ISOLATION OF MAJOR CELL-SURFACE GLYCOPROTEINS FROM NORMAL AND DIABETIC RAT-LIVER MEMBRANES

V. CHANDRAMOULI, Barbara LUNDT and James R. CARTER, jr
Department of Medicine, Case Western Reserve University School of Medicine and University Hospitals, Cleveland, OH, USA

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1. Introduction

The study of cell-surface glycoproteins has been facilitated by the use of lectins which bind saccharides in a highly specific manner [1]. In particular, lectin affinity chromatography has been used in the isolation of hormone and virus receptors and other membrane glycoproteins.

There is evidence to suggest that diabetes is associated with generalized changes in cell-surface glycoproteins. Changes in lectin-binding characteristics of liver-plasma membranes have been described in ob/ob and db/db mice [2] and by our laboratory in streptozotocin diabetic rats [3]. These changes could have important implications for a number of cellular functions.

To study these cell-surface changes in more detail, we have devised a method for the isolation of cell-surface glycoproteins by lectin affinity chromatography. The method involves initial desialation of the intact membranes, extraction of the glycoproteins with detergent-containing solutions, then passage over an affinity column containing *Ricinus communis* lectin. The glycoproteins can be recovered in good yield essentially free of glycolipid and non-carbohydrate containing proteins. Differences between glycoproteins from diabetic and control livers are described.

2. Materials and methods

Materials were obtained from the following sources: Sepharose 4B from Pharmacia Fine Chemicals; agarose polyacrylic hydrazide from Miles Labs; glutaraldehyde from Eastman Kodak; vibriocholera neuraminidase (500 units/ml) from Calbiochem-Behring Corp; galactose oxidase from AB Kabi (Sweden); tritiated sodium borohydride (8 Ci/mmol) and ACS (liquid scintillation cocktail) from Amersham Searle. Molecular weight standards and high purity chemicals for polyacrylamide gel electrophoresis were obtained from BioRad Labs. Streptozotocin was purchased from Sigma Chemical Co.

Male Sprague Dawley rats were made diabetic by the intravenous injection of streptozotocin (65 mg/kg) and kept 3–4 weeks before use. Liver-plasma membranes were prepared by the method in [4] and kept frozen at −80°C. Protein was estimated by the Lowry method [5] using crystalline bovine serum albumin as standard. Membrane samples were heated in 1 N NaOH for 30 min in a boiling water bath prior to protein determination.

Liver-plasma membranes were labeled by the method in [6]. Membrane suspensions (5–7 mg protein/ml) were incubated for 120 min at 37°C with neuraminidase, washed and resuspended. The suspended membranes were then either extracted directly or labeled prior to extraction as follows: the desialated membranes were incubated for 60 min at 37°C with galactose oxidase, washed and resuspended. The oxidized galactose residues were labeled by adding 500 μCi of NaB^3H_4, incubating for 30 min, then washing the membranes until no further radioactivity was eluted. Control samples, to correct for background radioactivity, were similarly handled except for omission of the galactose oxidase step.

Desialated liver-plasma membranes (labeled or unlabeled) were extracted twice with 1% Triton

Abbreviations: PAS, periodic acid-Schiff's reagent; SDS, sodium dodecyl sulfate

X-100 in Na-borate buffer (pH 8.0, 28 mM). The extracts were pooled, made 0.15 M with NaCl, and passed over the lectin affinity column (0.9 × 7.0 cm). The column was washed exhaustively with buffer containing 0.1% Triton X-100, then the glycoproteins eluted with 15 ml of the same buffer containing 5% lactose. The lactose eluates were dialyzed extensively and concentrated by vacuum dialysis.

Ricinus communis agglutinin (120 000 mol. wt) was isolated and purified by the method in [7]. The lectin was coupled either to CNBr-activated Sepharose 4B [7] or to glutaraldehyde-activated polyacrylic hydrazide Sepharose by the method in [8].

