting problems. Also, the occurring aggregation of insulin in the artificial delivery system forms a great problem [4].

Our approach is to design a self-regulating insulin delivery system based on the competitive binding of glucose and derivatized carbohydrate-coupled insulin to Concanavalin A (Con A), which has been demonstrated in the case of maltose-coupled insulin [5].

The glycosylated insulin is displaced from Con A by glucose in response to, and proportional to, the amount of glucose present in the blood. The release rate of insulin also depends on the binding affinity of the derivative to the lectin, and can be influenced by choice of the saccharide group in the glycosylated insulin.

The glycosylated insulin bound Con A has to be encapsulated with a suitable polymer membrane that is permeable to both glucose and insulin. Proper choice of the membrane criteria and the type of derivatized insulin would provide optimal insulin release rates necessary for the maintenance of normal blood glucose levels. A schematic diagram of the system is shown in Scheme 1.

This manuscript includes the preparation of eight insulin derivatives synthesized for



Scheme 1. Con A-glycosylated insulin derivative complex inside a microcapsule.

the use in insulin delivery systems. The compounds used for coupling with insulin were characterized by IR, NMR and MS/GC; glycosylated insulins were separated by chromatographic methods.

METHODS

Bovine insulin was obtained from Sigma Chemical Company (St. Louis, MO) and used as received. Maltose, D-glucosamine hydrochloride, *p*-nitrophenyl-α-D-glucopyranoside and *p*-nitrophenyl-α-D-mannopyranoside were products of Sigma Chemical Company. All materials were used without further purification.

Bio-gel P-6, 100—200 mesh (excl. M.Wt. 6,000) was purchased from Bio-Rad Laboratories (Richmond, CA). Con A-Sepharose 4B was obtained from Sigma Chemical Co. All other reagents were of acceptable purity. Melting points are uncorrected and were determined with a Thomas Hoover capillary melting point apparatus. UV absorption spectra were measured using a Beckman DU®-8 spectrophotometer and quartz cells (path length 10 mm). Infrared spectra were recorded on a Beckman Microlab 620MX computing infrared spectrophotometer. The samples were prepared as 0.5% (w/w) KBr pellets. NMR spectra were recorded on a JEOL JNM-FX 270 Fourier Transform NMR spectrometer. The samples (0.1 *M*) were dissolved in D_2O, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal reference.

Molecular weights of the synthesized intermediate carbohydrate derivatives were determined by LKB 9000S GC/MS interfaced to a DEC PDP 11/3K computer. The volatility of carbohydrate derivatives was enhanced by trimethylsilyl (TMS) derivatization on the hydroxyl, amine and carboxyl functional groups.

Unless otherwise stated, the phenol—sulfuric acid method [6] was used to determine the average degree of substitution of insulin with carbohydrate derivatives.

Maltose—insulin conjugate

Maltose, 4-*O*-α-D-glucopyranosyl-D-glucose (2 mmol, 685 mg), and bovine insulin (5 μmol, 28.5 mg) were dissolved in 5 ml of 0.1 *M* phosphate buffer (pH = 8.0) containing 0.02% sodium azide. Sodium cyanoborohydride (78.1 mg) was added, and the solution was stirred for five days at 37°C. The solution was then subjected to gel permeation chromatography (GPC) and affinity chromatography to remove unreacted maltose and insulin, respectively. The maltose—insulin conjugate (8.103 mg) was isolated by affinity chromatography eluting with a 0.02 *M* Tris buffer (pH 7.4) containing 0.1 *M* α-methyl-D-mannopyranoside. The degree of substitution was found to be 1.0 saccharide unit per insulin molecule as determined by the phenol- sulfuric acid method.

