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EPIDERMAL FUCOSYLATION OF CELL SURFACE GLYCOPROTEIN

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When Ulex europeus agglutinin I (UEA) conjugated with fluorescein isothiocyanate is applied to tissue sections from the cutaneous epidermis of the newborn rat, the lectin binds to the surfaces of cells in the layer immediately above the basal layer but not to the cells in the basal layer itself. The latter cells bind the isolectin I-B_4, from Griffonia simplicifolia (GS I-B_4). The addition of a fucosyl residue to the oligosaccharide of the glycoprotein found on the surface of the basal cell can account for the change in lectin-binding specificity which occurs as the basal cell moves toward the cutaneous surface and becomes a spinous cell. The epidermis of the newborn rat has the necessary transferase to convert a glycoprotein with binding-specificity for GS I-B_4 to binding specificity for UEA by adding a fucosyl residue from GDP-L-fucose.

Cell-surface glycoconjugates are altered during differentiation in the mammalian cutaneous epidermis (1-6) as in other differentiating and developing systems (7-18). These changes may have physiological significance since glycoconjugates have been implicated in various biological processes (19-25).

Lectins conjugated with fluorescein isothiocyanate (FITC) or rhodamine have been used to visualize changes in the structure of carbohydrate on the cell surface as the epidermal cell moves from the basal germinative layer through the spinous and granular layers and finally into the cornified layer (1, 2, 4, 5, 22, 26). For example in the epidermis of the newborn rat, the lectin, Griffonia simplicifolia I-B_4 (GS I-B_4) (α-D-galactopyranoside-binding specificity), binds to the cells in the basal layer, whereas Ulex europeus agglutinin I (UEA) (α-L-fucose-binding specificity) does not but binds to the cells in the layer immediately above (5). When tissue sections were treated with α-fucosidase prior to exposure to FITC - conjugated lectin, the UEA binding was eliminated and the surface binding of GS I-B_4 now extended throughout the spinous layer (27). This result and the observation that treatment of the tissue with α-galactosidase

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eliminated the binding of I-B_4 but did not result in binding of UEA suggest (27) that the addition of a fucosyl residue to the oligosaccharide of the glycoconjugates is responsible for the change in lectin-binding between the basal and spinous layer.

The present paper reports data indicating that a) some of the cell surface glycoproteins which have UEA-binding specificity can be converted to molecules which bind to GS I-B_4 by exposure to α-fucosidase, b) a transferase which can convert GS I-B_4-binding to UEA-binding glycoproteins is present in the epidermis of the newborn rat and c) GDP-L-fucose can serve as the fucosyl donor for this transferase.

METHODS

Isolation of glycoproteins. Glycoproteins were obtained from the surfaces of isolated basal or differentiated cells derived from the cutaneous epidermis of the newborn rat as previously described (27). These two fractions of glycoprotein were radiolabeled in the galactosyl residues of their carbohydrate moieties by the galactose oxidase/NaB^3H_4 technique (28) and purified by affinity chromatography on columns of Concanavalin A (Con A)-Sepharose 4B (purchased from Pharmacia, Biscataway, N.J.), UEA-Sepharose 4B and GS I-B_4-Sepharose 4B in that order (27, 29). Nonidet P-40 (NP-40) (0.5%) was present in all solutions during chromatography (30). Material specifically bound to Con A-Sepharose 4B was eluted with 0.5M α-methyl D-mannoside. Figure 1 shows the profiles of [^{3}H] glycoprotein observed when these two fractions were electrophoresed on 8% SDS-polyacrylamide slab gels (31) and analyzed for radioactivity by incubating gel slices (2 mm wide) in 3% Protosol/Econofluor (NEN) overnight at 45°C and counting in a liquid scintillation spectrometer. The 94K-fraction of each glycoprotein was used without further purification.

Treatment of glycoproteins with α-fucosidase or epidermal homogenate and isolation of product. The glycoprotein fraction specific for both UEA-and Con A- Sepharose 4B was dialyzed against 0.1M acetate buffer (pH 5.0) and then incubated with α-fucosidase (Boehringer Mannheim), 1.4 unit/ml, at 37°C. Aliquots were adjusted to pH 7.4 with phosphate buffered saline (PBS) containing 0.5% NP-40 and applied to the GS I-B_4 column to determine the extent of conversion from UEA- to GS I-B_4-binding specificity.

