Increase in Serum and Tissue Glycosyltransferases and Glycosidases in Tumor-bearing Rats

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SUMMARY

In this report, both galactosyltransferase and sialyltransferase were examined in the serum, liver, and tumor of rats with either 7,12-dimethylbenz(a)anthracene-induced mammary tumors that grew slowly or the transplantable Walker carcinomas 256 that grew rapidly. In rats bearing the 7,12-dimethylbenz(a)anthracene-induced mammary tumors, a twofold elevation in serum sialyltransferase activity was seen only when the tumor weight was in excess of 15 g. This was accompanied by an increase of about 60% in the liver sialyltransferase. No change in the levels of serum and liver galactosyltransferase was detected in these animals. In contrast, the increase in both serum and liver sialyltransferase and galactosyltransferase in rats bearing the transplantable Walker carcinomas paralleled the weight of the growing tumor mass. Moreover, the magnitude of increase of both enzymes in this group was significantly higher than that in rats carrying a similar load of 7,12-dimethylbenz(a)anthracene-induced mammary tumors. Examination of the substrate specificity of sialyltransferase in different tissues from tumor-bearing rats suggested that the serum enzyme resembled more closely the host liver enzyme. In addition, β-galactosidase and sialidase activities were found to be elevated only in the serum of rats carrying the Walker carcinomas, although there was no relationship between the increment of enzyme levels and the tumor mass in these animals. Thus, it seems that tumor growth rate could be 1 of the factors in determining the levels of serum glycosyltransferase and glycosidase activities.

INTRODUCTION

Neoplastic transformation of cells has been known to be accompanied by changes in the composition of plasma membrane glycoproteins. One example of such an alteration is an elevation in the level of sialic acid on the cell surface (26, 28). The importance of cell-surface glycoproteins is underscored by the increasing recognition of their role in modulating many membrane-mediated functions, such as cell-cell adhesion and agglutination, antibody-antigen interaction, hormone binding, etc. (7, 8). It is conceivable that changes in the glycocalyx could affect the behavior and therefore the invasive nature of the cancer cells. Another phenomenon that is related to the appearance of neoplasia is the detection of certain newly acquired serum glycoproteins that are associated with some human cancers. These include carcinoembryonic antigen of the digestive tract (10), α-fetoprotein for hepatic carcinoma (17), and specific antigens in human ovarian (2) and breast cancer (11). The use of these glycoprotein antigens as a diagnostic marker for cancer and disease recurrence has been widely investigated.

Glycoprotein synthesis is initiated by the sequential addition of individual monosaccharides to the parent protein molecule, catalyzed by a group of enzymes collectively known as glycosyltransferases. These enzymes are primarily located in the Golgi apparatus (22); however, recent ultrastructural evidence shows that they are also present on the cell surface (20). Presumably those in the Golgi are involved in the synthesis of glycoproteins or glycolipids that may either be secretory or subsequently translocated to the cell surface as structural or functional components of the plasma membrane. On the other hand, the respective formation of specific "ectoglycosyltransferases" and the appropriate glycoconjugate acceptors on the cell membrane have been implicated in the mechanism of cell adhesion and recognition (21).

In this report, we have examined 2 glycosyltransferases (sialyltransferase and galactosyltransferase) and 2 glycosidases (sialidase and β-galactosidase) in the serum, liver, and tumor of rats bearing either the DMBA3-induced mammary tumors or the Walker 256 carcinoma. The objective of this study is to determine whether measurement of enzymes in different tissues that are involved in glycoprotein metabolism may serve as an indicator of tumor proliferation.

MATERIALS AND METHODS

Materials. UDP-[U-14C]galactose (specific activity, 274 mCi/mmmole) and CMP-[4,5,6,7,8,9-14C]sialic acid (specific activity, 197 mCi/mmmole) were purchased from New England Nuclear, Boston, Mass. Fetuin was purchased from Grand Island Biological Co., Grand Island, N. Y. α1-Glycoprotein from human serum was a generous gift of the American National Red Cross, Bethesda, Md. Ovine submaxillary mucin, lactose, N-acetylgalactosaminylcysteine, and p-nitrophenoxygalactopyranoside were obtained from Sigma Chemical Co., St. Louis, Mo.