N-Succinyl-glucosamine (NSGA)

A solution of succinic anhydride (50 mmol, 5.005 g) in acetone (37.5 ml) was added to a solution of glucosamine hydrochloride (50 mmol, 10.78 g) in double distilled water (15 ml) containing triethylamine (50 mmol, 6.95 ml). After 4 hours stirring at room temperature, the solution was concentrated under reduced pressure until a viscous, yellowish residue was obtained. After the

TABLE 1

Physical properties of derivatives of glucosamine

Derivative	IR (cm^{-1})			NMR	MS/GC			Yield (%)	Melting point (°C)
	Amide I band	Amide II band	Carboxylic ν(C=O)	Observed ppm of spacer group	Calc. M.Wt.	Calc. M. Wt. of TMS derv.	Observed M −15		
NSGA	1650	1550	1700	2.63, 2.66	279.26	639.26 (5)[a]	624.3	39	174–175
NGGA	1650	1550	1700	1.86, 2.35, 2.39	293.27	653.27 (5)[a]	638.3	41	195–196

[a]Number in parenthesis is degree of substitution with TMS.

addition of three times the volume of glacial acetic acid a precipitate was obtained. The solid was collected by filtration and washed with ethanol and ether [7].

N-Glutaryl glucosamine (NGGA)

N-Glutaryl glucosamine was obtained using the same method with glutaric anhydride instead of succinic anhydride. The yield and physical properties of NSGA and NGGA are listed in Table 1.

(*N*-Succinyl/*N*-Glutaryl) glucosamine–insulin conjugates (NSGA–INS/NGGA–INS)

The coupling of compounds NSGA and NGGA to insulin was performed using a mixed anhydride method [8,9]. Bovine insulin (87.77 μmol, 500 mg) was dissolved in a 1:1 (v/v) mixture of water and dimethylformamide (200 ml total volume) which was precooled in an ice bath after adjustment of the pH to 9.5 with 0.1 *M* sodium hydroxide. Eight hundred micromoles of NSGA or NGGA were dissolved in DMF (3 ml) containing 800 μmol of tri-*N*-butylamine and isobutylchloroformate. After keeping this solution for 20 minutes at 0°C, an additional 1.6 mmol of tri-*N*-butylamine was added and the resulting solution was combined with the insulin solution. The reaction mixture was adjusted to pH 9.5 with 0.1 *M* NaOH, and kept for 1 hour at 0°C, and subsequently overnight at room temperature. The solution was dialyzed against distilled water for two days at 4°C, changing the water every 4 hours, to remove unreacted glucosamine derivatives. One hundred milligram of each glycosylated insulin were applied to the affinity column. After affinity chromatography the purified glycosylated insulin products (NSGA–INS/NGGA–INS) were obtained by lyophilization. The yields of the NSGA– and NGGA–insulin conjugate were 1.316 mg and 4.648 mg, respectively. Average degrees of substitution of insulin could not be determined due to the low absorptivity of the glycosylated insulins (NSGA and NGGA moieties) in the phenol–sulfuric acid test.

Woodward's Reagent K was also used in the coupling of NSGA and NGGA to insulin, but the very low yields made this method ineffective.

p-Aminophenyl-α-D-glucopyranoside (APG)

To a solution of *p*-nitrophenyl-α-D-glucopyranoside (NPG, 14 mmol, 4.214 g) in methanol (350 ml), ammonium formate (56 mmol, 3.54 g) and palladium on carbon (10%) (2 g) were added [10]. Nitrogen gas was led through the reaction mixture for 4 hours at room temperature. After filtration of the catalyst the solvent was evaporated at reduced pressure. Recrystallization from an ethanol–water mixture (50:1, v/v) gave APG in 71% yield, m.p. 169–170°C (lit. 158.5–159.5°C, ref. [11], 185–186°C, ref. [12]).

p-Aminophenyl-α-D-mannopyranoside (APM)

p-Aminophenyl-α-D-mannopyranoside (APM) was prepared according to the same method [10] as compound APG, starting from *p*-nitrophenyl-α-D-mannopyranoside

(NPM) in 91% yield, m.p. 150—153°C (lit. 164°C, ref. [13]).

p-Isothiocyanatophenyl-α-D-glucopyranoside (ITPG)

ITPG was obtained in 82% yield from APG by reaction with thiophosgene in 80% aqueous ethanol [14]. Excess thiophosgene was used because of its instability in water and alcohols, and because the intermediate aminothiocarbonylchloride can give thiourea derivatives in the presence of excess amine [15]. The ITPG prepared had a m.p. 185—187°C (lit. 184—186°C, ref. [14]).

