Intestinal Epithelial Cell Surface Membrane Glycoprotein Synthesis

II. GLYCOSYLTRANSFERASES AND ENDOGENOUS ACCEPTORS OF THE UNDIFFERENTIATED CELL SURFACE MEMBRANE

(Received for publication, October 2, 1972)

MILTON M. WEISER

From the Department of Medicine, Harvard Medical School and Medical Services (Gastrointestinal Unit), Massachusetts General Hospital, Boston, Massachusetts 02114

SUMMARY

The cell surface membranes of mitotically active crypt cells from rat intestinal epithelium were found to have glycosyltransferase activities and endogenous acceptors not found on the differentiating villus cells. Rat intestinal epithelial cells were isolated as intact cells from different levels of the villus and crypt areas representing sequential stages of cellular differentiation. Incubation of these cells with UDP-N-acetyl[1-14C]glucosamine, UDP-[U-14C]glucose, UDP-[1-3H]galactose, GDP-[U-14C]mannose, and GDP-[U-14C]fucose demonstrated up to a 10-fold greater incorporation into crypt cells as compared to villus cells. In marked contrast, incubation with CMP-[4,5,6,7,8,9-14C]sialic acid showed that radioactive sialic acid was preferably transferred to villus cell rather than to crypt cell surface. Effective transfer of sugars to exogenous acceptors gave further indication for the presence of galactosyltransferase enzyme activity on crypt cells rather than villus cells, and of sialyltransferase enzyme activity as being predominantly on villus cells. Neither glycosyltransferase activities nor endogenous acceptors were released into the incubation medium. Analysis of labeled endogenous product by dialysis, acid precipitation, differential solvent extraction, graded acid hydrolysis, high voltage electrophoresis, and descending chromatography suggested that most of the label was present as the original monosaccharide derived from the nucleotide sugar, and that it had been incorporated into a membrane-associated glycoprotein. Incubation of human fetal intestinal epithelial cells with UDP-[1-3H]galactose also showed galactosyltransferase enzyme activity to an exogenous as well as an endogenous acceptor. These results suggest that the plasma membrane of the mitotically active undifferentiated crypt cell and the fetal cell both contain (a) active glycosyltransferase enzymes and (b) acceptor sites which are glycoproteins with incomplete polysaccharide chains.

Evidence from tissue culture systems has suggested that cell surface membrane glycoproteins play a significant role in cell adhesion and contact inhibition and that alterations in these glycoproteins are related to the ability of a cell to enter mitosis or to exhibit changes in cell behavior as seen after viral transformation (1-4). In addition, Roth et al. (5) have demonstrated galactosyltransferase enzyme activity on cell surface membranes of embryonic chicken neural retina cells in tissue culture and they have suggested that these enzymes are involved in determining cell behavior. The fetal-embryonic cell membrane of neural retina cells from chicken embryo (6) and human fetal intestinal epithelial cells (7) could be further distinguished from the mature adult cell surface membrane by their differential agglutination with concanavalin A.

In the first paper of this series a gradient of cell surface membrane glycoprotein synthesis was demonstrated in rat intestinal epithelium. The gradient proceeded from very low rates in the undifferentiated crypt cell to constantly increasing rates for the differentiating villus cell. These data suggest that glycosylation of membrane-associated protein may be an integral part of the process of cell differentiation (8).

The present study extends these observations by examining the ability of isolated rat and human fetal intestinal epithelial cells to transfer labeled monosaccharides from nucleotide sugars to endogenous acceptors present on the cell surface and to exogenous acceptors. The surface membrane of the undifferentiated mitotically active crypt cell and of human fetal intestinal cells was characterized by active glycosyltransferase activity accompanied by the presence of appropriate endogenous acceptors. A significant exception was the sialyltransferase: endogenous acceptor system which appeared to be more a feature of the differentiating villus cell.

METHODS

Materials and General Methods—Radioactive materials were purchased from New England Nuclear and Amersham-Searle. Nonradioactive nucleotide sugars were bought from Sigma. Protein was determined by the method of Lowry et al. (9). The nonfasting Sprague-Dawley female rats (175 to 225 g) obtained from Holtzman Company were killed, the small intestine was
removed, and isolated intestinal cell preparations were made as previously described (8). Purified brush borders were prepared by the method of Forstner et al. (10). Human fetuses were obtained by hysterotomy with their length varying from 7 to 12 cm (crown to rump). Fetal intestinal isolated cell preparations were prepared as previously described (7). For estimation of glycosyltransferase activities the cells were washed and resuspended in 0.1 M cacodylate-HCl buffer, pH 7.2, with 0.154 M NaCl to a concentration of approximately 2 x 10^6 cells per ml.

