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PRELIMINARY CHARACTERIZATION OF CELL SURFACE GLYCOPROTEINS ASSOCIATED WITH EPIDERMAL DIFFERENTIATION IN THE NEWBORN RAT

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The cell surface of the newborn rat epidermis exhibits a discrete change in lectin-binding specificity from Griffonia simplicifolia I-B_4 (GS I-B_4), specific for α-D-galactosyl residues, to Ulex europeus agglutinin I (UEA), specific for α-L-fucose, as the cell leaves the basal layer and differentiates. It has been postulated that this change is a result of the addition of α-L-fucose to non-reducing terminal α-D-galactosyl residues on GS I-B_4-binding cell surface glycoproteins. If this were the sole nature of the conversion, the physical properties of isolated GS I-B_4- and UEA-bound glycoproteins should not differ greatly. UEA and GS I-B_4-binding glycoproteins isolated from newborn rat epidermis have similar charge properties and are similar in the nature of their carbohydrate moieties, supporting the proposed hypothesis.

The surface of cells in the newborn rat epidermis exhibits a discrete change in lectin binding specificity as the cell leaves the basal layer and differentiates (1). The isolectin Griffonia simplicifolia I-B_4 (GS I-B_4), specific for terminal α-D-galactosyl residues, shows preferential binding to basal cell surfaces. Ulex europeus agglutinin I (UEA), specific for α-L-fucose, preferentially binds to the surfaces of spinous and lower granular cells. A second lectin from Griffonia simplicifolia, GS II, specific for terminal N-acetyl-D-glucosamine, labels cornified cells. Recent evidence indicates that rat keratinocytes in culture display similar lectin-binding specificities (2).

Studies by Zieske and Bernstein (3) showed that when tissue sections were treated with α-L-fucosidase, UEA binding was eliminated and GS I-B_4 binding was present throughout the spinous layer. Treatment of tissue with α-D-galactosidase eliminated GS I-B_4 binding but did not result in the appearance of UEA binding. More recent data (4) indicated that some of the cell surface glycoproteins which have UEA-binding specificity can be converted to GS I-B_4-binding

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forms by treatment with α-L-fucosidase. The data further suggested that the epidermis of the newborn rat possesses the necessary transferase to convert GS I-B_4-binding glycoproteins to UEA-binding specificity.

It has been proposed (3) on the basis of the above evidence that the change from GS I-B_4 to UEA-binding specificity is a result of the addition of α-L-fucose so as to block non-reducing terminal α-D-galactosyl residues on GS I-B_4-binding cell surface glycoproteins. If this is indeed the nature of the conversion, one would expect that, with the exception of added fucose, the molecular properties of both protein and carbohydrate portions of GS I-B_4 and some UEA-bound glycoprotein should not differ greatly. Data from the preliminary characterization and comparison of UEA and GS I-B_4 binding glycoproteins isolated from newborn rat epidermis support this hypothesis on the basis of similar charge properties and similar nature of carbohydrate moieties.

METHODS

Isolation of Cell Surface Glycoproteins. Lectin-specific cell surface glycoproteins from 450 newborn rat skins were prepared and isolated as previously described (3). Cell surface radiolabeling was achieved by the galactose oxidase/NaB^3H_4 method of Gahmberg and Hakomori (5). From previous studies (4), an initial pass through a Concanavalin A (Con A) affinity column (Pharmacia, Piscataway, NJ) proved to be useful as a result of its ability to selectively bind a major group of glycoproteins having the apparent molecular weight of 96,000 daltons that contains specific glycoproteins also bound by GS I-B_4 or UEA affinity columns. This Con A - bound material was sequentially passed through UEA and GS I-B_4 columns attached in series, UEA first, then detached and eluted individually. The final yields of material isolated from the UEA and GS I-B_4 affinity columns were 560 ug and 270 ug protein respectively as determined by the method of Lowry (6). The glycoprotein fractions isolated from the UEA and GS I-B_4 affinity columns were subjected to analysis as described below.