p-(α-D-Glucopyranosyloxy) phenyl thiocarbamoyl insulin (GPT—INS)

The reaction of ITPG (313 μmol, 98 mg) with insulin (174 μmol, 1.0 g) was carried out without stirring during 19 hours at 5°C, and subsequently 13 hours at room temperature, in a pyridine—water (3:1, v/v) solution, with an initial pH of 8.0 [16]. The reaction mixture was evaporated to dryness under reduced pressure. Unreacted ITPG was removed by gel permeation chromatography. Unreacted insulin was removed by affinity chromatography. GPT—INS was obtained in 49% yield. The average degree of substitution was estimated from the UV absorption of this compound at 260 nm. A value of 1.4 saccharide units per insulin molecule was found, using the literature data reported for the phenylisothiocyanate derivative of insulin (ϵ = 15,000 l/mol cm at λ_{max} = 260 nm [16]).

p-(Succinylamido/glutarylamido) phenyl-α-D-glucopyranoside (SAPG/GAPG)

SAPG and GAPG were obtained by reacting APG with the appropriate anhydride as described for the glucosamine derivatives NSGA and NGGA. Yield and physical properties of SAPG and GAPG are listed in Table 2.

p-(Succinylamido/glutarylamido) phenyl-α-D-mannopyranoside (SAPM/GAPM)

SAPM and GAPM were obtained by reacting APM with the appropriate anhydride as described for the glucosamine derivatives NSGA and NGGA. Yield and physical properties of SAPM and GAPM are listed in Table 2.

Conjugates of SAPG, GAPG, SAPM, GAPM and insulin (SAPG—INS/GAPG—INS/SAPM—INS/GAPM—INS)

The sugar derivatives SAPG, GAPG, SAPM and GAPM were coupled to insulin via the mixed anhydride method as described for NSGA—INS and NGGA—INS. The yields of SAPG—INS, GAPG—INS, SAPM—INS and GAPM—INS were 63, 50, 70 and 70%, respectively. The average degree of substitution of insulin was found to be 2.3, 1.6, 2.5 and 2.0 saccharide units per insulin molecule, respectively, as determined by the phenol—sulfuric acid method [6].

TABLE 2

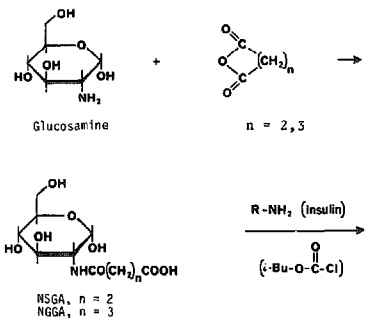
Physical properties of derivatives of APG and APM

Derivative	IR (cm^{-1})			NMR	MS/GC			Yield (%)	Melting point (°C)
	Amide I band	Amide II band	Carboxylic ν(C=O)	Observed ppm of spacer group	Calc. M.Wt.	Calc. M.Wt. of TMS derv.	Observed M−15		
SAPG	1670	1540	1730	2.71	371.34	803.34 (6)[a]	788.7	53	178—180
GAPG	1670	1540	1710	—	385.37	—	—	63	167—168
SAPM	1670	1550	1720	2.69	371.34	803.34 (6)[a]	788.5	67	65—66
GAPM	1670	1560	1690	—	385.37	—	—	75	134—136

[a] Number in parenthesis is degree of substitution with TMS.

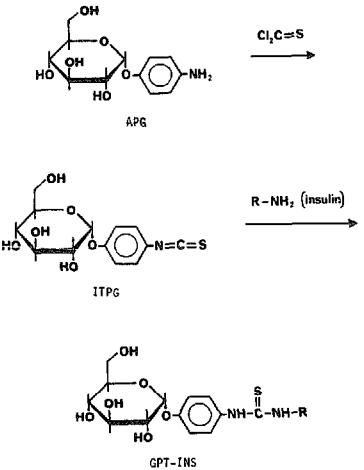
RESULTS AND DISCUSSION

The synthetic pathways for the preparation of glycosylated insulin derivatives are given in Schemes 2–4. The derivatized carbohydrate compounds NSGA, NGGA, SAPG, GAPG, SAPM and GAPM were obtained by reacting glucosamine, *p*-aminophenyl-α-D-glucopyranoside or *p*-aminophenyl-α-D-mannopyranoside with the appropriate acid anhydrides.



