INCREASE IN SERUM SIALYLTRANSFERASE IN TUMOR-BEARING RATS: THE ORIGIN AND NATURE OF THE INCREASED ENZYME

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In rats bearing a solid form of AH-109A hepatoma, serum asialofetuin sialyltransferase activity was significantly increased. In order to identify the source of the increased serum sialyltransferase, the asialofetuin sialyltransferase activities of normal and host liver, tumor, and normal and host serum were studied by phosphocellulose column chromatography. While normal and host (day 17) livers exhibited two peaks, namely, transferases I and II, which were previously shown to be the sialylated and unsialylated species, respectively, of β-galactoside α2→6 sialyltransferase, the tumor exhibited a single peak of the enzyme, which was a sialylated species (as was transferase I) but was eluted at a position clearly distinguishable from that of either transferase I or transferase II. Under these conditions, both normal and host (day 17) sera were found to contain transferase I but not the tumor-type enzyme. The results have been interpreted as indicating that in rats with AH-109A, the tumor is not the source of the increased serum sialyltransferase. In these rats as well as in normal rats, serum sialyltransferase appears to originate mainly from the liver, whose sialyltransferase activity was also increased in rats with AH-109A.

Key words: Sialyltransferase — Serum sialyltransferase — Asialofetuin — Rat hepatoma — Tumor-bearing rats

It is well documented that sialyltransferase (EC 2.4.99.1), as assayed with asialofetuin as an acceptor (asialofetuin sialyltransferase), is increased in the circulation of cancer patients6, 8, 11, 13, 14) and tumor-bearing animals2). The increase is striking in individuals with advanced metastasis1, 3, 10, 23, 24) or large tumor burden5, 9). What remains uncertain is the tissue origin of the increased serum sialyltransferase. While a concomitant increase in serum 5'-nucleotidase, a plasma membrane marker, has led to the suggestion that the increased serum sialyltransferase may be provided by tumor cells through plasma membrane shedding4, 10), other evidence favors the liver as the main source of increased serum sialyltransferase. In tumor-bearing rats, for instance, liver sialyltransferase is increased closely in parallel with serum sialyltransferase5, 9). To solve the problem of the tissue origin, qualitative comparisons of tumor, liver and serum sialyltransferases appear to be important. However previous studies have failed to give a clear-cut answer primarily because the tumor and liver enzymes were indistinguishable9, 18, 29).

During the course of investigating liver and hepatoma glycosyltransferases in the rat,18, 19, 27) we have found that a transplantable rat hepatoma AH-109A possesses asialofetuin sialyltransferase readily distinguishable from the corresponding enzyme of the liver by chromatography on phos-
The present study uses this finding to delineate the possible source of increased serum sialyltransferase in tumor-bearing animals.

**MATERIALS AND METHODS**

All preparative procedures described below were conducted at 0-4°C. Serum, liver and tumor samples were used immediately, but phosphocellulose column chromatography was often performed with samples stored at -70°C. No difference was observed between the fresh and stored samples.

**Materials**

The sources of CMP-[4,5,6,7,8,9-14C]NeuAc, fetuin, *Arthrobacter ureafaciens* neuraminidase and phosphocellulose were as described previously.19) CMP-NeuAc and asialofetuin were prepared as described previously.19) EGTA and PMSF were purchased from Sigma (St. Louis, Mo., U.S.A.) and TPCK and TLCK from Merck (Darmstadt, F.R.G.).

**Animals and Tumor**

Male Donryu rats (150-200 g) fed ad libitum were used. AH-109A, a strain of Yoshida ascites hepatoma of the rat, was inoculated subcutaneously into rats. The tumor grew as a solid mass and became palpable 5-6 days after inoculation.

**Serum Collection**

Blood was collected by cardiac puncture of animals anesthetized with ethyl ether, and allowed to clot, then serum was obtained by centrifugation.

**Tissue Extraction**

Animals were killed by decapitation. The liver and tumor were immediately excised and homogenized in 0.25M sucrose/1mM EDTA/10mM potassium phosphate (pH 6.8) as described previously.19) The homogenate was centrifuged at 600g for 10 min, and the supernatant was further centrifuged at 105,000g for 60 min. The 105,000g pellet was saved.

