Release of Glycosyltransferase and Glycosidase Activities from Normal and Transformed Cell Lines

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ABSTRACT

The release of galactosyltransferase, sialyltransferase, and several glycosidase activities into the growth media from several normal and transformed cell lines was examined. Six of the seven cell lines released galactosyltransferase into their culture media. Only the human leukemia CCRF-CEM cells failed to release demonstrable galactosyltransferase activity. Release of galactosyltransferase activity into the media closely paralleled the growth curves for all but the BHKpy cells. These cells continued to release peak levels of galactosyltransferase activity into the culture media after their growth had plateaued. Media galactosyltransferase activity was unaffected by Triton X-100 treatment and remained in the supernatant fraction of a 100,000 x g, 12-hr centrifugation, suggesting that the cells release galactosyltransferase in a soluble form. In contrast to galactosyltransferase activity, only one of the cell lines (L1210) released sialyltransferase activity in appreciable amounts. Even this level of activity was 20-fold less than that observed for galactosyltransferase in the media from L1210 cells. Of the nine glycosidase activities assayed, only N-acetylgalactosaminidase was observed in significant amounts in the media from all but the CCRF-CEM cells. However, N-acetylgalactosaminidase release did not correlate closely with cell growth. These findings suggest a relatively specific release of galactosyltransferase and N-acetylgalactosaminidase activities by cells in tissue culture. Moreover, the release of galactosyltransferase closely parallels cell growth. The significance of these released enzymes, especially to cell growth, has yet to be determined.

INTRODUCTION

Glycosyltransferases are enzymes that catalyze the transfer of monosaccharides from nucleotide sugars to oligosaccharide chains of glycoproteins or glycolipids. They appear to be located in the smooth endoplasmic reticulum (7, 31) and the Golgi apparatus (16, 32) where they participate in the biosynthesis of various glycoconjugates. A number of reports (17, 19, 20, 27, 33) have demonstrated recently the presence of glycosyltransferases on the plasma membrane of several different cell types, and it is thought that these cell surface enzymes may mediate cellular recognition and adhesion (13, 28). Elevated levels of glycosyltransferases have also been observed in a number of animal and human tumors as compared to normal tissue or cells (2, 5), as well as in the bloodstream of both animals and humans bearing metastatic tumors (2, 6, 15).

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trypan blue exclusion. Media were collected and centrifuged 2 times at 1500 x g prior to measurement of enzyme activities. All cell lines were routinely screened for Mycoplasma contamination.

Glycosyltransferase Assays. Sialyltransferase assays were performed according to procedures described previously by Klohs et al. (18). A typical assay medium (total volume, 100 µl) contained 50 µl of cell culture medium, 20 µl of desialylated fetuin (15 mg/ml), 10 µl of 0.1 mM MgCl₂, 10 µl of 1.0 M cacodylate buffer (pH 7.2), and 10 µl of 82.2 µM CMP-N-acetyl-[¹⁴C]neuraminic acid (specific radioactivity, 304 mCi/mmol; Amersham Corp., Arlington Heights, Ill.). The assay procedure for galactosyltransferase activities was similar to that described by Podolsky et al. (25). A stock solution of 714 µM UDP-galactose was prepared to a final specific activity of 6.1 Ci/mmol by using unlabeled UDP-galactose (Sigma Chemical Co., St. Louis, Mo.) and UDP-[¹⁴C]galactose (specific radioactivity, 12.3 Ci/mmol; New England Nuclear, Boston, Mass.). A typical assay medium (total volume, 100 µl) contained 50 µl of culture medium, 20 µl of fetuin minus sialic acid and galactose (15 mg/ml), 10 µl of 0.1 M MnCl₂, 10 µl of 1.0 M cacodylate buffer, pH 7.2, and 10 µl of stock UDP-galactose. Incubations for both glycosyltransferase activities were carried out at 37° in a shaker bath for 1 hr. The reaction was terminated by the addition of 2.0 ml of 1% phosphotungstic acid in 0.5 N HCl. Samples were washed twice in 10% trichloroacetic acid and once in 95% ethanol:ether (2:1, v/v) and counted in a Packard Tri-Carb liquid scintillation counter. Enzyme activities are calculated as the difference between the exogenous and endogenous activities.