Membrane and solubilized proteins were analyzed by polyacrylamide gel electrophoresis in SDS–dithiothreitol by the method in [9] using a stacker gel [10]. Gels were stained for protein with Coomassie blue and for carbohydrate with PAS.

3. Results and discussion

The present method for affinity chromatography relies upon removal of terminal sialyl residues with neuraminidase, exposing penultimate galactosyl residues, and then binding these to the *Ricinus communis* lectin which is specific for galactose. The glycoproteins can easily be labeled to high specific activity by introduction of a galactose oxidase-tritiated borohydride step. The glycoproteins are then eluted from the lectin column with lactose. Preliminary studies showed that ~80% of desialated fetuin could be recovered by this means; albumin was recovered essentially quantitatively in the initial washes, with < 1% appearing in the lactose eluates. Based upon experiments in which membrane glycoproteins were labeled with [^{3}H]borohydride after galactose oxidation, we estimate that 55–70% of the total membrane glycoprotein can be recovered in the lactose eluate from the affinity column. If the neuraminidase step is omitted, much less glycoprotein is recovered in the lactose eluate.

As shown in fig.1, the glycoproteins concentrated by this means represent a minor fraction (3.5–5.0%) of the total starting membrane protein. In addition, glycolipids present in the Triton X-100 extract pass through with the column washes. No significant PAS-staining material remained in the membrane residue after Triton X-100 extraction, and the only PAS-staining material in the column washes was the dense glycolipid band. One can thus achieve a selective concentration of the membrane glycoproteins freed of non-carbohydrate containing proteins and glycolipids.

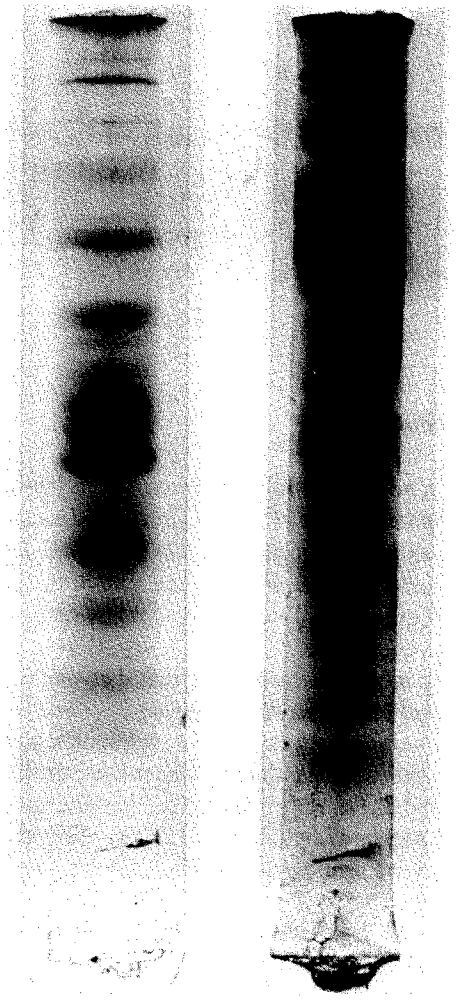

Fig.1. Polyacrylamide gel profiles of membrane proteins and purified glycoproteins. Rat-liver membranes were desialated, then analyzed directly by polyacrylamide gel electrophoresis as in section 2 (left). Membrane glycoproteins were extracted and purified by *Ricinus communis* lectin affinity columns as described, then analyzed similarly (right). Both gels are stained with Coomassie blue.

As compared to the majority of membrane proteins, the liver-cell surface glycoproteins are predominantly high molecular weight (fig.1), with the

major band having an est. mol. wt 94 000 and with a secondary band at 130 000. The lower molecular weight glycopeptides seen with Coomassie blue staining (fig.1) do not stain readily with PAS but can be detected by the more sensitive galactose oxidase labeling method.