Non-equilibrium pH Gradient Electrophoresis. Non-equilibrium pH gradient electrophoresis (NEpHGE) was carried out according to the procedure for the first dimension by O'Farrell et al (7). Electrophoresis was performed on a 4% polyacrylamide NEpHGE tube gel (13 cm) with 2% (v/v) pH 3.5-10 ampholines in a tube gel electrophoresis cell (Bio Rad). Radioactivity was analyzed by incubating tube gel slices (2 mm) in NCS tissue solubilizer (Amersham) overnight and counting in a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). Electrophoresis was for 4 h at 500 V for a total of 2000 Vh. ^{14}C-lactoglobulin A (pI 5.20) (NEN, Boston, MA) was included in each sample as an internal standard to align tube gels during slicing.

Alternatively, radiolabeled UEA and GS I-B_4-bound glycoproteins were treated with 5 U neuraminidase from Clostridium perfringens (Sigma) in 0.1 M sodium acetate buffer, pH 5.6, with 1 mM $CaCl_2$ and 1 mM phenylmethane sulfonyl fluoride (PMSF) for 6 h at 37 °C. PMSF was included throughout the incubation and analysis to minimize effects of protease contamination in the neuraminidase preparation. Samples were dialyzed and subject to NEpHGE as described above.

Alkaline Borohydride Treatment. Radiolabeled UEA and GS I-B_4-bound glycoproteins were treated with 0.05 M NaOH-1 M $NaBH_4$ at 50 °C for 16 h (8).

Gel filtration of alkaline borohydride treated material was performed on Sephadex G-50 (1.5 x 120 cm) and G-25 (1.0 x 40 cm) (Pharmacia) columns in 0.1 M sodium acetate buffer, pH 5.6, with 0.02% sodium azide (9). Fractions were collected (1.0-2.0 ml) and aliquots were counted in a liquid scintillation spectrometer.

To assess lectin binding characteristics of carbohydrate chains liberated from radiolabeled UEA and GS I-B_4-bound glycoproteins, "peak" fractions were isolated and exchanged with phosphate-buffered saline, pH 7.2, by gel filtration on Bio Gel P-10 (1.5 x 15 cm) (Bio Rad) after alkaline borohydride treatment. The pH of samples was adjusted to pH 7.2 and assayed on UEA/GS I-B_4 affinity columns (3).

RESULTS AND DISCUSSION

Isolation of Cell Surface Glycoproteins

The composition of epidermal cell populations subjected to surface radiolabeling (5) was approximately 55% basal cells and 45% differentiated cells based on morphologic criteria. The radiolabeled cell suspensions were solubilized with 0.5% NP-40 and glycoprotein fractions were isolated by sequential lectin affinity chromatography on Con A-Sepharose, UEA-Sepharose and GS I-B_4-Sepharose in that order.

It is presumed from previous data (10,11) that in the initial pass through a Con-A column, any tritium-labeled glycoproteins containing hybrid-type or complex-type sugar chains would be adsorbed (24% of originally labeled NP-40 solubilized material). Material isolated by UEA and GS I-B_4 affinity columns was 13.3% and 2.3% of the originally labeled NP-40 solubilized material, respectively.

Electrophoretic Analysis

Analysis of radiolabeled UEA and GS I-B_4-bound material by SDS-polyacrylamide slab gel electrophoresis revealed a common major fraction of cell surface glycoproteins of an apparent molecular weight of 96,000 daltons (4) (data not shown). A number of minor species of lower molecular weight were also identified in the UEA-bound fraction, suggesting the heterogeneous size of UEA-bound glycoproteins.

