Glycosyltransferase Changes upon Differentiation of CaCo-2 Human Colonic Adenocarcinoma Cells

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ABSTRACT

The spontaneous differentiation of CaCo-2 human colonic adenocarcinoma cells to enterocytes in culture is associated with a decrease in polylactosaminoglycans, particularly those attached to the lysosomal membrane glycoprotein h-lamp-1 (Yousakim et al., Cancer Res., 49: 6889-6895, 1989). To elucidate the biosynthetic mechanisms leading to these alterations we have compared glycosyltransferase activities that are involved in the synthesis of polylactosaminoglycans and of the N- and O-glycan structures that provide the framework for the attachment of these chains. Glycosyltransferase activities in cell homogenates obtained from undifferentiated and differentiated CaCo-2 cells were assayed by high pressure liquid chromatography separation of enzyme products. The β-galactosidase activities and extremely high pyrophosphatase activities in differentiated cells were effectively inhibited by 5 mM β-galactonolactone and 10 mM AMP, respectively. CaCo-2 cells contain most of the enzymes that are involved in N-glycan branching at [N-acetylgalactosamine (GlcNAc) transferases I to V] with the exception of GlcNAc transferase VI. The levels of GlcNAc transferase I activities were comparable in undifferentiated and differentiated cells, but GlcNAc transferase II to V activities were significantly increased upon differentiation. The enzyme activities that are directly involved in the synthesis of linear polylactosaminoglycans (Galβ4GlcNAcβ3-Repeating units), blood group I UDP-GlcNAc:Galβ3-R β3-GlcNAc transferase and UDP-Gal:GlcNAc β4-Gal transferase, were found at similar levels in undifferentiated and differentiated CaCo-2 cells. Since GlcNAc transferase III activity is known to increase in differentiated CaCo-2 cells, its increased activity in differentiated CaCo-2 cells may be partly responsible for the decreased synthesis of fucosylated polylactosaminoglycans. Differentiated cells showed a 2-fold increase in O-glycan core 2 UDP-GlcNAc:Galβ3Galaaco-R (GlcNAc to N-acetylgalactosamine (GalNAceβ6-GlcNAc transferase activity. In contrast, O-glycan core 1 UDP-Gal:Galaaco-R β3-Gal transferase activity was found decreased. Several enzymes that are found in homogenates from normal human colonic tissue are absent or barely detectable in CaCo-2 cells. These include blood group I UDP-GlcNAc:Galβ3Galaaco-R (GlcNAc to Gal) β6-GlcNAc transferase, O-glycan core 3 UDP-GlcNAc:Galaaco-R β3-GlcNAc transferase and O-glycan core 4 UDP-GlcNAc:Galaacoβ3Galaaco-R (GlcNAc to Galac) β6-GlcNAc transferase.

INTRODUCTION

CaCo-2 human colonic adenocarcinoma cells differentiate spontaneously into cells with the properties of enterocytes upon prolonged culturing (1). This differentiation is accompanied by the acquisition of a brush border and of hydrolytic enzymes typical of absorptive intestinal cells. CaCo-2 cells were previously shown to contain polylactosaminoglycans, which could be labeled with fucose and glucosamine. Although these are 

primarily associated with N-linked carbohydrate chains, a significant proportion of glucosamine-labeled glycopeptides were also found in alkali-labile O-linked oligosaccharides (2). The proportion of fucosylated polylactosaminoglycans known to carry oncodevelopmental antigens decreases significantly during differentiation (2). In particular, the lysosomal membrane protein h-lamp-1 was shown to be affected by these glycosylation changes (3).