**Phosphocellulose Column Chromatography**

The 105,000g pellet fraction from fresh or stored tissues was homogenized in 0.25M sucrose/1mM EDTA/10mM potassium phosphate (pH 6.8)/2% (w/v) Triton X-100 using a glass/Teflon homogenizer to solubilize sialyltransferase. The resulting homogenate was centrifuged at 105,000g for 60 min, and the supernatant was brought to 50% saturation in (NH₄)₂SO₄. After centrifugation at 15,000g for 10 min, the precipitate and floating pellicle were dissolved in 50mM potassium phosphate (pH 6.8)/1mM EDTA/0.01% Triton X-100 (buffer A) and applied to a Sephadex G-200 column (2.5 x 40 cm) previously equilibrated with buffer A. The column was eluted with buffer A, and fractions (4 ml) collected were assayed for asialofetuin sialyltransferase activity. The active fractions were pooled and applied to a phosphocellulose column (1.5 x 5 cm) previously equilibrated with buffer A. The column was washed with buffer A (100 ml) and eluted with a 0-0.3M NaCl linear gradient in 300 ml of buffer A, collecting 10 ml fractions at 20 ml/hr. The fractions were then assayed for asialofetuin sialyltransferase activity. When serum was the starting material, it was first diluted with an equal volume of 0.15M NaCl and brought to 50% saturation in (NH₄)₂SO₄. The precipitate formed was treated as above except that buffer A lacked Triton X-100.

**Treatment with Neuraminidase**

This was performed as described previously.19) In short, phosphocellulose fractions containing sialyltransferase were pooled; the pooled fractions were concentrated, adjusted to pH 5.5 and incubated with *A. ureafaciens* neuraminidase (2 units) at 37°C for 40 min; the mixture was neutralized, diluted and applied to a phosphocellulose column as described above.

**Assay**

Sialyltransferase was assayed by measuring the transfer of [14C]NeuAc from CMP-[14C]-NeuAc to asialofetuin using the procedure described previously.19) When the 105,000g pellet fraction was being assayed, it was suspended in 0.25 M sucrose/1mM EDTA/10 mM potassium phosphate (pH 6.8) and the assay mixture contained 0.1% Triton X-100. One unit of enzyme was defined as the amount which catalyzed the transfer of 1 nmol of sialic acid per hr. Protein content was estimated by the Lowry method.17)

**Determination of Km for CMP-NeuAc**

Sialyltransferase activity was assayed under the standard conditions except that the concentration of CMP-NeuAc was varied from 0.02 to 0.82mM. The data were then plotted double-reciprocally to obtain the $K_m$.

**RESULTS**

**Growth of Tumor**

AH-109A was inoculated into rats. A solid tumor then grew and on days 10 and 17, the wet weight of tumor mass was roughly 10 and 20 g, respectively. Animals died around day 24 when the tumor weight had reached almost 40 g. The tumor was apparently non-metastasizing.

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Level of Serum Sialyltransferase

Asialofetuin sialyltransferase activity was determined in the sera of rats at various times after tumor implantation; the results are shown in Fig. 1. After a slight fall on day 1, the level of sialyltransferase rose and kept rising until a few days before the death of the animal. At this point, the level was almost three times that found in age-matched controls. Sialyltransferase activity due to endogenous acceptors was insignificant in all cases, and mixing experiments using serum samples from control and tumor-bearing animals gave additive results. The rise in serum sialyltransferase activity shown here therefore reflects quantitative and/or qualitative alterations in serum sialyltransferase.