Transferase activity was assayed in cell homogenates made in 1.0 ml containing 0.8 ml of Minimal Essential Medium, and 0.5% NP-40, 2.5% BSA, 300 μM L-fucose and 1mM phenylmethylsulfonyl fluoride. The population used consisted of cells from the basal and lower spinous layers. Homogenization was performed in a Teflon-glass tissue grinder. Homogenates were incubated at 37°C with the addition of either GDP-[^{14}C]-L-fucose (1 uCi/ml) or GS I-B_4-bound [^{3}H]-glycoproteins which had been passed through a column of UEA-Sephadex 4B. The detergent was necessary for enzymatic activity. Reaction mixtures were dialyzed against 0.5% NP-40 in PBS and applied to the UEA column. Adsorbed material was eluted with 60 mM L-fucose.

RESULTS AND DISCUSSION

UEA-bound glycoprotein can be converted to GS I-B_4-bound glycoprotein by α-fucosidase in concordance with the results from previous histochemical studies

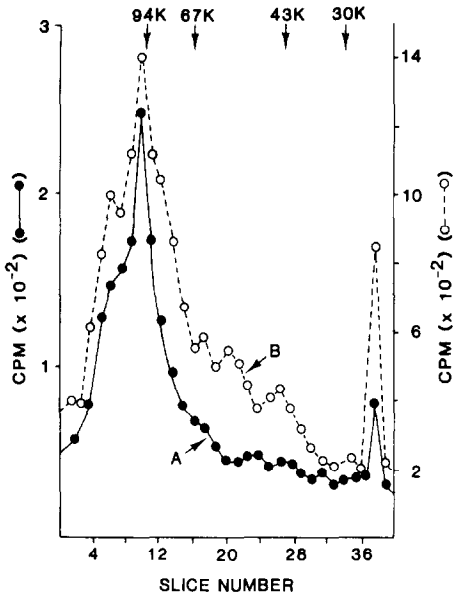

Figure 1: Polyacrylamide gel electrophoretic patterns of tritiated GS I-B_4-bound (A) and UEA-bound (B) glycoproteins. Preparation of samples by affinity column chromatography: (A) Adsorbed to and eluted from Con A-Sepharose 4B, passed through UEA-Sepharose and adsorbed to and eluted from GS I-B_4-Sepharose; (B) Adsorbed to and eluted from Con A-Sepharose 4B and UEA-Sepharose 4B in that order. Figures at the top of the graph note the positions of standard proteins with the indicated molecular weights.

(27). Figure 2 illustrates the time-course of this conversion as assayed by the appearance of glycoprotein with GS I-B_4-binding specificity using tritiated UEA-bound glycoprotein as substrate. Also indicated in Figure 2 is the release of

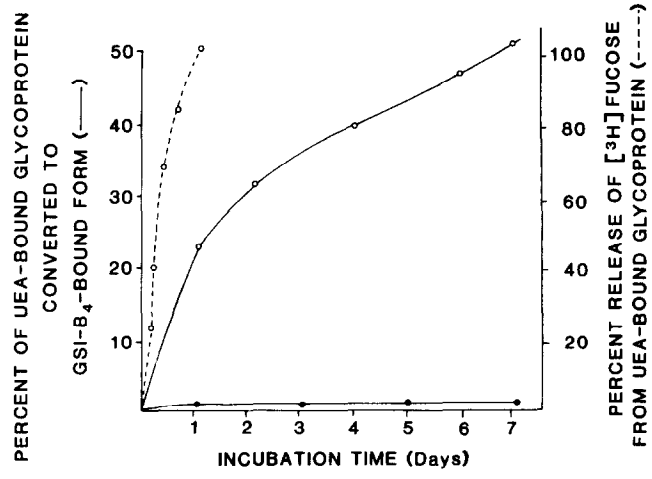

Figure 2: Conversion of UEA-bound glycoprotein to GS I-B_4-bound material catalyzed by α-fucosidase (0-0-0-0-). As a control, addition of 60 mM L-fucose was used to inhibit the enzyme (●-●-●-●-). Reaction monitored by affinity column chromatography.