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3 The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.
Sialic acid-free fetuin for sialyltransferase assay was prepared by mild acid hydrolysis (24). The removal of terminal sialic acid was confirmed by the thiobarbituric acid assay (27). Sialic acid and galactose-free fetuin for galactosyltransferase assay were prepared by the periodate oxidation-borohydride reduction method described by Spiro (25). N-[\textsuperscript{3}H]Acetylneuraminic acid of α,2-glycoprotein for sialidase assay was prepared according to the method described by Schauer et al. (23). The radioactive α,2-glycoprotein had a specific activity of 15 µCi/mg and was tested against Clostridium perfringens sialidase (Sigma). The amount of radioactivity released (in the form of A/-[\textsuperscript{3}H]acetylneuraminic acid analog) was proportional to the quantity of the purified bacterial enzyme added, showing that the modified α,2-[\textsuperscript{3}H]glycoprotein could be used as the appropriate substrate for the sialidase assay.

**Tumors.** Mammary tumors were induced in female Sprague-Dawley rats by a single injection i.v. of 5 mg DMBA in an emulsion when the animals were 55 to 60 days old. The lipid emulsion of DMBA was prepared by Dr. Paul Schurr of the Upjohn Company, Kalamazoo, Mich. The DMBA-induced mammary tumors grew relatively slowly, with a latent period of about 2 months before the tumors became palpable. In some rats, multiple tumors could be found. Walker 256 carcinomas were obtained by injecting a homogeneous mince of Walker tumor (suspended in Eagle’s medium) s.c. in female Sprague-Dawley rats. The Walker tumors grew rapidly as a solid mass, attaining a size of from 30 to 40 g in about 3 weeks.

**Serum Collection and Tissue Extraction.** Rats were anesthetized with ether, and blood was collected in 10-ml syringes via heart puncture without anticoagulant. RBC were removed by centrifugation, and serum samples were stored at −70° until ready for assay.

Liver and tumor samples were dropped in liquid nitrogen immediately after excision from the animals. They were then pulverized with a Thermovac pulverizer and stored at −70°. Prolonged storage of tissues at this temperature for 3 to 4 months had no effect on the activities of either sialyltransferase or galactosyltransferase. On the day of the enzyme assay, the pulverized tissue was homogenized in approximately 10 volumes of ice-cold 0.9% NaCl solution with a Potter-Elvehjem homogenizer. The crude homogenate was centrifuged at 1200 × g for 10 min to remove cell debris and nuclei. The supernatant fraction thus obtained was used for enzyme assays. Protein in serum and tissue extract was determined by the method of Lowry et al. (16).

**Enzyme Assays.** For the determination of sialyltransferase, the assay mixture contained 50 µl of serum or the same quantity of liver homogenate or tumor extract, 500 µg of desialated fetuin (sialic acid-free-fetuin), 1 n mole of CMP-\textsuperscript{[\textsuperscript{4}C]}sialic acid (444,000 dpm), 10 mM MnCl\textsubscript{2}, and 20 mM Tris buffer (pH 7.4) in a final volume of 150 µl. For the determination of galactosyltransferase, the assay mixture contained 20 µl of serum of tissue extract, 250 µg of sialic acid and galactose-free-fetuin, 3 n moles of UDP-galactose (22,000 dpm of UDP-\textsuperscript{[\textsuperscript{4}C]}galactose + unlabeled UDP-galactose), 10 mM MnCl\textsubscript{2}, and 20 mM Tris-maleate buffer (pH 6.8) in a total volume of 100 µl. In the case of liver and tumor samples, the presence of Triton X-100 in a final concentra-
RESULTS

Results in Table 1 show that there was no significant increase in serum sialyltransferase activity in rats bearing the DMBA-induced mammary tumors until the total tumor load in the animals was in the range of 10 to 20 g. The host liver sialyltransferase activity was elevated maximally about 60% in these same rats. The activity of this enzyme was measured in tumors (ranging from 0.5 to 18 g) and was found to be the same, 6.2 ± 0.4 pmoles/mg protein/hr, despite a wide variation in the size of the tumors. For comparison, the lactating mammary gland was also investigated. Although there was an abundance of enzyme activity in the lactating gland, 64.3 ± 0.6 pmoles/mg protein per hr, no increment in sialyltransferase was detected in the serum or liver of the host (Table 1). Examination of galactosyltransferase activities in these same rats bearing the DMBA-induced mammary tumors showed that there was no elevation in the enzyme activities in the serum, liver, and tumor when compared to the control animals.

Results presented in Table 2 disclose a positive correlation between the tumor weight and the increase in sialyltransferase levels in the serum and the liver in rats bearing the Walker 256 carcinomas. Thus, in rats carrying a tumor load of greater than 30 g, there was about a 9- to 10-fold increase in the serum enzyme activity and an increase of about 4-fold in the enzyme level in the host liver. It was interesting to note that as early as 5 days after tumor inoculation, at a time before the tumor became palpable, a slight but significant increase (about 55%) in both the serum and liver sialyltransferase activities was detected. There was no relationship between the specific activity of the enzyme in the tumor and the size of the tumor mass.