Charge properties were assessed by non-equilibrium pH gradient electrophoresis (NEpHGE) with pH 3.5-10 ampholines. It was reasoned that a difference in charge properties between UEA and GS I-B_4-bound glycoproteins would reflect changes other than the simple addition of α-L-fucose, including differences in

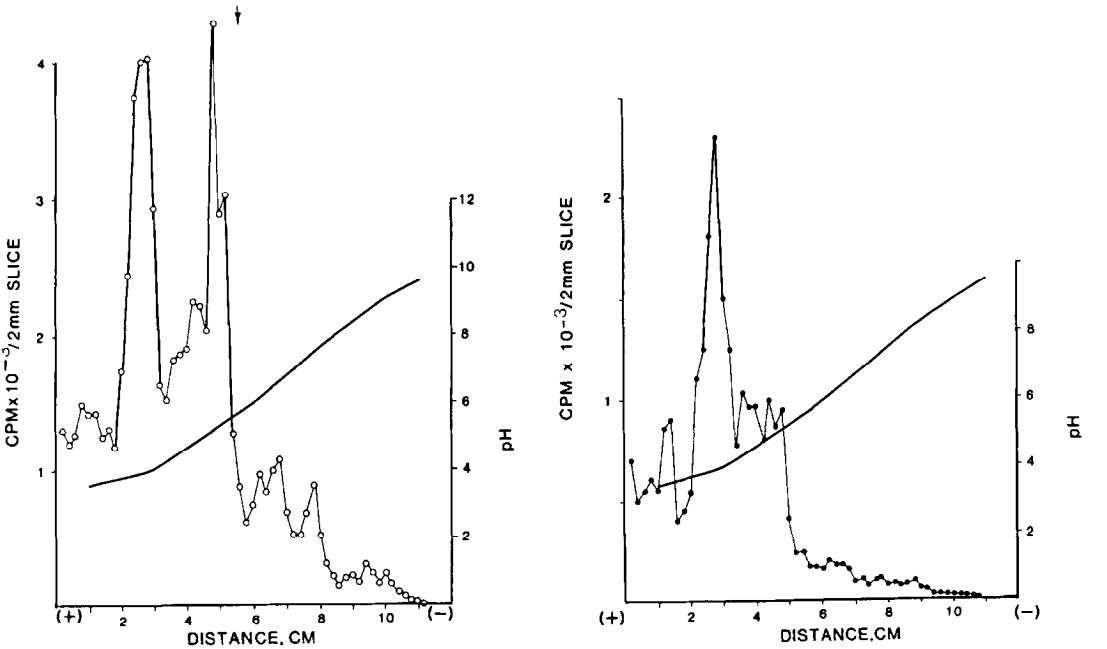

Figure 1. Non-equilibrium pH gradient electrophoresis (NEpHGE) of UEA and GS I-B_4-bound glycoproteins. 100,000 CPM of tritiated UEA (O-O-O) and GS I-B_4 (●-●-●)-bound glycoproteins were run on a 4% polyacrylamide NEpHGE gel (13 cm) with 2% (v/v) pH 3.5-10 ampholines. Radioactivity was determined. ^{14}C-Lactoglobulin A (arrow) is included as internal standard. Running conditions: 2000 Vh.

sialylation and/or de novo glycoprotein synthesis. Results on NEpHGE gels showed that UEA-bound material had two major peaks of radioactivity while GS I-B_4-bound material displayed only one major peak in the pH range 4-7 (Fig. 1). Comparison of NEpHGE gel profiles for UEA and GS I-B_4-bound material revealed a common major peak at pH 3.90. The common major peak represents 33% and 44% of total ^{3}H applied to the gel for UEA and GS-I-B_4-bound material respectively. Analysis on isoelectric focusing gels with pH 4-6.5 ampholines further supported similar charge properties between UEA and GS I-B_4-bound forms (data not shown).

Radiolabeled UEA and GS I-B_4-bound glycoproteins were treated with neuraminidase for a specific length of time and then subjected to NEpHGE as described. It was predicted that if UEA and GS-I-B_4-bound glycoproteins possessed similar amounts of sialic acid, both forms would exhibit a similar shift on NEpHGE following a controlled treatment with neuraminidase. Figure 2 shows a similar upward shift in pH of the common major peak of radioactivity for both