**Glycosyltransferase Enzyme Assay**—To a 0.1 ml suspension of intact cells in 0.1 M cacodylate-HCl buffer, pH 7.2, containing 0.154 M NaCl was added 0.01 ml of 0.1 M MnCl_2 and 0.01 ml of radioactive nucleotide sugars (see figures and tables for specific activities and concentrations). The cell suspension was then incubated at 37° for 45 or 90 min. Endogenous product was assayed in two ways. (a) The samples were precipitated by the addition of 2 ml of 5% cold trichloroacetic acid, filtered through glass fiber filters, 2.4 cm in diameter (Reeve Angel grade 394AH), and washed with 10 ml of cold 5% trichloroacetic acid followed by 10 ml of absolute ethanol. The filters were then placed in counting vials, dried in air, covered with 10 ml of a toluene-based scintillator (4 g of 2, 5diphenylosazole, 50 mg of 1,4-bis[2-(5-phenylosazoyl)]benzene, per liter of toluene made with Liquifluor from New England Nuclear), and counted in a liquid scintillation counter. (b) The reaction was stopped by placing the reaction mixture in ice and adding 0.08 ml of 2% sodium tetraborate. Aliquots of the total mixture were then placed on Whatman No. 3MM paper and subjected to high voltage paper electrophoresis, 2000 volts, 30 to 60 min, through glass fiber filters, 2.4 cm in diameter (Whatman No. 1) chromatography with (a) butanol-acetic acid-H_2O (3:2:1, 28 hours), and (b) butanol-pyridine-H_2O (6:4:3, 40 hours). The products of the galactosyltransferase and N-acetylglucosaminyltransferase activities were hydrolyzed with 1 M HCl, 10° for 18 hours under vacuum and the mixture deionized with 0.2 M diethylethylamine in chloroform. These hydrolytic products were also separated by sodium tetaborate high voltage electrophoresis as well as by descending paper chromatography with (c) pyridine-ethylacetate-H_2O-acetic acid (5:5:3:1, 24 hours) and (d) butanol-ethanol-H_2O (10:1:2, 5 days).

**RESULTS**

**Crypt-Villus Gradients of Glycosyltransferase: Endogenous Acceptor Activity**—In marked contrast to the results in the preceding paper with d-[1-^14C]glucosamine (8), incubation of intact cells with nucleotide sugars showed a much greater incorporation of labeled sugar into crypt cells as compared to villus cells (Fig. 1). The highest specific activity of incorporation was found in the bottom crypt cells; those cells also had the highest specific activity for thymidine kinase, an indicator of the mitotically active crypt cell. The one significant exception was with CMP-sialic acid. Incubation of cells with CMP-sialic acid demonstrated some incorporating activity in all cell types but most prominently in the villus rather than crypt cells.

The data shown in Fig 1 were measured as trichloroacetic acid-precipitable counts. However, data obtained after separation of products by high voltage paper electrophoresis gave
similar results and is illustrated in Fig. 2a. As expected, the endogenous product of cells incubated with UDP-[1-3H]galactose remained at the origin while UDP-[1-3H]galactose moved anodally.

Activity with Exogenous Acceptors—The glycosyltransferase activities were then examined with exogenous acceptors to see if the enzyme activities were present on all cell types and whether only the presence of endogenous acceptor determined the high activity of crypt cells. This appeared not to be the case since villus cells best transferred sialic acid from CMP-sialic acid to lactose and only crypt cells transferred galactose from UDP-galactose to N-acetylglucosamine forming lactosamine. Moreover, no labeled lactosamine was found in the cells isolated after incubation. Fig. 2 shows that the cells had only one radioactive product, an immobile glycoprotein, while the incubation medium contained no endogenous product but possessed all the exogenous product lactosamine. This confirmed that no endogenous product was released during the incubation and that most of the enzyme activity measured was probably on the cell surface rather than inside the cell or in both locations. Furthermore, when cells were preincubated and the preincubation medium separated and tested for galactosyltransferase activity using an exogenous acceptor no activity was detected, suggesting that the enzymatic activity was not released into the incubation medium. Thus this evidence, together with the fact that intact cells were used, suggested that both endogenous acceptor and the transferase enzyme activities were located on the cell surface.