Scheme 2. Schematic route from glucosamine to *N*-(succinyl/glutaryl) glucosamine—insulin conjugates.

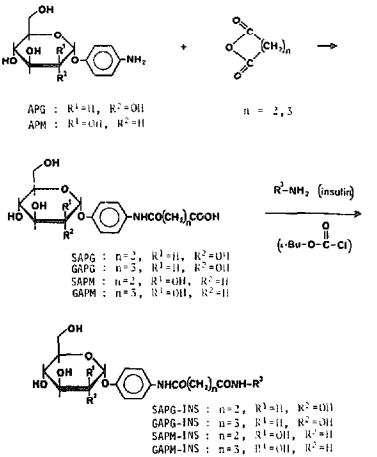
Spectroscopical and physical data are presented in Tables 1 and 2. The presence of the spacer groups in compounds NSGA and NGGA were confirmed by IR-absorption bands at 1550 cm^{-1} (amide II), 1650 cm^{-1} (amide I), and 1700 cm^{-1} (carboxyl group), the proton resonances in the NMR-spectrum



Scheme 3. Schematic route from *p*-aminophenyl-α-D-glucopyranoside to *p*-(α-D-glucopyranosyloxy)phenyl thiocarbamoyl insulin.

at δ = 2.63, 2.66 ppm (succinyl) or at δ = 1.86, 2.35, 2.39 ppm (glutaryl), and finally by the M–15 peak in the mass spectrum of the trimethylsilyl (TMS)-derivatized compounds. The structures of compounds SAPG, GAPG, SAPM and GAPM were confirmed in an analogous way.

methods. Maltose was directly coupled to insulin by reduction of the imine group obtained, using sodium cyanoborohydride as described by Brownlee and Cerami [5]. The maltose-insulin conjugate was obtained in 28% yield after purification by gel permeation chromatography and affinity chromatography using a Con A-Sepharose 4B column. The degree of substitution was found to be 1.0 as determined by phenol—sulfuric acid method.



Scheme 4. Schematic route from *p*-aminophenyl-α-D-glucopyranoside or *p*-aminophenyl-α-D-mannopyranoside to *p*-(succinylamido/glutarylamido)-phenyl-α-D-glucopyranosyl insulin or *p*-(succinylamido/glutarylamido)phenyl-α-D-mannopyranosyl insulin.

p-(α-D-Glucopyranosyloxy)phenyl thiocarbamoyl insulin (GPT—INS) was obtained by reacting ITPG with insulin in a pyridine—water solution (Scheme 3). After purification as mentioned before, GPT—INS was obtained in 49% yield. An affinity chromatogram of a mixture of insulin and glycosylated insulin is presented in Fig. 1. After the elution with a buffered α-methyl-D-mannopyranoside, GPT—INS (component 2) was obtained. Component 1 contains some glycosylated insulin, as indicated by a positive phenol—sulfuric acid test [6]. The average degree of substitution was calculated from UV-spectroscopy data. Values of 1.4 saccharide units per insulin molecule were found based on data reported for the phenylisothiocyanate derivative of insulin

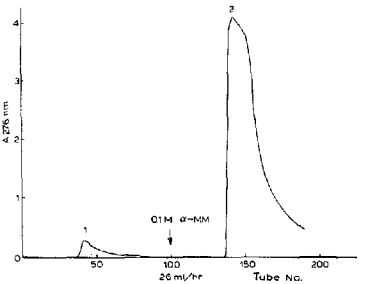


Fig. 1. The chromatographic separation of the reaction product (1 g) of insulin, treated with a two-fold molar amount of ITPG at pH 8.0, on a column of Con A-Sepharose 4B (2.5 × 60 cm). Initial eluent was buffer A (1 m*M* $MnCl_2$, 1 m*M* $CaCl_2$, 0.5 *M* NaCl and 0.02 *M* Tris, pH 7.4 at 4°C). At the point indicated by the arrow, the eluent was changed to buffer B (buffer A + 0.1 *M* α-methyl-D-mannopyranoside). Fractions (5 ml) were collected at a flow rate of 26 ml/h at 4°C. Component 1: unreacted insulin + GPT—INS; component 2: GPT—INS.