To elucidate the mechanisms responsible for the decrease in polylactosaminoglycans with differentiation, we compared the glycosyltransferase activities that are involved in the synthesis of polylactosaminoglycan chains (Fig. 1) and of the backbone structures of O- and N-linked oligosaccharides in UDP and CaCo-2 cells. The synthesis of core structures and of branches of N- and O-glycans requires distinct sets of glycosyltransferases (4, 5). However, most of the enzymes involved in the elongation of N- and O-glycans and in the addition of terminal antigens are the same for both types of glycans. We found increased activities for both O- and N-glycan branching enzymes and decreased activity of the transferase responsible for the synthesis of the O-glycan core 1 (Galβ3GalNAc-R) upon differentiation. Unexpectedly, the enzymes directly involved in polylactosaminoglycan biosynthesis, i.e., UDP-Gal:GlcNAc β4-Gal transferase (β4-Gal-T) and blood group I UDP-GlcNAc:Galβ4GlcNAc-R β3-GlcNAc transferase (β3-Gn-T), remain essentially unchanged with differentiation of CaCo-2 cells; blood group I UDP-GlcNAc:GlcNAcβ3Galβ-R (GlcNAc to Gal) β6-GlcNAc transferase, responsible for branching, was undetectable.

MATERIALS AND METHODS

Materials. AG 1-X8 (100-200 mesh, Cl− form) was purchased from Bio-Rad. Bovine serum albumin, Galβ1-4GlcNAc, AMP, Triton X-100, β-galactonolactone, Galβ3GalNAc-Bn, Galβ3Galβ-methyl, and GalNAc-Bn were purchased from Sigma. Acetonitrile (190 UV cutoff) was from Fisher Scientific Co. or Caledon Laboratories. UDP-N-[1-14C]acetylgalactosamine was synthesized as described previously (6) and diluted with UDP-GlcNAc from Sigma. UDP-[1-14C]galactose was purchased from Amersham and diluted with UDP-Gal from Sigma. Substrates and Standards. GlcNAcβ3-1-GalNAc-Bn, GlcNAcβ3-6 (Galβ1-3) GalNAc-Bn, GlcNAcβ3-6 (Galβ1-3) GalNAc-Bn, GlcNAcβ3-6 (Galβ1-3) GalNAc-Bn, and GlcNAcβ3-6 (Galβ1-3) GalNAc-Bn were synthesized by Dr. K. L. Matta, Buffalo, NY. GlcNAcβ3Manα6 (GlcNAcβ3Manα3) Manβ2-(CH2)3COOCH3 (2,5mco) was donated by Dr. O. Hinds-gaul, Edmonton, Alberta, Canada. GlcNAcβ6 (GlcNAcβ2) Manα6-methyl was provided by Dr. R. Shah, Toronto, Ontario, Canada. Manα6-
It was shown that the purified enzyme attaches GlcNAc in β2-linkage to Manβ₆(GlcNAcβ₂)Manβ₃Hex as prepared by Dr. H. Paulsen, Hamburg, Germany. Manβ₆(GlcNAcβ₂)Manβ₃Hex was synthesized by Dr. H. Paulsen, Hamburg, Germany. The activity synthesizing the branch of the blood group I antigen is absent from CaCo-2 cells but is high in normal human colon.

(Manβ₃)Manβ₆-hexyl was prepared from GlcNAcβ₁₂(Mannβ₆-hexyl with purified rat liver GlcNAc transferase I and subsequent purification by AG 1-X8 and Bio-Gel P-4. It was shown that the purified enzyme attaches GlcNAc in β2-linkage only to the Manβ₃ residue of the substrate. The purified enzyme attaches GlcNAc in β2-linkage only to the Manβ₃ residue of the substrate. GlcNAcβ₆-[¹⁴C]-GlcNAcβ₄ [GlcNAcβ₂] Manβ₆-hexyl was prepared from GlcNAcβ₆-GlcNAcβ₈ [GlcNAcβ₂] Manβ₆-hexyl with hen oviduct microsomal GlcNAc transferase VI (8). (Manβ₃)Manβ₆atic acid (NH₃) column (Waters carbohydrate analysis column). The enzyme was purified by incubation of GlcNAcβ₆-Galβ₃-methyl with pig gastric mucosa microsomal blood group I β6-GlcNAc transferase (9, 10) as follows: 3.2 μmol GlcNAcβ₆-Galβ₃-methyl was incubated with pig gastric mucosal microsomes (4.8 mg protein) for 2 h at 37°C in a total volume of 1.32 ml containing 2.62 mM UDP-[¹⁴C]Galactose (3287 dpm/nmol), 1.05% Triton X-100, 76 mM MES buffer (pH 7), and 76 mM GlcNAc. To stop the reaction, 8 ml 20 mM sodium tetraborate/1 mM EDTA, pH 9, and freezing. Mixtures were passed through Pasteur pipets filled with AG 1-X8, 100-200 mesh, Cl⁻ form. The column was washed with 52 ml enzyme reactions requiring MnCl₂.

