

Notes

[Chem. Pharm. Bull.]
[28(12):3670—3673(1980)]

SDS-polyacrylamide Gel Electrophoresis of the D-[5-³H]Glucose-binding Membranes in Rat Islets of Langerhans

KUNIO KOBAYASHI, KEIKO YOSHIDA, and AKIRA MATSUOKA

Department of Clinical Pathology and Clinical Laboratory, Hyogo College of Medicine¹⁾

(Received February 9, 1980)

The D-[5-³H]glucose-binding membrane proteins in rat islet homogenate and its F₂ fraction obtained by sucrose gradient ($d^{20}=1.00-1.18$) ultracentrifugation at 70000g for 2 hr were analyzed by SDS-polyacrylamide gel electrophoresis. Five components in the F₂ fraction, which contains membrane proteins related to glucose-induced insulin release, showed relative mobilities of around 0.75 on SDS gel stained with Coomassie brilliant blue (R-250), and had apparent molecular weights of 22000—30000. One component among them appeared to be a glycoprotein because it was sensitive to the periodic acid-Schiff reagent (PAS reagent).

Keywords—insulin release; B cell membrane; glucoreceptor; rat islet homogenate; D-[5-³H]glucose-binding membrane protein; SDS-polyacrylamide gel electrophoresis; glycoprotein

The primary role of the B cells of pancreatic islets is to secrete an appropriate amount of insulin in response to a particular blood glucose level. It has been speculated that the B cell membrane contain a glucose-recognition site (glucoreceptor) for glucose-induced insulin release based on many studies on insulin release using the anomers of D-glucose.²⁾ If the glucoreceptor is present on or in the B cell membrane, it may be possible to extract and characterize it. Recently, Price found that some hexoses which were stimulants for insulin release formed complexes with the solubilized cell membranes of dog pancreatic islets,³⁾ and Lernmark *et al.* tried to purify and characterize plasma membrane-rich fractions from rat pancreatic islets.⁴⁾ However, no distinction between the glucose-recognition site for insulin release (glucoreceptor) and that for glucose transport has been found so far.

In a preliminary study, we separated three kinds of plasma membrane fractions which could specifically bind D-glucose molecules from rat islet homogenate by ultracentrifugation on a sucrose gradient, and demonstrated that the membrane in the F₂ fraction ($d^{20}=1.06-1.12$) was closely related to insulin release.⁵⁾

In the present work, we further characterized the F₂ fraction by analysis based on SDS-polyacrylamide gel electrophoresis.

Materials and Methods

Reagents—All reagents used were of special grade. Acrylamide (for electrophoresis), N,N,N',N'-tetramethylethylenediamine, sodium dodecyl sulfate (SDS), N,N-methylene-bis-acrylamide, ammonium persulfate, β -mercaptoethanol, sucrose, periodic acid and fuchsin were products of Wako Pure Chemical

1) Location: 1-1, Mukogawa-cho Nishinomiya 633, Japan.

2) A. Niki, H. Niki, I. Miwa, and J. Okuda, *Science*, **186**, 150 (1974); G.M. Grodsky, R. Fanska, L. West, and M. Manning, *Science*, **186**, 536 (1974); K. Kobayashi, Y. Tsumura, H. Ise, S. Kagawa, Y. Shinomiya, M. Hata, and A. Matsuoka, *J. Japan Diab. Soc.*, **19**, 839 (1976).

3) S. Price, *Biochim. Biophys. Acta*, **318**, 459 (1973).

4) A. Lernmark, A. Nathaus, and D. Steiner, *J. Cell. Biol.*, **71**, 606 (1976).

5) K. Kobayashi, Y. Yoshida, Y. Tsumura, S. Kagawa, and A. Matsuoka, *J. Pharm. Dyn.*, **3**, 33 (1980).

Industries Ltd., Japan. Collagenase was purchased from Sigma Chemical Co., U.S.A.. Bovine serum albumin (fraction V) was obtained from Armour Laboratories, U.S.A.. Coomassie brilliant blue (R-250) was purchased from Nakarai Chemical Ltd., Japan. D-[5-³H]Glucose (12 Ci/mmol) and NCS® solubilizer were obtained from The Radiochemical Centre, Amersham, England. Reference proteins for SDS-gel calibration of molecular weight [trypsin inhibitor (MW: 21500), bovine serum albumin (MW: 68000) and (RNA) polymerase (α , MW: 39000, β , MW: 155000, β' , MW: 165000)] were obtained from Boehringer Mannheim, GmbH, Federal Republic of Germany. All other chemicals used for experiments were products of Wako Pure Chemical Industries Ltd., Japan.