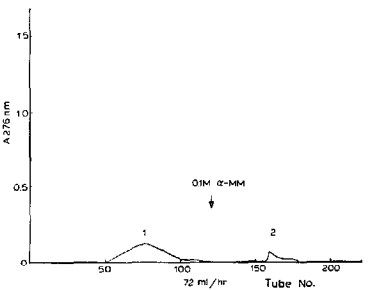


Fig. 2. The chromatographic separation of the lyophilized reaction product (100 mg) of insulin, treated with a nine-fold molar amount of NGGA at pH 9.5, on a column of Con A-Sepharose 4B (2.5 × 60 cm). Initial buffer was buffer A and at the point indicated by the arrow this eluent was changed to buffer B. Fractions (7 ml) were collected at a flow rate of 72 ml/h at 4°C. Component 1: unreacted insulin + NGGA—INS; component 2: NGGA—INS.

(ϵ = 15,000 l/mol cm at λ_{max} = 260 nm [16]).

Compounds of NSGA, NGGA, SAPG, GAPG, SAPM and GAPM containing spacer groups (succinyl or glutaryl) were coupled to insulin using a mixed anhydride method. The affinity chromatogram of the product obtained after coupling of NGGA to insulin is shown in Fig. 2. Although after elution with Tris buffer unreacted insulin was obtained in component 1, this fraction also contained glycosylated insulin (NGGA—INS), as shown by a positive phenol—sulfuric acid test. The occurrence of NGGA—INS together with unreacted insulin in component 1 can be explained by the low intrinsic binding affinity of the glucosamine moiety to Con A [17]. The degree of substitution of component 2, isolated by eluting with a solution of α-methyl-D-mannopyranoside, could not be determined due to the low absorptivity of NGGA in the phenol—sulfuric acid test.

After coupling of SAPG to insulin, the reaction products were again subjected to affinity chromatography using a Con A-Sepharose 4B column. In this case also

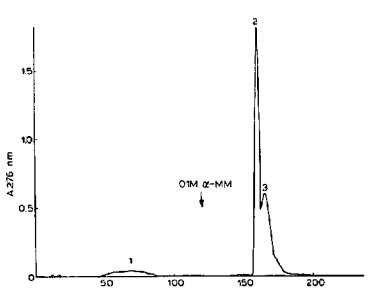


Fig. 3. The chromatographic separation of the lyophilized reaction product (100 mg) of insulin, treated with a nine-fold molar amount of SAPG at pH 9.5, on a column of Con A-Sepharose 4B (2.5 × 60 cm). Elution procedures were the same as described in Fig. 2. Component 1: unreacted insulin + SAPG—INS; component 2: disubstituted SAPG—INS; component 3: trisubstituted SAPG—INS.

component 1 contains insulin as well as SAPG—INS (Fig. 3). The presence of SAPG—INS was verified with the phenol—sulfuric acid method. Components 2 and 3 were combined and the mixture was dialyzed to remove α-methyl-D-mannopyranoside. Considering the larger amount of eluent required to elute component 3, it is expected that the degree of substitution of this compound is higher as compared to component 2. The average degree of substitution of the combined components 2 and 3 was 2.3 according to the phenol—sulfuric acid test. This number is in accordance with the assumption that component 2 is mainly disubstituted whereas component 3 is trisubstituted. In principle, three primary amino groups in insulin are available for substitution [18]. These groups are located in glycine (Gly A-1, pK_{app} 8.0), phenylalanine (Phe B-1, pK_{app} 6.7) and lysine (Lys B-29, pK_{app} 11.2). The coupling reactions were usually performed at a pH of 9.5 in order to prevent the denaturation of insulin and to maintain the ϵ-amino group (B-29) in the less reactive protonated state. However, trisubstituted glycosylated insulin probably still appeared in component 3. This might be explained by the fact that still 2% of the B-29 ϵ-amino groups are not protonated at pH 9.5 (based on the Henderson—Hasselbalch equation) and that the reaction was carried out with an excess of SAPG. It is expected that component 2 mainly consists of disubstituted glycosylated insulin.