We have previously shown changes in chemical composition [11] and receptor properties [3] of liver-plasma membranes from streptozotocin-diabetic rats. To extend these observations, we used *Ricinus communis* lectin affinity columns to isolate glycoproteins from liver-plasma membranes of control and diabetic rats, then analyzed the glycoproteins by polyacrylamide gel electrophoresis. In the diabetic membranes, there was a striking reduction of the predominant PAS-staining band (fig.2), and this band appeared more diffuse than that observed in control preparations. Since equal amounts of protein were applied to these gels, this appears to reflect primarily a reduction in carbohydrate content and possibly more heterogeneity in the sugar side chains. Similar results were obtained when the desialated glycoproteins were initially labeled with galactose oxidase and [^{3}H]borohydride before extraction from the membranes and isolation (fig.3). There is a marked reduction in incorporation of the label into the predominant glycoprotein from the diabetic membranes as compared to controls.

Since insulin binding to intact cells is not affected by removal of sialic acid [12], we looked for evidence of insulin 'receptors' in membrane extracts and

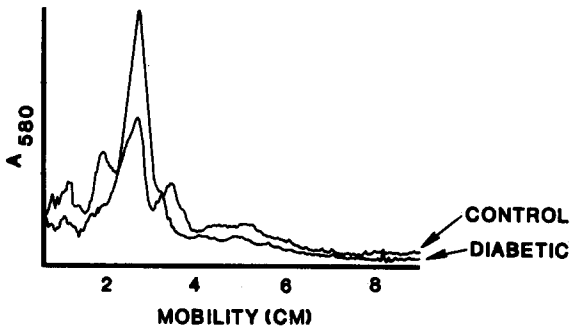

Fig.2. Glycoproteins from control and diabetic rat-liver membranes. Glycoproteins were purified and concentrated as described before polyacrylamide gel electrophoresis. The gels were stained with PAS and scanned at 580 nm. Similar amounts of protein were applied to each gel.

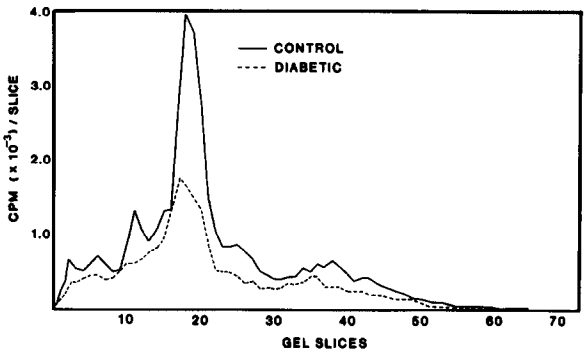

Fig.3. ^{3}H-Labeled glycoproteins from control and diabetic liver membranes. Glycoproteins were labeled, isolated, and subjected to polyacrylamide gel electrophoresis as described. The gels were sliced into 1 mm sections with a gel slicer, the slices dissolved and radioactivity determined. Similar amounts of protein were added to each gel.

lactose eluates using [^{125}I]insulin and polyethylene glycol precipitation [13]. As expected, significant amounts of 'receptor' were found in the initial Triton X-100 extract; however, only ~5–10% of this activity was recovered in the lactose eluates from the affinity column (data not shown), indicating either irreversible binding of receptor to the column or extensive denaturation during the purification step.

Somewhat similar lectin columns have been used by others. *Ricinus communis* columns were used [14] to separate desialated from native fetuin. Concanavalin A columns were used [15] to isolate cell-surface glycoproteins from hepatoma cells, but recoveries of only 20–30% reported. The present method appears to give good recoveries of all of the major glycoproteins present in the cell-surface membrane.

The procedure described here may prove useful as an initial step in glycoprotein isolation when terminal sialic acid residues are not essential to the glycoprotein being investigated. With it, we have extended our previous observations on changes in cell-surface glycoproteins in diabetes and can now investigate the nature of these changes in greater detail.

Acknowledgement

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