The structures of all substrates and standards were confirmed by nmr spectroscopy. Preparations of Cell Homogenates. CaCo-2 cells were maintained in culture as described previously (3). Cells were harvested after 4 days in culture for rapidly growing UD cells, or after 25 days for slow confluent D cells. Cells were washed three times in 0.9% NaCl solution followed by centrifugation. Pellets were frozen and thawed in 0.9% NaCl solution and centrifuged. The pellets were suspended in 0.25 M sucrose (1 ml/10⁶ cells), homogenized with a Potter-Elvehjem homogenizer, and stored at −70°C. Human colon homogenates were prepared from colon tissue obtained from tissue resections of normal colon mucosa adjacent to colon cancer; tissue was hand homogenized in 0.25 M sucrose.

Protein Determinations. Protein was determined by the Bio-Rad method using bovine serum albumin as the standard.

Nuclear Magnetic Resonance. Samples were prepared by exchanging twice with 99.8% D₂O (Aldrich) and twice with 99.96% D₂O (Merck, Sharpe and Dohme). Samples were dissolved in 99.96% D₂O as the internal standard. Proton nmr spectra were recorded at the Toronto Carbohydrate Research Centre with a Bruker 500-MHz spectrometer. The acetone signal was set at 2.225 ppm.

High Pressure Liquid Chromatography. HPLC separations were carried out with an LKB or a Waters system as described (12, 13). Acetonitrile/water mixtures were used as the mobile phase for all columns at a flow rate of 1 ml/min. To separate enzyme products with mco, hexyl, benzyl, and methyl groups, a reverse phase C₁₈ column was used. Free reducing sugars or methylglycosides were separated on a propylamine (NH₃) column (Waters carbohydrate analysis column). Elution of compounds was monitored by measuring the absorbance at 195 nm and counting the radioactivity of collected fractions (12).

Assays for GlcNAc Transferases (Table 1). The standard assay mixture for GlcNAc transferases contained the following ingredients in a total volume of 40 μl: 0.125 M GlcNAc, 0.125 M MES, pH 7; 0.125% Triton X-100; 12.5 mM MnCl₂; 10 mM AMP; 5 mM γ-galactonolactone (only for substrates with terminal Gal at the nonreducing end as indicated in Table 1); 0.87 mM UDP-[¹⁴C]Galactose (5486 dpm/nmol); substrate (as indicated in Table 1); and 10 μl cell homogenate (0.057 or 0.068 mg protein/ml for D or UD cells, respectively). β6-GlcNAc transferases that do not require MnCl₂ (GlcNAc transferase V, blood group I, core 2, and core 4 β6-GlcNAc transferases) were measured in the absence of exogenous MnCl₂ to reduce any enzyme reactions requiring MnCl₂. Mixtures were incubated for 1 h at 37°C. Reactions were stopped by the addition of 400 μl 20 mM sodium tetraborate/1 mM EDTA, pH 9, and freezing. Mixtures were passed through Pasteur pipets filled with AG 1-X8, 100–200 mesh, Cl⁻ form. After these were washed with 2.6 ml water, eluates were lyophilized, taken up in 200 μl water, and stored at −20°C. We used 100 μl for HPLC analysis. Results from assays lacking the acceptor were routinely subtracted in the calculations of activities.