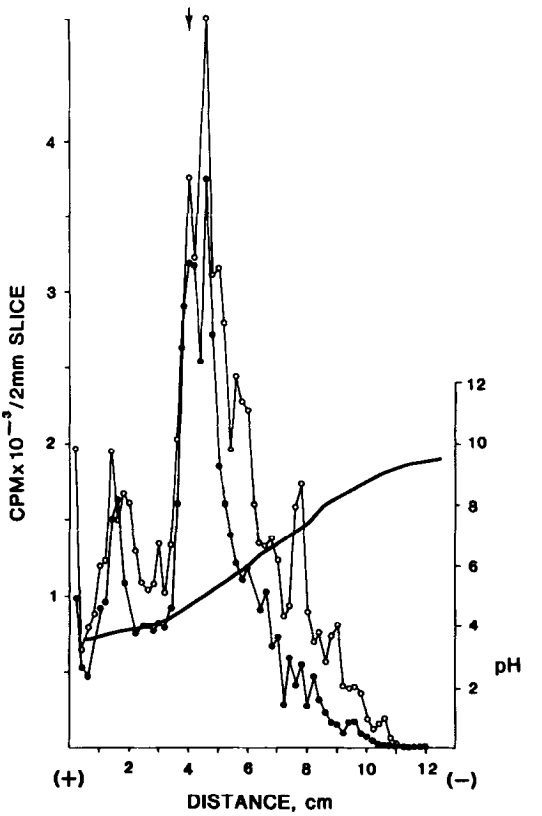

Figure 2. Non-equilibrium pH gradient electrophoresis (NEpHGE) of UEA (O-O-O) and GS I-B_4 (●-●-●)-bound glycoproteins after treatment with neuraminidase. 100,000 CPM of tritiated UEA and GS I-B_4-bound glycoproteins were treated with neuraminidase as described in Methods and then subjected to NEpHGE with pH 3.5-10 ampholines. Arrow represents ^{14}C-Lactoglobulin A internal standard.

UEA and GS I-B_4-bound forms. This indicated the presence of similar number of sialic acid residues on UEA and GS I-B_4-bound glycoproteins.

Alkaline Borohydride Treatment

Using glycoproteins of known structure, Ogata and Lloyd (9) showed that mild alkaline borohydride treatment efficiently released both O- and N- linked oligosaccharides, together with a small proportion of N-glycopeptides. The usefulness of such a procedure for the analysis of N- and O- linked carbohydrate moieties of glycoproteins was illustrated by the application of this technique to a cell surface differentiation antigen of human kidney cells. To determine the nature of the oligosaccharide portions of UEA and GS I-B_4-bound glycoproteins, both forms were subjected to mild alkaline borohydride treatment and analyzed by gel filtration and lectin affinity chromatography.

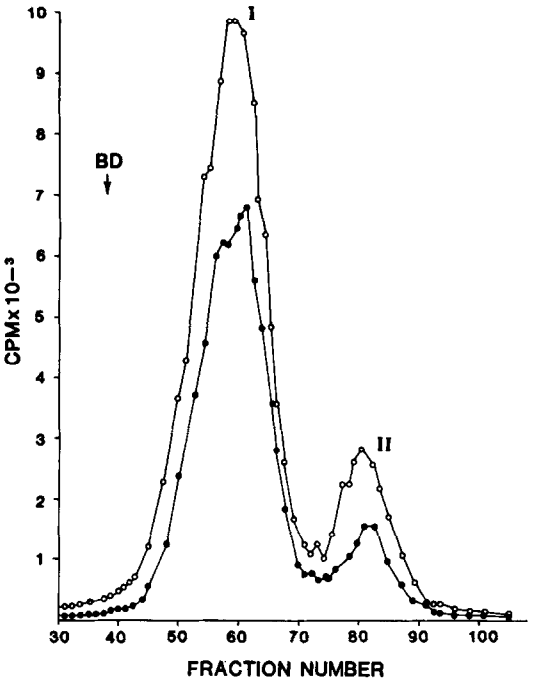

Figure 3. Gel filtration (Sephadex G-50) profiles of tritiated UEA (O-O-O-) and GS I-B_4 (●-●-●)-bound glycoproteins after mild alkaline borohydride treatment. Fraction size: 2.0 ml. BD: Blue Dextran; I: Major peak; II: Minor Peak.