Sialyltransferase Activities of Host Liver and Tumor

Asialofetuin sialyltransferase was also assayed in liver and tumor tissues from rats with AH-109A. In the experiments shown in Table I, the 105,000g pellet fraction rather than whole homogenate was used as the enzyme source since the enzyme activity was located almost exclusively in this fraction.19) After a lag of several days, the liver sialyltransferase level rose as the tumor grew and reached a plateau around day 15, where the activity was almost twice that found in the control. In terms of the activity per mg protein, the tumor was always much below the liver value, but tumor activity almost tripled between days 10 and 24 (Table I), and the tumor mass more than tripled during the same period (see above). Thus, in rats with AH-109A, the liver and the tumor are likely candidates for the source of increased serum sialyltransferase.

Sialyltransferase of Liver as Studied by Chromatography on Phosphocellulose

In accordance with the previous observation,19) asialofetuin sialyltransferase of normal rat liver was separated into transferases I and II by chromatography on phosphocellulose (Fig. 2a). In the previous
These transferases were purified to near homogeneity and both were found to be \( \beta \)-galactoside \( \alpha 2 \rightarrow 6 \) sialyltransferase, the enzyme that transfers sialic acid to position 6 of the terminal \( \beta \)-galactoside residues of asparagine-linked oligosaccharide chains. Treatment of these transferases with neuraminidase as well as autocatalytic sialylation of transferase II further revealed that transferase I is a sialylated while transferase II is an unsialylated glycoprotein. It can be seen in Fig. 2a that after neuraminidase treatment, the elution position of transferase I shifted toward that of II.

**Sialyltransferases of Host Liver and Tumor** Rats bearing AH-109A were sacrificed on day 17, and asialofetuin sialyltransferases of liver and tumor were chromatographed on phosphocellulose. The enzyme of host liver emerged from the column in the same manner as that of normal liver except that the transferase I/II activity ratio was much greater (Fig. 2b). The elution profile of tumor enzyme, on the other hand, was distinct from the liver profile in that the majority of the activity emerged as a single peak at a position between those of transferases I and II (Fig. 2c). The slower elution of the tumor enzyme as compared to liver transferase I, however, was not due simply to less complete sialylation, since neuraminidase treatment of the tumor enzyme yielded a desialylated product whose elution position was still behind that of desialylated transferase I (Fig. 2c). Inclusion of a mixture of protease inhibitors (2mM EGTA, 0.1mM PMSF, 0.1mM TPCK and 0.1mM TLCK) in the extraction medium did not alter the elution profile of the tumor enzyme, indicating that the tumor pattern shown here was not a consequence of *in vitro* proteolytic attack.

**Serum Sialyltransferase of Normal Rats** Asialofetuin sialyltransferase of serum from normal rats exhibited a single peak of activity which co-migrated with liver transferase I on phosphocellulose column chromatography (Fig. 2d). After neuraminidase treatment, this peak shifted toward the position of transferase II, where no enzyme activity could be detected in the original

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**Fig. 2. Chromatography of asialofetuin sialyltransferases on phosphocellulose**

Samples for chromatography were prepared from approximately 20 g of normal liver, 10 g of host liver, 40 g of tumor and 50 ml each of normal and host serum. After chromatography, column fractions were assayed for asialofetuin sialyltransferase (a). a, normal liver; b, host liver; c, tumor; d, normal serum; and e, host serum. Liver transferase I, tumor transferase and transferases from normal and host serum thus isolated were individually treated with *A. ureafaciens* neuraminidase, rechromatographed and assayed for asialofetuin sialyltransferase (O).
serum (Fig. 2d). Since unsialylated glycoproteins are rapidly cleared from the blood by the liver,\(^7\) one would not expect any appreciable amount of transferase II in normal serum. All these results are consistent with the view that in normal rats, the liver is the main source of serum sialyltransferase.\(^1,16,20,29\)

**Serum Sialyltransferase of Rats with AH-109A** Serum collected from rats with AH-109A on day 17 had almost twice as much asialofetuin sialyltransferase as control serum (Fig. 1). When chromatographed on phosphocellulose, however, the serum exhibited only a single peak of activity co-migrating with transferase I, and did not show detectable tumor-type enzyme (Fig. 2e). That the peak was in fact transferase I was verified by its total shift toward transferase II upon neuraminidase treatment (Fig. 2e). The same results were obtained when rats were examined at the end stage of tumor growth (day 23) (data not shown). Even in animals with large tumor burden, therefore, the tumor does not seem to release sialyltransferase into the blood.