Assays for Gal Transferases (Table 1). The assays for Gal transferases were carried out as described for the GlcNAc transferases, except that the assay mixtures contained 1.86 mM UDP-[U-¹³C]Galactose (3266 dpm/nmol) instead of UDP-GlcNAc and 5 mM α-galactonolactone, and the addition of 0.125 M GlcNAc was omitted.

Assays for β-Galactosidase. β-Galactosidase was assayed according to the method of Distler and Jourdain (14) in phosphate-citrate buffer (pH 4.3) or MES buffer (pH 5 to 8) with Galβ₃-p-nitrophenyl as the substrate and 30 min incubation time. Enzyme Kinetics. Km and Vₘ₉₉ values were determined from 5 to 6 different substrate concentrations by linear double reciprocal Lineeweaver-Burk plots.

RESULTS

Glycosyltransferases that assemble and elongate the branches of N-linked oligosaccharides and O-glycan cores were assayed in UD and D CaCo-2 cells. The enzymes; structures of their substrates and products; the HPLC conditions of product separation, including retention times; and enzyme activities are summarized in Table 1. The assays were complicated by high pyrophosphatase activities observed especially in D cells. These activities were calculated from the total amount of free radioactive sugars arising from the breakdown of radioactive UDP-Gal or UDP-GlcNAc during the incubation. Without the addition of AMP, UD cells exhibited reasonable levels (<5%) of breakdown; however, pyrophosphatases in D cells cleaved more than 80% of the nucleotide sugar donor. It was thus essential to add 10 mM AMP as a pyrophosphatase inhibitor to the assay. Under these conditions, less than 2% of the nucleotide sugars in both cell populations were degraded during a 1-h incubation. The absorbance scans of HPLC graphs indicated that the

Fig. 1. Pathways of polylactosaminoglycan synthesis. The enzymes involved in the synthesis of the blood group i antigen attached to R (R = N-linked or O-linked oligosaccharides) are present in CaCo-2 cells. The β6-GlcNAc transferase activity synthesizing the branch of the blood group I antigen is absent from CaCo-2 cells but is high in normal human colon.
GLYCOSYLTRANSFERASES IN CaCo-2 CELLS

