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HIGH EXPRESSION OF AN N-ACETYLGLUCOSAMINYLTRANSFERASE III IN 3'-Methyl DAB-INDUCED HEPATOMA AND ASCITES HEPATOMA+

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Summary: Ascites hepatoma AH-66 and 3'-Me-DAB-induced hepatoma of rats contain highly active N-acetylglucosaminyltransferase III (GnT-III) which catalyzes the addition of N-acetylglucosamine through a ß1-4 linkage (bisecting N-acetylglucosamine) to the ß-linked mannose of the trimannosyl core of asparagine-linked sugar chains, whereas normal rat liver contains very little. The high activity was also detected in fetal rat liver, newborn rat liver, hyperplastic nodules and various transplantable hepatomas.

Previous studies by our group indicated that γ-glutamyl transpeptidase purified from AH-66 ascites hepatoma cells has a ß1-4 linked N-acetylglucosamine residue, whereas the enzyme from normal rat liver does not (1-2). These data suggest that hepatoma tissues contain a highly active N-acetylglucosaminyl-transferase III (GnT-III), which catalyzes the addition of N-acetylglucosamine through a ß1-4 linkage to the ß-linked mannose of the trimannosyl core of asparagine-linked sugar chains (3). The GnT-III catalyzes the following reaction.

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Abbreviations used are: GnT, N-acetylglucosaminyltransferase; HPLC, high performance liquid chromatography; PA-, pyridylamino-; MES, 2-(N-morpholino)-ethanesulfonic acid; UDP-GlcNAc, uridine 5'-diphospho-N-acetylglucosamine; 3'-Me-DAB, 3'-methyl-4-dimethylamino-azobenzene.

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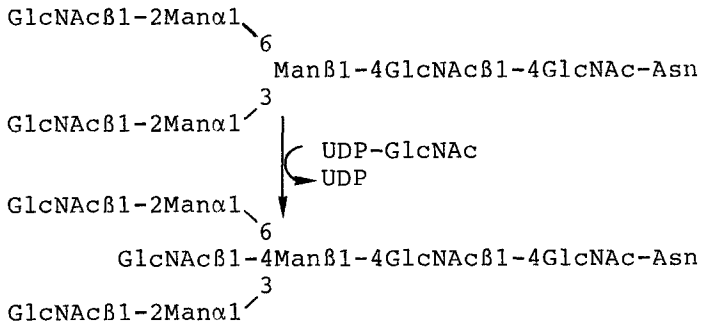

The inserted N-acetylglucosamine is called a "bisecting N-acetylglucosamine". In our previous studies, we developed a simple and convenient method for the assay of GnT-III involving the fluorescent pyridylamino derivatives of sugar chains as acceptor substrates, the product being detected on an HPLC column (4). This method made it possible to assay the GnT-III activities of various crude tissues. In the present paper we report that the GnT-III is dramatically induced in ascites hepatoma cells and 3'-Me-DAB induced hepatomas as well as in fetal or newborn rat liver.

MATERIALS AND METHODS

Reagents

Pyridylaminated N-acetylglucosamine was kindly supplied from Dr. S. Hase, Osaka University Faculty of Science, Japan. Human transferrin was kindly donated by Green Cross Co. Ltd. Japan.

Animals and hepatoma tissues

Male and pregnant female rats of the Sprague-Dawley strain, weighing 150-250 g, were used in this experiment. Fetal, newborn and adult rat livers, and adult rat kidneys were obtained from the above rats. Regenerating livers were obtained at 24 h and 72 h after partial hepatectomy (removal of approximately 70 % of the liver mass) according to the method of Higgins and Anderson (5). Hepatomas were induced by feeding 0.03 % 3'Me-DAB as described previously (6). Transplantable AH-66 and AH-130 ascites hepatoma cells and Yoshida sarcomas were supplied by the Institute of Tuberculosis and Cancer, Tohoku University, and were used 7 days after inoculation. Hyperplastic nodules were obtained by the method of Farber and Solt (7).

Crude tissue extracts

The various rat livers and hepatoma tissues were homogenized in 4 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose with an Ultra-Turrax homogenizer (Ika-Werk, West Germany). After centrifugation at 900 x g

for 10 min, the supernatants were collected and used as the crude enzyme preparations.

Preparation of pyridylaminated sugar chains as substrates

GnT-III activity was assayed using a fluorescence-labeled pyridylaminated biantennary sugar chain as substrate. The sugar chains as the substrate were obtained from human serum transferrin as described (4,8). The structure of the substrate was determined by ^{1}H-NMR to be, GlcNAcß1-2Manα1-6(GlcNAcß1-2Manα1-3)Manß1-4GlcNAcß1-4GlcNAc-PA, where PA denotes the pyridylaminated sugar chain, as described(4).

Assaying of GnT-III activity

The standard incubation mixture contained 770 µM substrate, 0.125 M MES, pH 6.25, 10 mM $MnCl_2$, 200 mM N-acetylglucosamine, 0.5% (v/v) Triton X-100, 20 mM UDP-GlcNAc and enzyme protein in a final volume of 50 µl. After incubation at 37°C for 1 h, the reaction was stopped by the addition of 10 µl of 2 % sodium tetraborate-0.25 M EDTA. Then the product was separated by HPLC on a TSK-GEL ODS-80TM column (4.6 X 150 mm; Toyo Soda Co.), using 0.1 M ammonium acetate buffer, pH 4.0, containing 0.3 % n-butanol as the mobile phase, and then determined with a fluorescence photometer. The product was characterized with ^{1}H-NMR as described (4). The amount of product was estimated with a fluorescence intensity using pyridylaminated N-acetylglucosamine as a standard. The specific activity of the enzyme was expressed as pmol of N-acetylglucosamine transferred/mg protein/h. Protein concentration was determined with a Bio-Rad protein assay kit (Bio-Rad, Richmond, California) using bovine albumin as the standard.