Pyrophosphatase and Hydrolase Activities. To determine the breakdown of CMP-sialic acid and UDP-galactose during transferase assays, incubation mixtures were deproteinized by the addition of 1.0 ml of 95% ethanol. The precipitate was removed by centrifugation, and the supernatant material was chromatographed on S & S orange ribbon paper (Schleicher & Schuell, Keene, N. H.) using a solvent system composed of 95% ethanol:1 M ammonium acetate (7:3, v/v). Radioactivity was located on the chromatogram using a Packard Model 7201 radiochromatogram scanner. Areas on the chromatogram showing radioactivity were removed and counted as described earlier.

Glycosidase Assays. Enzyme incubation mixtures consisted of 0.05 M citrate buffer (pH 4.5), 3.3 mM p-nitrophenylglycoside, and 200 µl of culture medium in a total volume of 1.0 ml. The reactions were performed at 37° for 90 min and terminated by the addition of 0.5 ml of 50 mM glycine, pH 10.5. The amount of product formed was measured in a Beckman Model 25 spectrophotometer at a wavelength of 400 nm.

Determination of Cellular Breakdown. As a measure of cellular disruption, culture media were assayed for succinic-INT-reductase and lactic dehydrogenase activities following procedures described by Morré (22) for the reductase and Wroblewski and LaDue (38) for lactic dehydrogenase.

All experiments were performed at least twice, and individual assays were performed in triplicate.

RESULTS
The release of galactosyltransferase and sialyltransferase into the media from BHK, BHKpy, MDA-MB-231, HTC, W138, L1210, and CCRF-CEM cells was determined over a 4-day growth period (Tables 1 and 2). All cell lines released significant galactosyltransferase into their media over 4 days of growth except for the human leukemia cell line CCRF-CEM (Table 1). Galactosyltransferase activity was barely detectable in the media from these leukemic cells which contrasts with the mouse leukemic cell line, L1210, where substantial galactosyltransferase activity was observed. With the exception of BHKpy cells (and CCRF-CEM cells), release of galactosyltransferase from the various cells closely paralleled the growth curve for these cell types (Chart 1). BHK cells released the greatest amount of galactosyltransferase of all the cells tested (Table 1). This was true not only in regard to the total galactosyltransferase activity detected in the culture media, but when expressed on a per cell basis, each BHK cell was shown to release more galactosyltransferase activity than any of the other cell types studied (Table 3). The human breast tumor cells, MDA-MB-231, as well as BHKpy cells, also released substantial galactosyltransferase activity into the culture media (Tables 1 and 3). The L1210, W138, and HTC cells, however, discharged one-fourth to one-fifth the galactosyltransferase activity as that observed for the BHK or MDA-MB-231 cells (Table 3). The release of galactosyltransferase activity for all the cells studied was relatively constant between 48 and 96 hr with the exception of the BHKpy cells (Table 3). The growth of these cells plateaued between 72 and 96 hr, yet the level of media galactosyltransferase from these cells continued to increase. This observation is reflected in the data from Table 3.
tosyltransferase assay media had no effect on either enzyme from several cell types was added to their growth media. These same cells. No inhibition of sialyltransferase activity was observed in the culture medium. Samples of media were taken at 5, 24, 48, 72, and 96 hr. Of the 9 glycosidase activities studied (α-D-galactosidase, β-D-galactosidase, α-D-glucosidase, β-D-glucosidase, α-D-mannosidase, α-L-fucosidase, β-L-fucosidase, N-acetylglucosaminidase, and N-acetylgalactosaminidase), only mannosidase, N-acetylgalactosaminidase, and N-acetylgalactosaminidase were detected in the media during the 4-day growth of these cells. Elevations in the former 2 enzyme activities over 96 hr, however, appeared insignificant. However, a definite increase in N-acetylglucosaminidase activity was observed in the culture media during the 96-hr growth of 6 of the 7 cell lines (Table 4). Only in the growth media from the human leukemic CCRF-CEM cells were we unable to detect N-acetylgalactosaminidase activity (as well as any other glycosidase activity determined).