Table 1 Glycosyltransferase changes upon differentiation of CaCo-2 cells: summary of enzymes assayed in CaCo-2 cells, structures of substrates and products, HPLC conditions, and enzyme activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Product</th>
<th>Retentionb time (min)</th>
<th>Col.</th>
<th>% AN</th>
<th>Activity (nmol/h/mg)</th>
</tr>
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<tbody>
<tr>
<td>Gn-T I</td>
<td>0.5 mM Mo6(Mo3) M6-hex</td>
<td>Mo6(Gn32Mo3) M6-hex</td>
<td>35</td>
<td>C18</td>
<td>12</td>
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<td>12.2</td>
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<td>Gn-T II</td>
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<td>[2,2+F]</td>
<td>14</td>
<td>NH2</td>
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<tr>
<td></td>
<td>1 mM [2,2mco]</td>
<td>[bis2,2mco]</td>
<td>25</td>
<td>C18</td>
<td>16</td>
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<tr>
<td></td>
<td>0.5 mM [26,24]</td>
<td>[bis26,24]</td>
<td>38</td>
<td>C18</td>
<td>16</td>
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<td></td>
<td>1 mM [2,2mco]</td>
<td>[2,2mco]</td>
<td>65</td>
<td>NH2</td>
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<tr>
<td>Gn-T IV</td>
<td>1 mM [2,2mco]</td>
<td>[bis2,2mco]</td>
<td>25</td>
<td>C18</td>
<td>16</td>
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<tr>
<td></td>
<td>0.2 mM [2,24]</td>
<td>[2,24mco]</td>
<td>26</td>
<td>C18</td>
<td>16</td>
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<td></td>
<td>4 mM Gn6(Gn2) Ma-me</td>
<td>Gn6[Gn64Gn64(Gn2) Ma-me</td>
<td>12</td>
<td>C18</td>
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<td>Core 1 β3-Gal-T</td>
<td>2 mM Gaα-Bn</td>
<td>Gaβ3Gaα-Bn</td>
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<td>C18</td>
<td>10</td>
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<td>2 mM Gb3Gaα-Bn</td>
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<td>C18</td>
<td>10</td>
<td>38.3</td>
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<tr>
<td>Core 4 β6-Gn-T</td>
<td>2 mM Gb3Gaα-Bn</td>
<td>Gb3Gaα-Bn</td>
<td>26</td>
<td>C18</td>
<td>10</td>
<td>8.9</td>
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<tr>
<td>Elongation β3-Gn-T</td>
<td>4 mM Gb3Gaα-Bn</td>
<td>Gb3Gaα-Bn</td>
<td>32</td>
<td>C18</td>
<td>8</td>
<td>0.99</td>
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<tr>
<td>i β3-Gn-T</td>
<td>4 mM Gb4Gn</td>
<td>Gb4Gn</td>
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<td>NH2</td>
<td>86</td>
<td>0.81</td>
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<td>I β6-Gn-T</td>
<td>2 mM Gb3Gβ-methyl</td>
<td>Gb3Gβ-methyl</td>
<td>18</td>
<td>NH2</td>
<td>86</td>
<td>0.81</td>
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<tr>
<td>β4-Gal-T</td>
<td>2 mM Gb4Gn</td>
<td>Gb4Gn</td>
<td>16</td>
<td>NH2</td>
<td>84</td>
<td>50.3</td>
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*Enzyme (5) abbreviations are: Gn-T I, II, III, IV, V, VI; UDP-GlcNAc: Man-R GlcNAc transferases I, II, III, IV, V, VI (8, 12, 15, 22); core 1 β3-Gal-T, core 1 UDP-GalGalNAc-R β3-Gal-T (33, 34); core 2 β6-Gn-T, core 2 UDP-GlcNAcGalβ3 GalNAc-R β6-GlcNAc-T (35, 36, 37); core 3 β3-Gn-T, core 3 UDP-GlcNAcGalNAc-R β3-GlcNAc-T (6); core 4 β6-Gn-T, core 4 UDP-GlcNAcGalβ3 GalNAc-R β6-GlcNAc-T (6); elongation β3-Gn-T, elongation UDP-GlcNAcGalβ3 (R,6) GalNAc-R, β3-GlcNAc-T (16, 17); i β3-Gn-T, blood group i UDP-GlcNAcGalβ3 GlcNAc-R β3-GlcNAc-T (19); I β6-Gn-T, blood group I UDP-GlcNAcGalβ3 GlcNAc-R β6-GlcNAc-T (10); β4-Gal-T, UDP-GalGalNAc β4-Gal-T (26). Enzymes were assayed at least in duplicate determinations as described in "Materials and Methods." AN, acetonitrile; Bn, benzyl; hex, hexyl; G, Glc; Ga, Gal; D-galactose; GA, A'-acetyl-D-galactosamine; Gn, N-acetyl-D-glucosamine; M, D-mannose; me, methyl; mc, (CH2)6COOCH3; ND, not detected; T, transferase.