Cell Surface Versus Total Activity—As shown again in Table I, intact crypt cells have a significantly greater N-acetylglucosaminyltransferase:endogenous acceptor activity than intact villus cells. Furthermore, the difference in glycosyltransferase:endogenous acceptor activity between crypt and villus cells persisted after homogenization. The increase in activity observed after homogenization was similar for both cells suggesting that the presence of glycosyltransferase:endogenous acceptor activity on the crypt cell surface membrane largely accounted for its greater total activity.

Enhancement of Galactosyltransferase Activity by Preincubation with UDP-N-acetylglucosamine

Assay system was as described under "Methods" except where cells were preincubated, the cells were separated by centrifugation, resuspended in buffer, and the other nucleotide added. Assay time for each nucleotide alone was 45 min.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ratio of Gal to aGlc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Each nucleotide alone UDP-[1-3H]Gal or UDP-ac[1-14C]Gln</td>
<td>0.73</td>
</tr>
<tr>
<td>UDP-[1-3H]Gal first, then UDP-ac[1-14C]Gln</td>
<td>1.00</td>
</tr>
<tr>
<td>UDP-ac[1-14C]Gln first, then UDP-[1-3H]Gal</td>
<td>2.27</td>
</tr>
</tbody>
</table>

When crypt cells were first incubated with UDP-[1-3H]galactose, and then incubated with UDP-N-acetyl[1-14C]glucosamine no significant increase of incorporation into trichloroacetic acid-precipitated material was observed over that seen with each nucleotide alone. However, prior incubation of cells with UDP-N-acetyl[1-14C]glucosamine markedly increased the subsequent incorporation of [1-3H]galactose from UDP-[1-3H]galactose. This suggested that more acceptor sites became available for galactose incorporation with the insertion into the glycoproteins of N-acetylglucosamine, possibly indicating that galactose was mainly transferred to glycoproteins having a terminal N-acetylglucosamine residue.

Fetal Cell Surface—It has been suggested that embryonic tissue is characterized by altered cell surface membrane glycoproteins (6). Further support for a difference in human fetal intestinal epithelial cell surface membrane glycoproteins was observed when intact human fetal intestinal epithelial cells were tested for galactosyltransferase:endogenous acceptor activity (Fig. 3). The cells appeared to have galactosyltransferase ac-
Fig. 3. Human fetal intestinal epithelial cell surface galactosyltransferase activity. Human fetal intestinal epithelial cells were prepared as isolated cell preparations and incubated with UDP-[1-14C]galactose with and without exogenous acceptor, N-acetylglucosamine. As in Fig. 2 the reaction was stopped by the addition of 0.08 ml of 2% sodium tetraborate and cells separated from incubation medium by centrifugation. Cells were washed with cacodylate buffer, resuspended in 0.1 ml of buffer and 0.05 ml was applied to Whatman No. 3MM for high voltage electrophoresis as described under “Methods.” Values in parentheses represent net incorporation (counts per min) for the entire peak, i.e., minus zero time control, of equivalent aliquots from equivalent cell concentrations. Again, labeled endogenous acceptor appears mainly with the cell pellet and labeled exogenous acceptor appears in the cell medium.

<table>
<thead>
<tr>
<th>Table III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stialyltransferase activity of intact villus cells</strong></td>
</tr>
<tr>
<td>Assay was as described under “Methods” with separation of products by 0.1% sodium tetraborate high voltage electrophoresis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestinal villus cells</th>
<th>Endogenous product</th>
<th>Exogenous product</th>
<th>Exogenous + endogenous product</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lactose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With lactose (25 mM)</td>
<td>2,084</td>
<td>3,806</td>
<td>3,806</td>
</tr>
<tr>
<td>CMP-[4-3H]sialic acid</td>
<td>3,806 (18,742)</td>
<td>1,172 (1,114)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Expected values.

Activity to both endogenous and exogenous acceptor (N-acetylglucosamine) and, as with the adult rat intestinal epithelial crypt cells, the labeled endogenous acceptor was strictly associated with the cells while the exogenous acceptor was labeled and remained in the incubation medium.