RESULTS AND DISCUSSION

GnT-III activities in fetal, newborn and adult livers of rats

The GnT-III activities of various rat livers and kidneys were assayed. As shown in Fig. 1, the enzymatic product after incubation with every tissue extract examined showed the same retention times at 10 min, indicating that all these tissues had GnT-III activities. The amount of the enzymatic product observed on HPLC was very high in rat fetal liver and newborn liver (24 h and 72 h after birth) as compared to in adult liver. In regenerating rat liver the enzyme activity was increased as compared to in normal liver.

GnT-III activities in transplantable and 3'-Me-DAB induced hepatomas

The GnT-III activities in various hepatomas were assayed. AH-66 ascites hepatoma and 3'-Me-DAB induced hepatomas

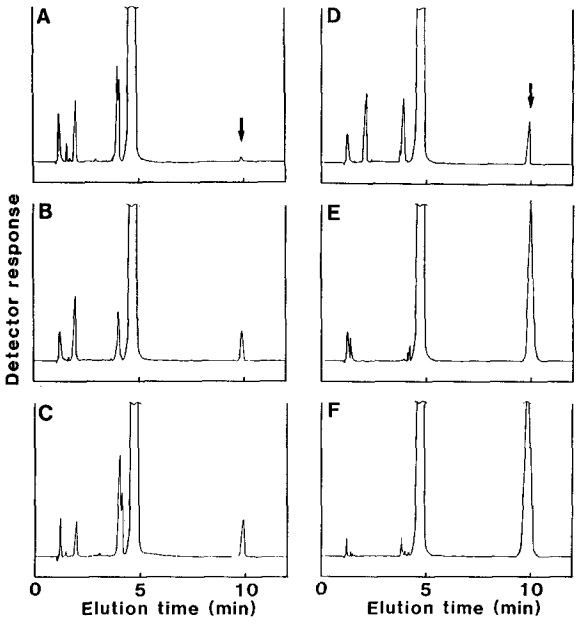

Figure 1. HPLC patterns of various rat livers and hepatomas. The enzymatic products were separated on a reverse-phase HPLC column as described under "Materials and Methods." Crude tissue extracts from various livers and hepatomas were incubated in the enzyme assay mixture at 37°C for 1 h and then an aliquot (10 µl) of each mixture was subjected to HPLC as described under "Materials and Methods." A, extract from normal rat liver; B, extract from fetal rat liver; c, extract from normal newborn rat liver; D, extract from hyperplastic nodules; E, extract from 3'-Me-DAB-induced hepatoma; F, extract from an AH-66 ascites hepatoma. The product as indicated by an arrow was eluted at 10 min was identified by comparing the elution times of a standard on HPLC which had been characterized by ^{1}H-NMR (5). A major peak eluted at 5 min was found to be unreacted substrates.

showed extremely high activities. On the other hand, the GnT-III activity in noninvolved adjacent tissues to the 3'-Me-DAB induced hepatomas were relatively low as compared to that in the hepatomas. Hyperplastic nodules showed relatively high activity as compared to normal rat liver.

Table I summarizes the data as to the GnT-III activities in various livers and hepatoma tissues.

In normal rat liver the enzyme activity is very low. This coincides with the absence of a bisecting N-acetylglucosamine residue in the γ-glutamyl transpeptidase from normal rat liver. Whether or not the bisecting N-acetylglucosamine residue exists in the γ-glutamyl transpeptidase from hyperplastic nodules, precancerous

Table I. GnT-III activities in various rat livers and hepatomas

Samples		GnT-III activities (pmol/mg protein/h)
Adult rat liver	(5)	28 ± 1.3
Newborn rat liver, 24 h	(pooled)	212
Newborn rat liver, 72 h	(pooled)	146
Fetal rat liver, 19 days	(pooled)	162
Regenerating rat liver, 72 h	(3)	105 ± 35
Hyperplastic nodule	(1)	321
Primary hepatomas induced by 3'-Me-DAB	(9)	1930 ± 540
Non-involved adjacent tissues to the hepatomas	(9)	234 ± 33
Transplantable hepatomas		
AH-66	(1)	2220
AH-130	(2)	405
Yoshida sarcoma	(1)	245

Values are means ± standard error; numbers in parenthesis indicate the numbers of specimens examined.

lesion is unclear at present. The present result suggests that a bisecting N-acetylglucosamine residue should also be present in the γ-glutamyl transpeptidase from hyperplastic nodules. The expression of GnT-III activity in precancerous tissues as well as in malignant tissues may be useful for detecting and monitoring a presumed precancerous stage as well as a malignant stage. Very recently, Narashimhan et al. (9) reported that the GnT-III is highly expressed in hepatic nodules promoted by orotic acids during the stage of experimental hepatomas in rats suggesting that the hyperplastic nodules contain the bisecting N-acetyl-glucosamine residues.

Previous studies in our laboratory indicated that polyclonal and monoclonal antibodies raised against purified

human γ-glutamyl transpeptidases were useful for the diagnosis and monitoring for the neoplastic diseases including hepatoma (10,11) and a possible presence of bisecting N-acetylglucosamine in human γ-glutamyl transpeptidase was suggested (2). This suggests that activation of GnT-III also occurs in the human hepatoma tissues.

Moreover, whether or not increased GnT-III is associated with a generalized introduction of bisecting N-acetylglucosamine in the membrane proteins of the hepatoma or is restricted to γ-glutamyl transpeptidase is an interesting problem. We are now studying along these lines.

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