* Retention times may change according to the HPLC system and the age of the column.
* Col., HPLC column; C18, silica-bound C18; NH2, silica-bound propylamine.
* Activity includes minor subsequent reactions by Gn-T III, IV, and V.
* Reducing oligosaccharides elute as two peaks on reverse phase columns.
* 5 mM γ-galactonolactone was added to the assay mixture.

substrate Galβ3GalNAcα-Bn was degraded by the action of β-galactosidases in D cells to GalNAcα-Bn. Degradation of Galβ3GalNAcα-Bn was barely detectable in UD cells. β-Galactosidase activity measured with Galβ-p-nitrophenyl substrate in D was 250% of the activity in UD cells. The optimal pH of β-galactosidase activity in both UD and D cells was pH 4.3 or below. This indicates that the β-galactosidases probably originate from the lysosomes. However, the addition of 2.5 or 5 mm


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- γ-galactonolactone inhibited breakdown of Galβ3GalNAc-Bn (Fig. 2) and concomitantly increased the activity of core 1 β3-Gal-T, while the core 2 β6-GlcNAc-T remained unchanged. 5 mM γ-galactonolactone was therefore routinely added to all Gal transferase assays and enzyme assays involving substrates with terminal Gal.

- Activities of β4-Gal and core 1 β3-Gal-T, the core 2, core 4, and blood group I β6-GlcNAc-T, and core 3 β3-GlcNAc-T were present in all five samples of normal human colonic homogenate, but there were considerable variations of the levels of all six transferase activities between samples. This may be due to the fact that colonic homogenates represent heterogeneous cell populations. Three of these activities (Table 1), i.e., core 3 β3-GlcNAc-T, core 4 β6-GlcNAc-T, and blood group 1 β6-GlcNAc-T, were absent from CaCo-2 cells or present at only very low levels. Fig. 3 shows that the blood group I β6-GlcNAc-T has high activity in the normal human colon but is absent from CaCo-2 cells.

- Of the GlcNAc transferases that attach the branches to N-linked oligosaccharides, activities of GlcNAc-T I to V were detectable in CaCo-2 cells while GlcNAc-T VI was absent (Table 1).

- GlcNAc-T I activities were high (11 to 12 nmol/h/mg) and at a similar level in UD and D cells. GlcNAc-T II, III, IV, and V activities appeared to be increased in D cells (Table 1). GlcNAc-T II was increased 2-fold using [0,2+F] as the substrate. In the absence of GlcNAc-T VI, the tetraantennary [26,24] oligosaccharide is a specific substrate for GlcNAc-T III and showed a 2-fold increase of this enzyme in D cells (Table 1). [2,2mco] was used as the substrate to measure both GlcNAc-T III and GlcNAc-T IV activities in the same assay (Fig. 4). GlcNAc-T III was increased more than 3-fold, and GlcNAc-T IV was increased more than 7-fold in D cells (Table 1).

- GlcNAc-T V assays were carried out in the absence of additional MnCl₂ to reduce the action of GlcNAc transferase III, using [2,24] as the substrate. The assays indicated an almost 3-fold increase of GlcNAc-T V in D cells (Table 1). However, low levels of the product of GlcNAc-T III action, [bis2,24], were also detected (not shown).

- The enzymes assembling the repeating units of polyglactosaminoglycans, i /i3-GlcNAc-T and 04-Gal-T, were found at a comparable level in UD and D cells (0.81 and 0.62 nmol/h/mg for the i β3-GlcNAc-T and 50.3 and 58.8 nmol/h/mg for the β4-Gal-T) (Table 1). Enzyme kinetics for the β4-Gal-T indicates similar Kₘ (6.3 mM for UD and 7.1 mM for D) and Vₘₐₓ (167 nmol/h/mg for UD and 250 nmol/h/mg for D) values.

- The core 1 β3-Gal-T that synthesizes O-glycan core 1 (Galβ3 GalNAc-), using GalNAc-Bn as the substrate, showed a re-

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Fig. 2. Core 2 β6-GlcNAc transferase assay by HPLC using Galβ3GalNAc-Bn as the substrate and D cells as the enzyme source. Assays were carried out as described in "Materials and Methods" for GlcNAc-T. Enzyme product was separated on a reverse phase C18 column using acetonitrile/water = 10/90 as the mobile phase at 1 ml/min. There is no significant breakdown of the substrate to GalNAc-Bn.