Figure 3 shows the gel filtration profiles on Sephadex G-50 of the products generated from UEA and GS I-B_4-bound glycoproteins after mild alkaline borohydride treatment. Two common peaks of radioactivity (I and II) were obtained. The major peak for both forms (Peak I) was excluded on a Sephadex G-25 column, suggesting a molecular weight (dextran) of between 5000-10,000. Peak I represented 85% of the radioactivity eluted on Sephadex G-50. It is believed that Peak I represents oligosaccharides and glycopeptides released from both UEA and GS I-B_4-bound forms by alkaline borohydride treatment.

To assess lectin-binding characteristics of material released by exposure to alkaline-borohydride, the material in Peak I for both forms was isolated by gel filtration on Bio Gel P-10 and assayed on UEA/GS I-B_4 affinity columns as described previously (3). It was possible that the liberated material might represent non-specific labile chains which did not exhibit either UEA or GS I-B_4-binding properties but were labeled by the galactose oxidase/NaB^3H_4 method. Results showed that Peak I for UEA and GS I-B_4-bound glycoproteins was bound by

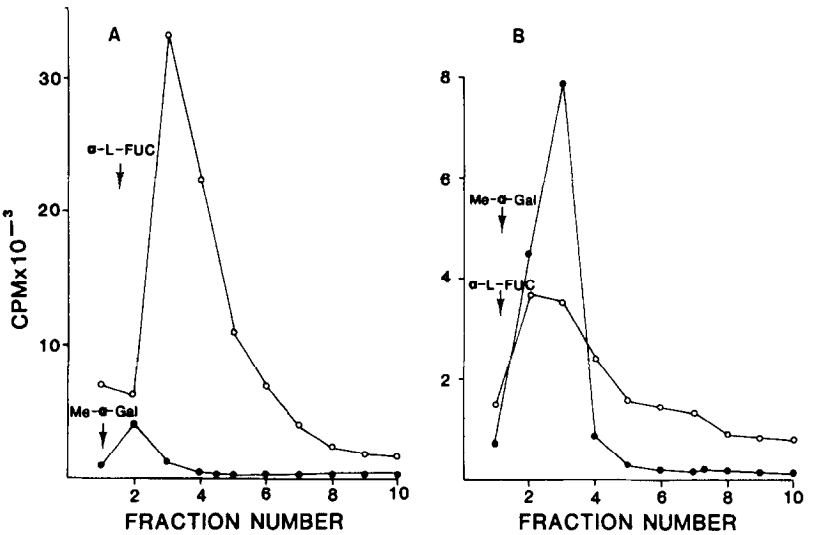

Figure 4. Elution profiles on lectin affinity columns of carbohydrate chains released from tritiated UEA (A) and GS I-B_4-bound (B) glycoproteins after mild treatment with alkaline borohydride (Peak I material). (O-O-O) UEA affinity column; (●-●-●) GS I-B_4 affinity column. Material was applied to UEA and GS I-B_4 columns attached in series, UEA column first, then detached and eluted individually. Profiles represent 70-80% of total applied ^3H.

the respective lectin affinity columns (Fig. 4A and 4B). Peak I for GS I-B_4-bound glycoproteins also exhibited a significant amount of UEA-binding specificity (Fig. 4B). When untreated UEA-bound material is chromatographed on the GS I-B_4 affinity column, about one-third also exhibits GS I-B_4-binding properties (data not shown). The material exhibiting both lectin-binding characteristics may be intermediates (incomplete chains) in the conversion from GS I-B_4 to UEA-binding specificity.

Further study will determine the exact nature of such carbohydrate chains or glycopeptides. At present, this study is complicated by the low yields of UEA and GS I-B_4-specific material. Further work will initially be directed towards purification and structural analysis of the major peak of radioactivity common to both forms on NEpHGE.

These studies, undertaken to compare UEA and GS I-B_4-binding glycoproteins isolated from newborn rat epidermis on the basis of charge properties and the nature of carbohydrate moieties, are consistent with the hypothesis that some UEA and GS I-B_4-bound GP differ simply by the addition of α-L-fucose.

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