**Stialyltransferase: Endogenous Acceptor Activity**—This activity appeared largely confined to the differentiating cell in the villus area with the lowest activity at the bottom of the crypts (Fig. 1). This transferase activity also appeared to be available for activity with lactose as the exogenous acceptor suggesting a cell surface membrane location (Table III). Part of the differentiated villus cell surface membrane, the brush border or microvilli, can be isolated as a relatively purified preparation (10). Sialyltransferase: endogenous acceptor activity was found to be present in these brush border preparations but there was a decrease in specific activity rather than an increase. However, when exogenous acceptor was used the specific activity appeared to be similar to that of the whole cells. Since brush border purification represents a 25-fold purification of a potent β-galactosidase, it may be that the brush border transferase specific activity was actually much higher.

**Mixing Experiments**—Mixing experiments with crypt and villus cells were done to detect any endogenous acceptors on villus cells that could be glycosylated by crypt cell surface membrane glycosyltransferase enzyme activity. It had already been determined that activity was linear with the amount of cells. The results (Table IV) indicated that not only was there no increase...
in the amount of labeled endogenous product with the addition of villus cells to crypt cells, but that there was a decrease from the expected value (in parentheses) for the number of crypt cells present in the assay. Again the only exception was with CPM-sialic acid. There was evidence of increased nucleotide sugar breakdown in the presence of villus cells but this constituted less than 20% of the labeled nucleotide sugar and may explain some but not all of the decrease in labeled endogenous product. In addition, prelabeling of crypt cells followed by incubation with villus cells did not indicate destruction of crypt cell endogenous acceptor by villus cells. Two explanations appear possible: (a) there was no endogenous acceptor present on villus cell surface, and (b) presence of villus cells physically interfered with the accessibility of crypt cell endogenous acceptor with crypt cell enzyme activity. The second explanation suggests that the glycosyltransferase: endogenous acceptor activities were the result of interactions between two crypt cells, one contributing the endogenous acceptor and the other the enzyme activity, and would indicate spatial separation on the cell surface of the enzyme from the acceptor.

Product Identification—The labeled endogenous products were found to be: (a) precipitated by trichloroacetic acid, (b) not dialyzable, (c) immobile during high voltage electrophoresis, and (d) not extracted by chloroform:methanol (2:1) after repeated back washing of each layer. Most of the labeled material precipitated at the chloroform:methanol-H2O interface with no more than 10% in the upper layer and none in the lower chloroform layer. This suggested that the labeled sugar was largely incorporated into glycoproteins rather than glycolipids. The demonstration that as much as 10% of the counts were extracted into the methanol-H2O (upper) layer suggested that some known complex glycolipids were also being labeled since they are extractable into this layer (11), but this was not further investigated at this time. The endogenous products of the sialyltransferase, galactosyltransferase, and N-acetylgalactosaminyltransferase reactions were further identified by examining the products of acid hydrolysis. Fig. 4 illustrates that the label incorporated into endogenous product with CMP-[4,5,6,7,8,9-3H]sialic acid as substrate was sialic acid after mild acid hydrolysis the radioactive material on electrophoresis and chromatography migrated with authentic sialic acid. Similarly, in Figs. 5 and 6, and after strong acid hydrolysis, the radioactive material was galactose and glucosamine for the respective galactosyl- and N-acetylgalactosaminyltransferase reactions.

**Discussion**

In the preceding paper of this series a method was described for the isolation of intact intestinal epithelial cells from different areas of villus and crypt zones (8). It was also demonstrated that simple monosaccharides such as N-glucosamine or L-fucose were rapidly incorporated in vivo and in vitro into membrane-associated glycoproteins of the upper villus cells but not into the crypt cells (8). This suggested that with villus cell differentiation there was a concomitant increase in membrane-associated glycoprotein synthesis. This raised the question as to whether the membrane glycoproteins of the undifferentiated cell might be "incomplete." Roth et al. recently reported the presence of active glycosyltransferase activity on the cell surface of chicken embryo neural retina cells (5). Consequently, intact isolated intestinal epithelial cells were incubated with labeled nucleotide sugars and evaluated for expressed surface membrane glycosyltransferase enzyme activity and endogenous acceptors.