**DISCUSSION**

The mechanism by which glycosyltransferases, especially those elevated activities observed in the sera from patients with certain cancers (10–12, 14), samples from the media of both normal and tumor cell types were assayed for glycosidase activities over the 96-hr cell growth period. Of the 9 glycosidase activities studied (α-D-galactosidase, β-D-galactosidase, α-D-glucosidase, β-D-glucosidase, α-D-mannosidase, α-L-fucosidase, β-L-fucosidase, N-acetylglucosaminidase, and N-acetylgalactosaminidase), only mannosidase, N-acetylgalactosaminidase, and N-acetylgalactosaminidase were detected in the medium during the 4-day growth of these cells. Elevations in the former 2 enzyme activities over 96 hr, however, appeared insignificant. However, a definite increase in N-acetylglucosaminidase activity was observed in the culture media during the 96-hr growth of 6 of the 7 cell lines (Table 4). Only in the growth media from the human leukemic CCRF-CEM cells were we unable to detect N-acetylgalactosaminidase activity (as well as any other glycosidase activity determined).

**Table 3**

<table>
<thead>
<tr>
<th>Specific activity (pmol galactose × 10⁻⁷/cell/hr)</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
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<tbody>
<tr>
<td>BHK</td>
<td>11.9</td>
<td>9.72</td>
<td>12.5</td>
</tr>
<tr>
<td>BHKpy</td>
<td>3.0</td>
<td>4.78</td>
<td>9.6</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>10.0</td>
<td>8.77</td>
<td>8.6</td>
</tr>
<tr>
<td>HTC</td>
<td>1.2</td>
<td>1.4</td>
<td>1.14</td>
</tr>
<tr>
<td>W138</td>
<td>2.1</td>
<td>3.25</td>
<td>2.8</td>
</tr>
<tr>
<td>L1210</td>
<td>1.5</td>
<td>2.3</td>
<td>2.6</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>0.17</td>
<td>0.25</td>
<td>0.25</td>
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**Table 4**

<table>
<thead>
<tr>
<th>Specific activity (nmol/mI/hr)</th>
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<tr>
<td>Cell type</td>
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<tr>
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</tr>
<tr>
<td>BHK</td>
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<tr>
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<td>L1210</td>
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The release of galactosyltransferase (β-→α) and sialyltransferase (α-→β) activities into the medium during growth of BHK cells. Both enzyme activities were determined as described in Tables 1 and 2. Cell growth and the release of galactosyltransferase as exemplified with BHK cells were also observed with MDA-MB-231, HTC, L1210, and W138 cells.

**Chart 1.** The release of galactosyltransferase (β-→α) and sialyltransferase (α-→β) activities into the culture medium over the 96-hr cell growth period. Of the 9 glycosidase activities studied (α-D-galactosidase, β-D-galactosidase, α-D-glucosidase, β-D-glucosidase, α-D-mannosidase, α-L-fucosidase, β-L-fucosidase, N-acetylglucosaminidase, and N-acetylgalactosaminidase), only mannosidase, N-acetylgalactosaminidase, and N-acetylgalactosaminidase were detected in the medium during the 4-day growth of these cells. Elevations in the former 2 enzyme activities over 96 hr, however, appeared insignificant. However, a definite increase in N-acetylglucosaminidase activity was observed in the culture media during the 96-hr growth of 6 of the 7 cell lines (Table 4). Only in the growth media from the human leukemic CCRF-CEM cells were we unable to detect N-acetylgalactosaminidase activity (as well as any other glycosidase activity determined).

**Table 4**

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<tr>
<td>Cell type</td>
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greater glycosyltransferase activities were found in the media from cultured human fetal colonic cell lines than from several colonic tumor cell types. In addition, while LaMont et al. (19) found greater galactosyltransferase activity in the media from the transformed cells, NILpy, than in the media from normal NIL cells, we observed higher glycosyltransferase activity in the media from normal BHK cells than in the media from the transformed BHKPy cells.