The galactosyltransferase activities in rats bearing the Walker tumors (Table 3) showed a similar increase in both serum and liver enzyme activities that paralleled the growth of the tumors. Although the pattern of increase in galactosyltransferase was similar to that of the sialyltransferase activities, the magnitude of the increase was not as great.

In order to rule out the possibility that the increase in serum glycosyltransferase activities in rats bearing Walker carcinomas was not due to the presence of an “activator” in the circulation of the host, we performed mixed-assay experiments involving serum samples from both control and experimental animals in the same reaction tube. The data in Table 4 demonstrate that the elevation in serum glycosyltransferase activity is a reflection of an increase in enzyme proteins, since varying mixtures of aliquots of control and experimental samples yielded results that were additive in nature. Dialysis of the serum samples for several hr before the assay also did not alter the enzyme activities.

We also investigated a possible difference in the biochemical characteristics of the sialyltransferases assayed in different tissues and tumors. Experiments with various substrates were carried out to test the property of this particular enzyme. The acceptors for sialyltransferase assay selected for this experiment included the macromolecules α1-glycoprotein, ovine submaxillary mucin, and a low-molecular-weight compound such as lactose. The data are shown in Table 5. It should be pointed out that results for both serum and liver samples from the tumor-bearing rats showed almost identical findings. This suggested that the serum and liver sialyltransferases had the same substrate specificity. Moreover, the serum and liver enzymes from normal control rats were killed 5 days postpartum.

Statistics. Results were given as mean ± S.E. Statistical comparisons were determined by Student’s t test. Differences between means were considered significant when the p value was less than 0.05.
Glycosyltransferase and Glycosidase in Tumor-bearing Rat

Rats were found to have similar substrate specificities when compared to those of tumor-bearing rats (results not shown). The sialyltransferase assayed in the DMBA-induced mammary tumors had a lower activity with desialated α₁-glycoprotein and ovine submaxillary mucin but a much higher activity with lactose when compared to the enzymes in both serum and liver. In contrast, the Walker tumor sialyltransferase seemed to be very active in the sialation of all 3 substrates examined.

Since both sialyltransferase and galactosyltransferase activities were determined on the basis of the amount of sugars transferred to the appropriate acceptor, changes in sialidase and galactosidase activities could lead to erroneous estimations. For this reason, both of these glycosidase activities were studied in the course of this investigation. It was found that both sialidase and β-galactosidase activities were undetectable at pH's of 7.4 and 6.8, the optimal pH's for the assay of sialyltransferase and galactosyltransferase, respectively. It was therefore concluded that the presence of glycosidases could not possibly interfere with the outcome of the glycosyltransferase assays. However, a different picture emerged when the measurements were carried out at pH 4.5. Results are shown in Table 6.

In rats carrying the DMBA-induced mammary tumors, no change in sialidase or β-galactosidase was detected in the serum and liver of the hosts. The glycosidase activities in the tumor, however, were higher than that found in the mammary gland. In contrast, serum from rats carrying the Walker carcinomas showed an increase in both sialidase and β-galactosidase, although the degree of elevation was not related at all to the tumor mass. Again, no change in liver glycosidases was observed in these animals.

**DISCUSSION**

This study shows that, in rats carrying the DMBA-induced mammary tumors, an increase in serum and liver sialyltransferases is observed only when the tumor mass is above 15 g, thus confirming the results reported earlier by Bosmann and Hilf (5). In contrast, both serum and liver glycosyltransferases as well as serum glycosidases show a marked elevation in rats bearing the transplantable Walker carcinoma. These results suggest that the level of the enzyme activities may be a function of the growth rate of the tumors since DMBA-induced mammary tumors grow very slowly, whereas the transplantable Walker carcinoma is a fast-growing tumor. Bosmann et al. (6) reported higher serum sialyltransferase levels in rats bearing the rapidly growing Novikoff hepatomas in comparison to the slower-growing Reuber H35 hepatomas.

Recently, Bernacki and Kim (1) examined several transplantable mammary tumors, including both metastasizing and nonmetastasizing lesions. They found that rats with metastasizing tumors showed about a 2-fold elevation in their serum sialyltransferase activity compared with the normal rats or rats with various nonmetastasizing tumors. The magnitude of increase of the serum sialyltransferase in rats with either the DMBA-induced mammary tumor or the Walker transplantable carcinoma reported in this paper was much higher, even though both tumors were nonmetastasizing. The discrepancy between these 2 studies could be due to the total tumor burden of the animals. Thus, it is possible that several factors, including tumor load, growth rate, and type of tumor, as well as the metastatic potential of the tumor, could be important in determining the serum glycosyltransferase levels.