Fig. 3. Blood group I β6-GlcNAc transferase assay by HPLC using GlcNAcβ6Galβ-methyl as the substrate, and human colonic homogenate (A) and differentiated CaCo-2 cells (B) as the enzyme source. Assays were carried out as described in "Materials and Methods" for GlcNAc-T, in the absence of MnCl₂. Enzyme product was separated on an amine column using acetonitrile/water = 86/14 as the mobile phase at 1 ml/min. The radioactive standard for the enzyme product, [14C]GlcNAcβ6 [GlcNAcβ3] Galβ-methyl eluted at 64 min (assays B). Human colonic homogenate shows good activity, but CaCo-2 cells lack the blood group I β6-GlcNAc transferase.

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diction in D cells (Table 1). Enzyme kinetics revealed similar
$K_M$ for the enzyme in UD and D (0.83 and 0.74 mm respectively), but a reduction in the $V_{\text{max}}$, from 50 nmol/h/mg in UD to 29.4 nmol/h/mg in D. In contrast, the core 2 06-GlcNAc-T activity that synthesizes core 2 (GlcNAc/36 [Gal/33] GalNAc), using Gal/33 GalNAcO-Bn as the substrate, was increased in differentiated D cells (Table 1; Fig. 2). While the $K_M$ values were similar for both UD and D cells (0.36 and 0.43 mm respectively), the $V_{\text{max}}$ increased from 9.6 nmol/h/mg in UD to 21.3 nmol/h/mg in D cells.

Only very low levels of the 06-GlcNAc-T that elongates core 1 and core 2 (16, 17) were found in CaCo-2 cells (Table 1). The activity that synthesizes GlcNAcO6GalNAc-R could not be demonstrated in either normal human colon or CaCo-2 cells.

**DISCUSSION**

To test the possibility that the decreased proportion of fucosylated polylactosaminoglycans previously observed with differentiation of CaCo-2 cells (2, 3) is due to changes in the branching patterns of N- and O-glycans, we have assayed the glycosyltransferases that build and elongate the four common O-glycan cores and the branches of N-linked glycans (4, 18). Upon differentiation, we found a general increase in O- and N-glycan branching enzyme activities, while the enzymes directly involved in the synthesis of the repeating linear polylactosaminoglycan units; i.e., the activities of 04-Gal-T and blood group $\alpha$2,6-GalNAc-T, were found to be unchanged. These observations are rather unexpected since it has generally been assumed that the synthesis of polylactosaminoglycans is primarily regulated by the activities of these glycosyltransferases. Lee et al. (20) found a correlation between the blood group 06-GlcNAc-T and blood group 03-Gal-T and the increase in polylactosaminoglycan chains during granulocytic differentiation of HL 60 cells. However, HL 60 cells have also been reported to lose their polylactosaminoglycan chains upon monocoytoid differentiation (21).

The activity of GlcNAc-T III, the enzyme that introduces the bisecting GlcNAc linked to the Man residue of N-linked oligosaccharides (22), was elevated in differentiated CaCo-2 cells, along with the other branching enzymes. It has been shown that the presence of the bisected GlcNAc has a profound effect on oligosaccharide conformation (23) and blocks further branching (24), lectin binding (25), and galactosylation (26). Bisected structures have also been found in differentiated HL 60 cells associated with decreased high molecular weight oligosaccharide chains (21). It seems possible, therefore, that the increased GlcNAc-T III activity observed in differentiated cells prevents subsequent reactions, including elongation to polylactosaminoglycans. GlcNAc-T III may thus be an important regulator of polylactosaminoglycan synthesis. Other factors may also be involved. As protein determinants are possible biosynthetic controlling factors (23, 27, 28), proteins may carry their individual carbohydrate structures. It is possible that proteins undergo qualitative and quantitative changes upon differentiation, and these changes influence lactosaminoglycan synthesis. However, the decrease in polylactosaminoglycans with differentiation was clearly demonstrated for a specific glycoprotein, h-lamp-1 (3). This decrease was not due to a change in the amount of protein but to glycosylation changes. Another possibility for the decrease in fucosylated polylactosaminoglycans may be increased degradation of these chains in D, changes in the subcellular organization or intracellular transport, or changes in the activity of glycosyltransferases that add terminal carbohydrate residues. Sialyltransferases have been reported to increase upon differentiation of rat intestinal cells (29), HL 60 cells (30), and human myeloblasts (31). If polylactosaminoglycan chains became hyper-sialylated or increasingly substituted with blood group antigens, extension would likely be inhibited. However, for Chinese hamster ovary cells it has been suggested that the addition of terminal $\alpha$3-Gal and $\alpha$3-sialic acid does not compete with polylactosaminoglycan synthesis, probably because these reactions take place in different subcellular compartments (32). The subcellular distribution of terminal transferases in CaCo-2 cells is not known.