In view of our present understanding of glycoconjugate biosynthesis, it is intriguing that only one (L1210) (Chart 6) of the 7 cell lines studied released significant amounts of sialyltransferase into the culture media. If the addition of galactose to the growing end of an oligosaccharide chain is followed by attachment of sialic acid to the galactose, one might expect the glycosyltransferases responsible for these reactions to be in close proximity to one another and represent part of a complex of enzymes collectively referred to as a multiglycosyltransferase system (28). If such a complex of glycosyltransferases does occur within the cell, the selective release of galactosyltransferase into the growth media by 5 of 7 cell lines suggests that a different pathway for galactosyltransferase release may exist. Perhaps, in certain cells, the galactosyltransferase may be more loosely bound to the membranes of the cells than is sialyltransferase, rendering it easier for dissociation at the cell surface. This might explain the insoluble nature of the galactosyltransferase found in the culture media. Alternatively, the fate of these enzymes may be different due to a selective reabsorption or recycling of sialyltransferase by these cells.

A distinctive feature of the human leukemia CCRF-CEM cells was their inability to release into their growth media significant sialyltransferase, galactosyltransferase, or N-acetylglucosaminidase activities. These were the only cells in our studies unable to shed or secrete the latter 2 enzymes. The significance of this observation, however, remains unclear. The murine leukemic cell line, L1210, not only released galactosyltransferase and N-acetylglucosaminidase activity, but this was the only cell line to release appreciable levels of sialyltransferase activity into the culture media, although this activity was one-twentieth the galactosyltransferase activity we found in the same media. Other cells have been reported recently (35) to release sialyltransferase activity into their culture media, and like L1210 cells, the sialyltransferase activity was considerably less than that activity observed for galactosyltransferase in the same culture media (35). In view of recent studies (2, 6, 15) reporting that the elevations of serum sialyltransferase activity observed in animals and patients with various cancers may originate from the tumor tissue, we found it surprising that the L1210 cells were the only transformed cell line in our study to release sialyltransferase activity. We have observed, for instance, no significant sialyltransferase activity in the media of the human breast tumor-derived MDA-MB-231 cells (Table 2) or a human breast tumor-derived cell line, MCF-7 (data not shown), yet increases in sialyltransferase activity have been demonstrated in the sera from animals and patients with malignant mammary tumors (2, 15). Conceivably, as has been suggested previously (14, 15, 17), these observed increases in serum sialyltransferase activity from cancer patients may be due to liver involvement in the diseased state. In contrast to our findings for HTC cells, a recent report by Liu et al. (21) observed that 2 human hepatoma cell lines released significant levels of sialyltransferase activity into the culture media, and the amount of sialyltransferase activity in the media from normal (Chang) liver cells more closely resembled that which we observed in all our cell lines except L1210 cells.

In both the normal and transformed cells, we failed to detect galactosidase, glucosidase, and fucosidase, and relatively minimal mannosidase and N-acetylgalactosaminidase activities were found in their culture media. In accord with previous findings for the mouse leukemic L5178Y cell line (4), we observed appreciable N-acetylgalactosaminidase activity in the growth media for all of the cell lines except CCRF-CEM cells, although there appears to be no correlation between transformation and the amount of enzyme in the media. Recent studies (36, 37) have suggested that, under in vitro conditions, extracellular glycosidase activity from certain cells may be (at least in part) of nonsylososomal origin. Our findings with N-acetylgalactosaminidase lend support to this notion. The high levels of N-acetylgalactosaminidase activity (relative to other glycosidase activities) found in the culture media suggest that this lysosomal enzyme may be secreted into the media (at least in part) by a pathway such as that described for more traditionally associated secretory enzymes (9).

In conclusion, we have demonstrated a selective release of galactosyltransferase by several different normal and transformed tissue culture cell lines. We have also demonstrated significantly greater N-acetylgalactosaminidase activity in these culture media than other glycosidase activities tested. The release of these enzymes in this limited study does not appear to be correlated with neoplastic transformation. As yet, it is unclear how these enzymes are released from these cells. Studies are presently in progress to determine if these enzymes are secreted by these cells or are shed from the cell surface. Finally, in view of numerous reports demonstrating elevated sialyltransferase activities in patients with cancer, the failure to detect sialyltransferase activity in the culture media of 6 of the 7 cell lines is important. Perhaps in vitro conditions inhibit sialyltransferase activity release by these cells. We believe that these results do emphasize the need to more critically examine the nature of serum sialyltransferase activity in an effort to identify the source of these elevations in activity observed in many cancer patients.

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2614  CANCER RESEARCH VOL. 41
Enzyme Activity in Cultured-Cell Medium