[^{3}H] fucose from UEA-bound glycoprotein containing the tracer. The UEA-bound glycoprotein fraction appears to be heterogenous metabolically as well as structurally since the release of fucose and the appearance of GS I-B_4-binding in these experiments occurred at different rates and to different extents. Figure 3 shows the formation of tritiated UEA-binding material when a tracer level of ^{3}H-glycoprotein isolated by adsorption on GS I-B_4-Sepharose 4B was incubated at 37^{o}C with a homogenate of cutaneous epidermal cells. No tritiated UEA-binding material was formed in the absence of homogenate. Figure 4 shows the results of a similar experiment in which GDP-[^{14}C]-L-fucose was used as the fucosyl donor, unlabeled glycoprotein (isolated on GS I-B_4-Sepharose 4B) was the acceptor and the reaction was monitored by the appearance of tritiated material with binding-specificity for UEA-Sepharose 4B.

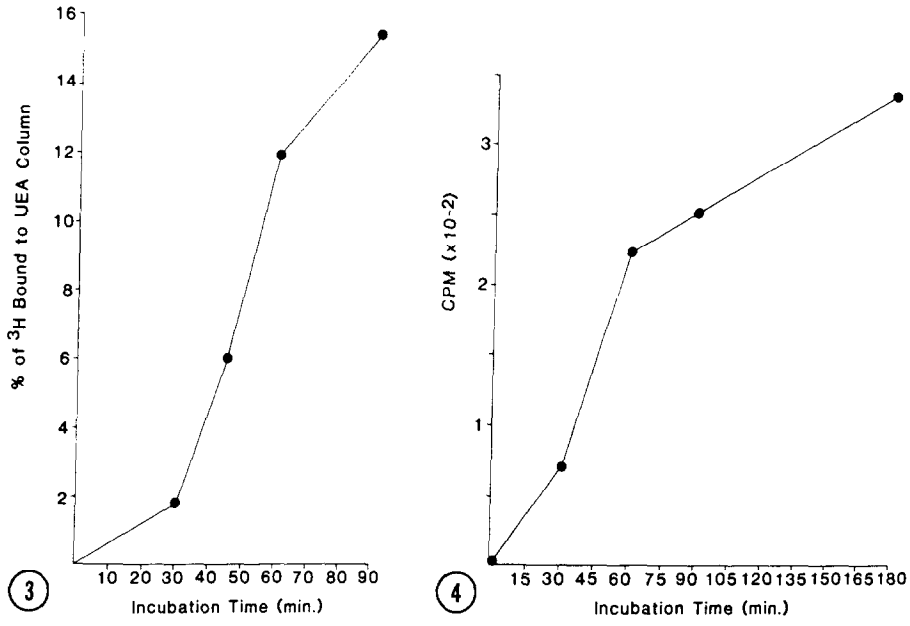

Figure 3: Conversion of GS I-B_4-bound [^{3}H] glycoprotein to tritiated UEA-bound material by a homogenate of cells from the lower cutaneous epidermis of the newborn rat. See METHODS for analytical procedures used. Net % of ^{3}H incorporated was determined by subtracting % of ^{3}H bound in mixtures incubated without homogenate from % ^{3}H bound in mixtures incubated with cell homogenate.

Figure 4: Conversion of GS I-B_4-bound glycoprotein to UEA-bound [^{14}C] glycoprotein using GDP-[^{14}C]-L-fucose as the fucosyl donor. Reaction carried out in the presence of 60 mM L-fucose to block the action of fucosidase present in the homogenate. See METHODS for analytical procedures used.

Clearly the epidermis contains the necessary transferase to convert glycoconjugates which bind with GS I-B_4 into molecules which have UEA-binding specificity. Although GDP-L-fucose can serve as the fucosyl donor, other sugar nucleotides may be more efficient. GDP-L-fucose was the only labeled sugar nucleotide available for these experiments.

By using lectins only change in the nonreducing domain of the oligosaccharide chain is monitored. Changes in other parts of the molecule could also occur physiologically. A determination of the entire primary structure of the two types of glycoprotein is in progress to address this uncertainty. The location of the transferase in the tissue and the mechanism of its regulation in context of the differentiative process are also being studied.