Isolation of Rat Islets of Langerhans—Pancreatic islets of Langerhans were isolated from overnight-fasted Sprague-Dawley rats weighing 180–250 g by the method of Lacy and Kostianovsky.⁶⁾

Preparations of Islet Homogenate and Plasma Membranes—The preparations of islet homogenate and plasma membranes were carried out by the procedures described in our previous report.⁵⁾ In brief, 700–1000 fresh islets were homogenized in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.2), and then three kinds of plasma membrane fractions (F_1 : $d^{20}=1.14$ – 1.18 , F_2 : $d^{20}=1.06$ – 1.12 , F_3 : $d^{20}=1.02$ – 1.05) were obtained from the islet homogenate by continuous sucrose gradient ($d^{20}=1.00$ – 1.18) centrifugation at 70000g for 2 hr.

Preparation of D-[5-³H]Glucose-binding Membranes Solubilized with SDS⁵⁾—The islet homogenate was ultracentrifuged at 70000g for 2 hr. Each fraction was diluted with 0.5 ml of 50 mM Tris-HCl buffer (pH 7.2), then centrifuged at 10000g for 30 min. Each pellet was suspended in 0.6 ml of 0.1 M Tris-HCl buffer (pH 7.2) containing 10 mM CaCl₂, 10 mM MgCl₂ and D-[5-³H]glucose (1000 cpm). The suspension was kept at room temperature for 1 hr, then centrifuged at 4° for 30 min at 10000g. The pellet was solubilized by placing it in 30 μ l of 20 mM Tris-HCl buffer (pH 8.0) containing 1% SDS, 20% sucrose and 2% mercaptoethanol at room temperature for 15 min. The solution (20 μ l) containing membrane proteins solubilized with SDS was subjected to SDS-polyacrylamide gel electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis—SDS-Polyacrylamide gel electrophoresis was carried out by the method of Fairbanks *et al.* with several modifications.⁷⁾ A gel column (0.8 \times 7 cm) containing 7.5% (w/v) acrylamide was used for the electrophoresis. After the application of 20 μ l of 0.04 M Tris-HCl buffer (pH 7.2) containing 5% sucrose and 0.001% Coomassie brilliant blue (R-250) (tracking dye), 20 μ l of sample solution containing solubilized-islet homogenate or fractionated membrane proteins was applied on top of the gel column and overlaid with an electrophoresis buffer (0.1 M phosphate buffer, pH 7.2) up to the rim of the tube. The electrophoresis was run at 6 mm per column until the tracking dye had migrated to the end of the gel column (about 2 hr) at 4°. The gel was stained with 0.025% Coomassie brilliant blue (R-250) for protein⁷⁾ and with periodic acid-Schiff reagent (PAS reagent) for glycoprotein.⁸⁾ In another experiment each gel column was cut into 2.5 mm slice. Each slice was kept overnight at room temperature in 10 ml of scintillation fluid (4 g of DPO, 0.1 g of POPOP and 100 ml of NCS® solubilizer per liter of toluene) in a scintillation vial, and then the radioactivity was counted in a scintillation counter (Tri-carb 2450, Packard Instruments, U.S.A.).

Results and Discussion

Fig. 1 shows the distribution of D-[5-³H]glucose-binding membrane proteins of rat islet homogenate on SDS-polyacrylamide gel electrophoresis. Four main peaks (I–IV) were observed (relative mobilities: I, <0.10; II, 0.15–0.25; III, 0.45–0.55; IV, 0.65–0.90). Peak I was determined to be unbound D-[5-³H]glucose from the electrophoretic mobility of D-[5-³H]-glucose alone. Peak IV was judged to be D-[5-³H]-glucose bound with the membrane proteins in F_2 fraction from the electrophoretic pattern, as shown in Fig. 2.

Typical electrophoretic banding patterns on SDS gels stained with Coomassie brilliant blue(R-250) and PAS reagent of the membrane proteins from the islet homogenate are shown in Fig. 1. The schematic banding patterns were prepared by direct visual inspection of stained gels. Thirteen prominent bands were observed on the gel column. If faintly stained bands are added, even more can be counted. The banding pattern on the gel column stained with PAS reagent had five pink bands (relative mobilities: 0.30, 0.45, 0.60, 0.73 and 1.00). Since the electrophoretic mobility and PAS-sensitivity of the broad band at the gel front were similar to those of the glycolipid fraction from human erythrocyte membranes,⁹⁾ it was con-

6) P.E. Lacy and M. Kostianovsky, *Diabetes*, **16**, 35 (1967).