The results presented here gain significance when compared with those of the preceding paper (8). Since almost all surface membrane hydrolytic enzymes have been associated with the villus cell brush border (10), it might have been expected that activity with nucleotide sugars might be attributed to breakdown of these compounds. Thus, if any of the glycosyltransferase activity were actually due to nucleotide sugar hydrolysis with absorption of the sugar it would have been expected to be a property of the villus cell, which was not the case. Even if the crypt
probably represented surface membrane activity. The villus and crypt cells appeared to be similar and the marked glycosyltransferase: endogenous acceptor activities for both proteins (8). Furthermore, as shown in Table I the intracellular voltage electrophoresis; f1, descending chromatography, System 3; C, descending chromatography, System 4. 

It could be argued that what was demonstrated in these experiments was the isolation of cells actively synthesizing new cell membrane in an early phase of differentiation and not, necessarily, a prerequisite cell membrane alteration for mitosis. Under these circumstances one might expect that there would be some order by which specific sugars were added. The data in Table II showing enhancement of galactosyltransferase activity by preincubation with UDP-N-acetylglucosamine suggest that the order was N-acetylglucosamine followed by galactose. Thus, either during or after mitosis, the cell surface membrane glycoproteins of the mitotically active crypt cells were characteristically incomplete and contained active glycosyltransferase enzymes. In contrast, the mixing experiments (Table IV) suggested that the villus cell surface membrane glycoproteins were either completed before insertion into the membrane or inaccessible since these villus cells had not demonstrated available endogenous acceptor activity when enzyme activity was present (as the crypt cell surface membrane).

One important exception was the sialyltransferase activity which was highest with villus cells and lowest with crypt cells (Fig. 1). Since sialic acid terminates chain growth when joined to a N-acetylgalactosamine residue (4) but is also the sugar with the most labile linkage (4), it might be argued that the presence of sialyltransferase activity on the surface membrane is for constant repair. The presence of sialic acid on membrane glycoproteins has been given greater functional significance than that of other sugars (12–17). Sialic acid is one of the factors which appears to give to the membrane its net negative electrical charge (18). The presence of increased or decreased sialic acid residues on tumor and transformed cell membranes has been debated for a number of years but the most recent studies (19, 20) have shown a decreased membrane sialic acid content after viral transformation. No direct evidence has been presented for any sialic acid-mediated control of cell turnover.

Cell surface membrane glycoproteins are believed to be incomplete during mitosis, in early sparse growth of tissue culture cells, and after viral transformation (1–3). The properties of decreased cell adhesion and contact inhibition under these conditions of cell growth or transformation may be due to these alterations in membrane glycoprotein. In fact, Roseman (4) has proposed that surface membrane glycosyltransferases interact with incomplete glycoproteins of another cell membrane and thereby produce specific cell adhesive forces. The results described in the present report are compatible with experiments in tissue culture systems (1–3) and with chicken embryo cells (5). However, there is no evidence that intestinal epithelial crypt...
cells or fetal intestinal cells are less adhesive to adjacent cells or to the basement membrane. In fact, it was easier to obtain isolated epithelial cell preparations of single cells with villus cells than with crypt cells which remained in clumps. This was not true for fetal cells which were easily prepared as isolated cells. Thus, at least for the intestinal epithelial system, there was no consistent relationship between cell adhesive forces and the incompleteness of surface membrane glycoproteins.

It has been suggested that the cell surface membrane is actually an outgrowth from the Golgi membrane and, therefore, would have glycosyltransferases imbedded in its unit membrane structure (21, 22). As long as the cell is relatively primitive and rapidly dividing, requiring rapid synthesis of new surface membranes, it will have a surface membrane which retains the characteristics of its source, the Golgi membrane. It would then follow that one explanation for finding glycosyltransferase activity on crypt and fetal cell surface membranes may be that the "early" cell surface membrane is, in fact, externalized Golgi membrane. Regardless of the origin, the presence of these enzymes and of incompletely glycosylated glycoproteins on the cell surface membrane appears to be a consistent and valuable identifying feature of the undifferentiated cell state.

Acknowledgments—I thank Miss Irene Kozdrowski for valuable technical assistance. I especially thank Dr. Kurt J. Isselbacher for his support in this work, as well as his critical review of this manuscript.

REFERENCES
Intestinal Epithelial Cell Surface Membrane Glycoprotein Synthesis: II.
GLYCOSYLTRANSFERASES AND ENDOGENOUS ACCEPTORS OF THE
UNDIFFERENTIATED CELL SURFACE MEMBRANE
Milton M. Weiser


Access the most updated version of this article at http://www.jbc.org/content/248/7/2542

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/248/7/2542.full.html#ref-list-1