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REFERENCES

1. Nieland, M.L. (1973) J. Invest. Dermatol., 60, 61-66.
2. Hashimoto, K., King, L.E., Yamanishi, Y., Beachey, E.H., and Maeyens, E.J. (1974) J. Invest. Dermatol., 62, 423-435.
3. Van Lis, J.M.J. and Kalsbeek, G.L. (1975) Brit. J. Dermatol., 92, 27-35.
4. Holt, P.J.A., Anglin, J.H., and Nordquist, R.E. (1979) Br. J. Dermatol., 100, 237-244.
5. Brabec, R.K., Peters, B.P., Bernstein, I.A., Gray, R.H., and Goldstein, I.J. (1980) Proc. Natl. Acad. Sci., U.S.A., 77, 477-479.
6. Nemanic, M.K., Whitehead, J.S., and Elias, P.M. (1983) J. Histochem. Cytochem., 31, 887-897.
7. Geltosky, J.E., Siu, C.H. and Lerner, R.A. (1976) Cell, 8, 391-396.
8. West, C.M., McMahon, D., and Molday, R.S. (1978) J. Biol. Chem., 253, 1716-1724.
9. Breuer, W., and Siu, C.H. (1981) Proc. Natl. Acad. Sci., U.S.A., 78, 2115-2119.
10. Etzler, M.E., and Branstrator, M.L. (1974) J. Cell. Biol. 62, 329-343.
11. Essner, E., Schreiber, J., and Griewski, R.A. (1978) J. Histochem. Cytochem., 26, 452-458.
12. Freeman, J.H., Lotan, R., and Kim, Y.S. (1980) Lab. Invest., 42, 405-412.
13. Raedler, E., Raedler, A., and Feldhaus, S. (1981) Anat. Embryol., 162, 21-28.
14. Maylie-Pfenninger, M.F., and Jamieson, J.D. (1980) J. Cell. Biol., 86, 96-103.
15. Reisner, Y., Ravid, A., and Sharon, N. (1976) Biochem. Biophys. Res. Commun., 72, 1585-1591.
16. Reisner, Y., Sharon, N., and Haron-Ghera, N. (1980) Proc. Natl. Acad. Sci., U.S.A., 77, 2244-2246.
17. Rose, M.L. Birbeck, M.S.C., Wallis, V.J., Forrester, J.A., and Davies, A.J.S. (1980) Nature, 284, 364-366.

18. Dabelsteen, E., Vedtofte, P., Hakomori, S. and Young, W.W. (1982) J. Invest. Dermatol., 79, 3-7.
19. Heifetz, A., and Lennarz, W.J. (1979) J. Biol. Chem., 254, 6119-6127.
20. Surani, M.A.H. (1979) Cell, 18, 217-227.
21. Duskin, D., and Bernstein, P. (1977) J. Biol. Chem., 252, 955-962.
22. Olden, K., Pratt, R.M., and Yamada, K.M. (1978) Cell, 13, 461-473.
23. Hickman, S., Kulczcki, Jr., A., Lynch, R.G., and Kornfeld, S., (1977) J. Biol. Chem. 252, 4402-4408.
24. Leavitt, R., Schlesinger, S., and Kornfeld, S. (1977) J. Biol. Chem. 252, 9018-9023.
25. Firestone, G.L., and Heath, E.C., (1981) J. Biol. Chem. 256, 1404-1411.
26. Nemanic, M.K. and Elias, P.M. (1979) J. Cell. Biol., 83, 46. (Abstr.)
27. Zieske, J.D., and Bernstein, I.A. (1982) J. Cell. Biol., 95, 626-637.
28. Gahmberg, C.G., and Hakomori, S., (1973) J. Biol. Chem., 248, 4311-4317.
29. Affinity Chromatography, Principles and Methods, Pharmacia, Uppsala, Sweden (1979).
30. Lotan, R., and Nicolson, G. L. (1979) Biochim. Biophys. Acta, 559, 329-376.
31. Canal SAGE Kit, Miles Laboratories, Inc., Elkhart, Indiana (1980).