7) G. Fairbanks, T.L. Steck, and D.F.H. Wallach, *Biochemistry*, **10**, 2606 (1971).

8) R.H. Zacharius, T.E. Zell, J.H. Morrison, and J.J. Wodlock, *Anal. Biochem.*, **30**, 148 (1969).

9) K.L. Carraway, A. Lam, D. Kobylka, and J. Huggins, *Anal. Biochem.*, **45**, 325 (1972); M. Kasahara and P.C. Hinkle, *J. Biol. Chem.*, **252**, 7384 (1977).

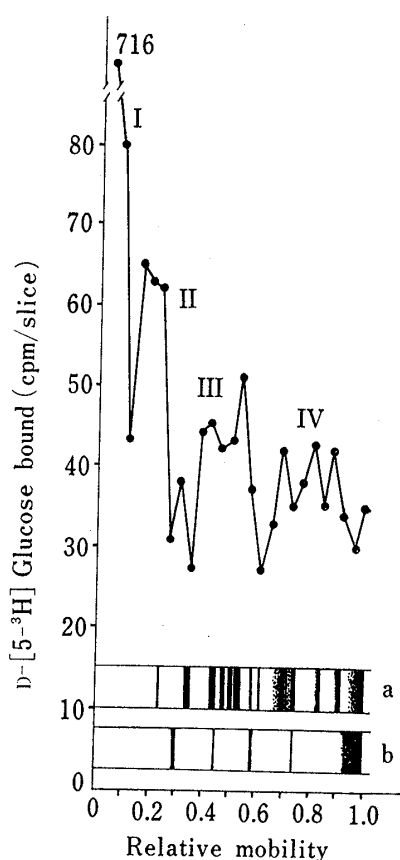


Fig. 1. Electrophoretic Pattern of Islet Homogenate on SDS Gel

Each experiment was performed with about 700 islets. The schematic banding patterns are based on SDS gels stained with Coomassie brilliant blue (R-250) (a) and PAS reagent (b).

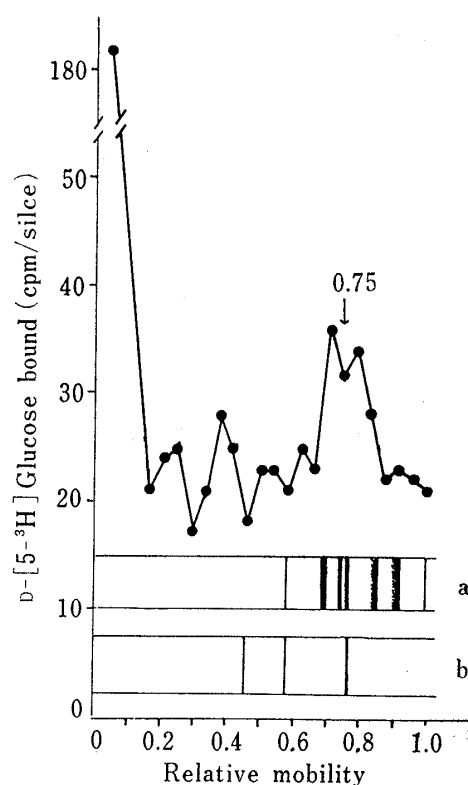


Fig. 2. Electrophoretic Pattern of the F_2 Fraction on SDS Gel

The graph represents a typical one of three experiments. Each experiment was performed by using about 1000 islets. The schematic banding patterns were derived from SDS gels stained with Coomassie brilliant blue (R-250) (a) and PAS reagent (b).

sidered to be a component containing glycolipid.

An electrophoretic banding pattern of the plasma membrane-containing fraction of the rat islet homogenate on SDS gel stained with Coomassie brilliant blue (R-250) was reported by Lernmark *et al.*⁴⁾ Although thirteen bands were detected on the stained gel column, no further characterization or discussion was carried out.