\(K_m\) for CMP-NeuAc The origin of serum asialofetuin sialyltransferase was also studied by comparing the \(K_m\) values for CMP-NeuAc of sialyltransferases isolated from various sources by chromatography on phosphocellulose. Liver transferase I, normal serum enzyme and serum enzyme from rats with AH-109A (day 17) gave values of 0.055, 0.054 and 0.055 mM, respectively, thereby lending support to the view that serum sialyltransferase is identical to liver transferase I in both normal and tumor-bearing animals. The \(K_m\) for CMP-NeuAc of the tumor enzyme, on the other hand, was found to be 0.068 mM.

**DISCUSSION**

Asialofetuin sialyltransferase of AH-109A hepatoma can be distinguished from liver sialyltransferases by chromatography on phosphocellulose (Fig. 2). Though its molecular basis remains unknown, this difference has been utilized to delineate the source of serum sialyltransferase in rats bearing AH-109A. Since the serum did not contain detectable tumor-type sialyltransferase, it has been concluded that in these animals, the tumor is not the source of increased serum sialyltransferase. Primary rat hepatomas previously studied in this laboratory, on the other hand, cannot serve as such a model since, unlike AH-109A, their asialofetuin sialyltransferase is identical to liver transferase I.\(^19\) The present study has also demonstrated that in both normal rats and rats with AH-109A, serum sialyltransferase is the same enzyme as liver transferase I: the serum and liver enzymes were identical in elution behavior from phosphocellulose, effect of neuraminidase treatment and \(K_m\) for CMP-NeuAc.

Although the molecular basis for the chromatographic difference of AH-109A transferase from liver transferase I has not as yet been elucidated, the difference is not due simply to differing sialic acid content, because a chromatographic difference still persisted even after desialylation by neuraminidase. The finding that AH-109A lacks the unsialylated species of sialyltransferase (Fig. 2c) is of interest, since the same phenomenon was observed for primary hepatomas\(^19\) and this has been related to hypersialylation,\(^27\) known to be a characteristic feature of cancer cell membrane.\(^28\) From the desialylation experiments, it is possible to consider that the two enzymes may differ in protein moiety. However, the tumor enzyme appears to catalyze the same reaction as transferase I, since both enzymes synthesized \(\alpha2\rightarrow6\) sialyllactose at equally high rates (data not shown). The question of why the two types of hepatoma possess different sialyltransferase is also unresolved. Here, it is of interest that AH-109A possesses a small fraction of activity eluting from phosphocellulose close to transferase I (Fig. 2c).
Although the contribution of other sources such as blood cells may not be excluded, the liver has been considered to be the main source of serum glycosyltransferases. The present study has demonstrated that in the serum of rats with AH-109A, sialyltransferase identical to liver transferase I is increased almost in parallel with sialyltransferase in the liver, thereby suggesting strongly that increased serum sialyltransferase originated largely, if not entirely, from the liver. We may speculate that the increase in sialyltransferase in the liver is an immediate cause of the increase in serum sialyltransferase. In host liver, the conversion of transferase II to transferase I appears to be accelerated (see Fig. 2b). This could be an additional factor causing the increased serum sialyltransferase, since the serum contains only transferase I.

These speculations, however, raise another important question of how a tumor growing at a distant locus can elevate the level of sialyltransferase in the liver. Tanaka and co-workers have demonstrated that in tumor-bearing rats, increase in pyruvate kinase (type M2) and ornithine decarboxylase in the liver is due to protein factors released from the tumor. A similar mechanism may function to enhance the formation of β-galactoside 2→6 sialyltransferase in the liver, but the final conclusion must await the results of future investigations.

After investigating only one type of rat tumor, we cannot claim that increased serum sialyltransferase in all malignant tumors is exclusively from the liver. A form of asialofetuin sialyltransferase not present in control serum has been reported in the serum of patients with breast cancer. The possibility still remains that certain tumors may discharge their own sialyltransferase into the blood.