The tissue origin of the serum glycosyltransferase remains to be clarified. Kim et al. (14) have suggested that in a normal situation, the majority may originate from blood cells, liver, or mammary gland. Recent evidence presented by Fraser and Mookerjea (9) seems to indicate that the serum galactosyltransferase may be the conversion product of a higher-molecular-weight liver enzyme. On the other hand, the abnormal levels of serum glycosyltransferases and glycosidases in the host with cancer has been attributed to "leakage" of the enzymes from either intact or dying neoplastic cells and/or as a consequence of "shedding" of the tumor plasma membrane (5, 13, 29). In the present investigation, a substrate specificity study showed that, in tumor-bearing rats, the serum sialyltransferase closely resembled the liver enzyme. Although the evidence is far from definitive, it nonetheless raises the possibility that the se-
Table 5
Substrate specificity of sialyltransferase activities of different tissues from tumor-bearing rats

<table>
<thead>
<tr>
<th>Exogenous acceptor*</th>
<th>Enzyme activities (%)</th>
<th>Rats with DMBA tumors</th>
<th>Rats with Walker tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>Liver</td>
</tr>
<tr>
<td>Sialic acid-free fetuin &amp;</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sialic acid- and galactose-free fetuin</td>
<td>2.7</td>
<td>3.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Sialic acid-free α1-glycoprotein</td>
<td>89.1</td>
<td>93.2</td>
<td>36.2</td>
</tr>
<tr>
<td>Sialic acid-free ovine Submaxillary mucin</td>
<td>14.6</td>
<td>17.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.2</td>
<td>8.7</td>
<td>42.7</td>
</tr>
</tbody>
</table>

* The preparation of these modified glycoproteins was described in "Materials and Methods."

Enzyme activities were expressed as percentages of an arbitrary reference measurement. The sialyltransferase activity determined with desialated fetuin (sialic acid-free fetuin) as the exogenous acceptor was designated as such a reference measurement and was set as 100%.

Table 6
Tissue glycosidase activities in tumor-bearing rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Tissue</th>
<th>β-Galactosidase*</th>
<th>Sialidase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Serum</td>
<td>2.6 ± 0.2</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>168 ± 12</td>
<td>48.1 ± 3.9</td>
</tr>
<tr>
<td>Postlactating</td>
<td>Serum</td>
<td>2.7 ± 0.3</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Involuting Mammary Gland</td>
<td>656 ± 59</td>
<td>124 ± 15</td>
</tr>
<tr>
<td>DMBA-treated</td>
<td>Serum</td>
<td>3.1 ± 0.4</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>149 ± 13</td>
<td>53.5 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>Mammary gland</td>
<td>125 ± 10</td>
<td>31.2 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>169-786</td>
<td>44.5-95.3</td>
</tr>
<tr>
<td></td>
<td>(317)*</td>
<td>61.5</td>
<td></td>
</tr>
<tr>
<td>With Walker carcinomas</td>
<td>Serum</td>
<td>5.5-23.8</td>
<td>2.8-8.1</td>
</tr>
<tr>
<td></td>
<td>(12.6)</td>
<td>(4.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>172 ± 14</td>
<td>55.1 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>183-352</td>
<td>38.3-56.7</td>
</tr>
<tr>
<td></td>
<td>(243)</td>
<td>(41.6)</td>
<td></td>
</tr>
</tbody>
</table>

* nmol of p-nitrophenyl galactose hydrolyzed per mg protein per hr.
* pmoles of N-[3H]acetylneuraminic acid analog released per mg protein per hr.
* Mean ± S.E.
* Range of values obtained.
* Numbers in parentheses, mean.

...
Bhattacharya et al. (3) have demonstrated an increase in serum galactosyltransferase levels in patients with ovarian cancer. Weiser et al. (29) have made the interesting observation of an isoenzyme of galactosyltransferase in the serum in 43 of 58 patients with various types of cancer. Moreover, the quantity of this isoenzyme is significantly higher in patients with widespread metastasis.

Altogether, it seems that the growth of a tumor can lead to other physiological events that are remote from the primary tumor sites, namely, the elevation of certain host liver glycosyltransferases. Moreover, the neoplastic tissues are also characterized by increased glycosidase enzymes. It would be interesting to know whether these changes could affect the immunological response of the host or the invasive nature of the tumor. The use of serum glycosyltransferase and glycosidase as markers of tumor proliferation seems a promising project for further investigation.

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