We previously reported that the membrane proteins in F_2 fraction⁵⁾ had the following properties; 1) specific binding activity for D -glucose or several other sugars which could stimulate insulin release. 2) activity to release from B granules by interaction with B granules. 3) moderate inhibitory effects of alloxan and phlorizin on D -glucose-binding. In the present work, D -[5 - 3 H]glucose-binding membrane proteins in F_2 fraction were analyzed by SDS-polyacrylamide gel electrophoresis. Fig. 2 shows the distribution of D -[5 - 3 H]glucose-binding membrane proteins of the F_2 fraction. One broad peak other than the peak of unbound D -[5 - 3 H]glucose was observed on SDS gel at a relative mobility of around 0.75 (0.70–0.90). The banding pattern of the membrane proteins of F_2 fraction on SDS gel stained with Coomassie brilliant blue (R-250) consisted of seven bands. There were five bands corresponding to the peak IV of D -[5 - 3 H]glucose-binding protein at a relative mobility of around 0.75. Therefore, peak IV included at least five kinds of membrane proteins which were different from each other in molecular weight. Moreover, the banding pattern on SDS gel stained with PAS reagent consisted of three bands at the relative mobilities of 0.45, 0.58 and 0.76. This result suggests that there are three kinds of membrane proteins which are probably glycoproteins in the F_2 fraction. One component among them had a relative mobility of 0.76 on SDS gel.

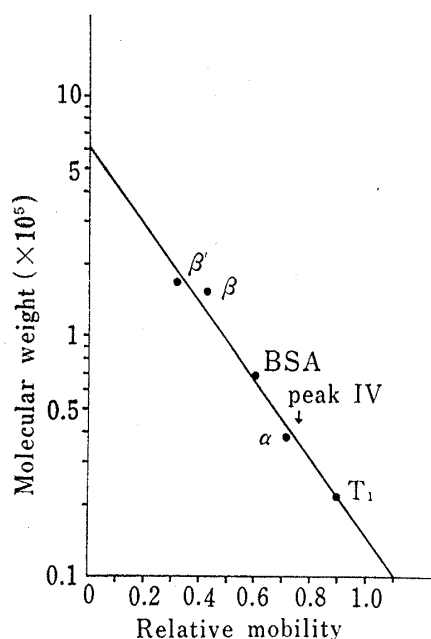


Fig. 3. Calibration of Molecular Weight

SDS-polyacrylamide gel electrophoresis of a mixture containing five kinds of reference proteins was carried out under the same conditions as before, and the SDS gel was stained with Coomassie brilliant blue(R-250). Each point is the mean of three experiments.

Consequently, it was found that the main D-[5-³H]glucose-binding components in the F₂ fraction consisted of five membrane proteins, including glycoprotein, with a molecular weight range of 22000—30000. However, further purification of the F₂ fraction will be necessary to characterize and confirm the existence of glucoreceptors on or in the B cell membrane in rat islets of Langerhans.

The calibration of molecular weight was performed by SDS-polyacrylamide gel electrophoresis with a mixture containing five reference proteins; trypsin inhibitor (T₁), bovine serum albumin(BSA) and RNA-polymerase(α , β and β') (Fig. 3). The molecular weights of the main components in F₂ fraction were estimated to be in the range of 22000—30000.

We could observe many bands on SDS gels stained with Coomassie brilliant blue (R-250) in electrophoresis of both F₁ and F₃ fractions, but we failed to distinguish clearly a peak of D-[5-³H]-glucose-binding membrane proteins on each electrophoretic pattern, and peaks of II or III could not be matched to those of F₁ and F₃ fractions on SDS gel. The radioactivity in each gel slice was very low in the present experiments, leading to relatively high errors and poor reproducibility of results, so labelled glucose which has a higher specific activity is required. Moreover, the conditions of solubilization of membrane proteins and polyacrylamide gel electrophoresis require improvement in order to avoid a decrease in D-[5-³H]glucose-binding with membrane protein.

[Chem. Pharm. Bull.]
28(12)3673—3678(1980)

Simultaneous Determination of Acid Dissociation Constants and True Partition Coefficients by Analysis of the Apparent Partition Coefficients. II. Dibasic Acid and Diacidic Base

TANEKAZU KUBOTA^{1a)} and KIYOSHI EZUMI¹⁾

Shionogi Research Laboratories, Shionogi and Co., Ltd.¹⁾

(Received May 10, 1980)

The simultaneous determination of acid dissociation constants (pK_a) and true partition coefficients (P) of a dibasic acid and a diacidic base was achieved by analyzing the pH dependence of the apparent partition coefficient (P_a). Equations derived theoretically show a parabolic relation of P_a to $(H^+)^{-1}$ and (H^+) for a dibasic acid and a diacidic base, respectively, (H^+) being the proton concentration. Theoretical considerations indicate

1) Location: Fukushima-ku, Osaka, 553, Japan; a) Present address: Gifu College of Pharmacy, 6-1, Mitahora-higashi 5 chome, Gifu, 502, Japan. Inquiries should be addressed to T.K.