In previous work, it was concluded that the polylactosaminoglycans of CaCo-2 cells were highly branched, based upon the pattern of products obtained following endo-0-galactosidase digestion (2). It is possible that the mixture of products obtained following endo-0-galactosidase digestion resulted from incomplete degradation of linear polylactosaminoglycans due to extensive substitutions with fucose residues. No branching activity to form the blood group I antigen (10) was found in the present study, although there was considerable core 2 06-GlcNAc-T activity. The 06-GlcNAc-T synthesizing the I antigen is present in normal human colon tissue homogenate which consists of a mixture of different cell populations. The activities synthesizing core 2, I antigen, and core 4 are probably due to the same enzyme in mucin-secreting tissues (9). Apparently, this 06-GlcNAc-T in CaCo-2 cells is similar to the enzyme in human leukocytes (13) but has a different specificity than the enzyme from pig gastric or human colonic mucosa inasmuch as it lacks the activities to synthesize core 4 and I antigen.

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The 06-GlcNAc-T activity that increases O-glycan core 1 (Gal/33 GalNAco-) (18, 33, 34) in UD cells is reduced to about one-half upon differentiation in D cells. Enzyme kinetics suggests that the same enzyme is expressed in both UD and D cells but that there is less enzyme protein in D cells. Core 2 06-GlcNAc-T [synthesizing core 2, GlcNAcO6 (Gal/33) GalNAco-] (35, 36, 37) is significantly increased in differentiated CaCo-2 cells, probably due to an increase in enzyme protein. The core 2 06-GlcNAc-T has previously been found to be significantly elevated in the less mature granulocytes and blast cells from
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patients with chronic and acute myelogenous leukemia (13). Piller et al. (38) reported a dramatic increase in core 2 β6-GlcNAc-T activity upon activation of lymphocytes by anti-CD3 antibody or interleukin 2. This enzyme appears to be developmentally regulated and may be important in the differentiation process. CaCo-2 cells have only low levels of the β6-GlcNAc-T-synthesizing core 4 (GlcNAcβ1→3 GalNAc-) (6) which in various mucin-secreting tissues, including human colon, accompanies core 2 β6-GlcNAc-T activities at fairly high levels. It is possible that two β6-GlcNAc-T exist with different specificities, and at least one of them is regulated during differentiation. Alternatively, tissues may contain endogenous factors regulating enzyme specificity.

O-Glycan cores are usually made by the attachment of a 3-linked residue prior to introducing the 6-linked branch (18). Human glycoproteins have been reported to contain O-glycans with the GlcNAcβ3 GalNAc- structure (39, 40). In normal colon tissue (6), suggests that this enzyme is turned off in colon adenocarcinoma cells. However, these cells, along with the GlcNAcβ1→6 GalNAc- structure (39, 40). In normal regulating enzyme specificity.

It is possible that two β6-GlcNAc-T exist with different levels. It is possible that two β6-GlcNAc-T exist with different specificities, and at least one of them is regulated during differentiation. Alternatively, tissues may contain endogenous factors regulating enzyme specificity.

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