Figure 4 shows the elution profile for the product formed after reacting insulin with SAPM. This chromatogram is similar as compared to that of SAPG—INS (Fig. 3). However, in this case component 1 proved to be unreacted free insulin as shown by the negative result with the phenol—sulfuric acid test. This indicates that SAPM—INS has a high affinity to Con A, which makes SAPM—INS a suitable candidate for the design of insulin delivery systems. After dialysis and lyophilization of the combined components 2 and 3, the average degree of substitution turned out

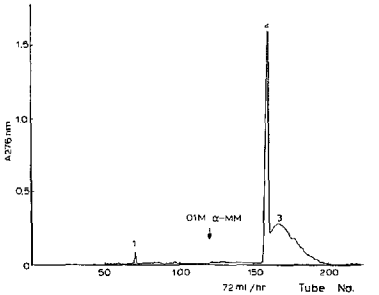


Fig. 4. The chromatographic separation of the lyophilized reaction product (100 mg) of insulin, treated with a nine-fold molar amount of SAPM at pH 9.5, on a column of Con A-Sepharose 4B (2.5 × 60 cm). Elution procedures were the same as those described in Fig. 2. Component 1: unreacted insulin; component 2: disubstituted SAPM—INS; component 3: trisubstituted SAPM—INS.

to be 2.5, as determined by the phenol—sulfuric acid test.

The results of the affinity chromatography of the glycosylated insulins are in accordance with expectations based on the binding of different carbohydrates with Con A as described by Goldstein et al. [17,19].

The typical yields and average degrees of substitution of the glycosylated insulins are presented in Table 3. It is interesting to note that the aggregation of the synthesized glycosylated insulins appeared to be substantially reduced as compared to native insulin. This is caused by the substitution of the A-1, B-1 and B-29 amino groups present in insulin. Blood sugar depression tests in rats show that all glycosylated insulin derivatives are biologically active when compared with native insulin except maltose—insulin.

Brownlee and Cerami [5,20] demonstrated that the release of maltose—insulin conjugate bound to Con A depends on glucose concentration. However, the coupling method requires a long incubation time; therefore, stability of insulin for five days incubation at 37°C remains in question, although it showed a high biological activity of maltose—insulin conjugate. Furthermore, the reductive amination resulting in one intact α-D-glucopyranosyl ring structure of maltose (disaccharide) after coupling may lead the maltose—insulin to have the same or very close binding affinity to Con A compared to glucose. This indicates that maltose—insulin can be released at any glucose concentration (even a hypoglycemic condition). The synthesized *p*-aminophenyl-α-D-glucopyranoside (APG)— and *p*-aminophenyl-α-D-mannopyranoside (APM)—insulin conjugate with or without spacer will provide high specific binding affinity to Con A due to the presence of APG and APM in the conjugates.

p-Nitrophenyl-α-D-glucopyranoside (NPG) and *p*-nitrophenyl-α-D-mannopyranoside (NPM) were used as starting materials because of minimal cost. Catalytic hydrogenation was selected for the reduction of NPG and NPM since conversion to the amine was efficient and free from side reactions.

We utilized the mixed anhydride method in most procedures for glycosylation of primary amine groups of insulin due to high reaction rates at low temperatures and the high purity of products with good yield. The low yield of NSGA and NGGA—INS is

TABLE 3

Yields and average degrees of substitution of the glycosylated insulins

Insulin derivative	Yield (%)	Degree of substitution[a]
Maltose—insulin	28	1.0
NSGA—INS	negligible	—[b]
NGGA—INS	negligible	—[b]
GPT—INS	49	1.5
SAPG—INS	63	2.3
GAPG—INS	50	1.6
SAPM—INS	70	2.5
GAPM—INS	70	2.0

[a] Average number of saccharide units per insulin molecule.

[b] Could not be determined by the phenol—sulfuric acid method.

attributed to the low binding affinity of the glucosamine moiety, not to coupling procedures.

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