UDP-GalNAc: GalNAc-mucin α-*N*-acetylgalactosamine transferase activity in human intestinal cancerous tissues

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GalNAc transferase activities of 6 human intestinal cancerous tissues were examined using bovine submaxillary gland mucin and its desialylated derivative, asialomucin, as acceptors. A Triton X-100 extract of these tissues was used as an enzyme source. All the tissues examined had GalNAc transferase that catalyzes the transfer of GalNAc from UDP-GalNAc to serine or threonine residues of the polypeptide chain. One of 6 specimens showed in addition UDP-GalNAc:GalNAc-mucin α-GalNAc transferase activity, synthesizing a disaccharide unit, GalNAcα→GalNAc, when asialomucin was used as an acceptor. This carbohydrate structure was deduced on the basis of results of gel filtration, exoglycosidase digestion, and high-voltage paper electrophoresis.

GalNAc transferase *Human intestinal cancerous tissue* *Bovine submaxillary gland mucin* *O-Glycosidically linked sugar chain*

1. INTRODUCTION

The carbohydrate moieties of mucins are often altered on malignant transformation of cells [1–3]. The alterations should be ascribed to the changes in activities of glycosyltransferases and/or glycosidases that are responsible for the biosynthesis or degradation of the sugar units of such glycoproteins [2].

In our previous reports [4,5], the structures of the major sugar units of the mucins produced by a human rectal adenocarcinoma were determined including a novel structure, Siaα2→6(GalNAcα1→3)GalNAcol. To search for the GalNAc transferase that should be involved in the biosynthesis of this sugar chain, 6 human intestinal cancerous tissues were examined with respect to their GalNAc transferase activities using bovine submaxillary gland mucin and its desialylated derivative, asialomucin, as acceptors. Here we report the occurrence of an α-GalNAc transferase in one of the tissues that transfers [1-^{14}C]GalNAc from UDP-[1-^{14}C]GalNAc to asialomucin (its major sugar unit: GalNAc→Ser(Thr)) to produce disaccharide, [1-^{14}C]GalNAcα→GalNAc(→Ser or Thr), indicating that this enzyme may be responsible for the biosynthesis of the above unique oligosaccharide.

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2. MATERIALS AND METHODS

2.1. *Materials*

UDP-[1-^{14}C]GalNAc (54 mCi/mmol) was purchased from the Radiochemical Centre, Amersham. UDP-GalNAc was a generous gift from Professor S. Suzuki of Nagoya University. *N*-Acetylgalactosaminitol was prepared in our laboratory by conventional procedures. GalNAcα1→3GalNAcol was prepared from a human rectal adenocarcinoma glycoprotein [5]. Sephadex G-50 (fine) and Sephadex G-25 (superfine) were obtained from Pharmacia, Uppsala, and AG 50WX8 (H^+ form, 200–400 mesh) was from Bio-Rad, Richmond. α-*N*-Acetylgalactosaminidase from *Charonia lampas* was purchased from

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Seikagaku Kogy, Tokyo. Human intestinal cancerous tissues were dissected free from all surrounding normal tissues and stored at −80°C before use.

2.2. *Preparation of acceptors*

The major mucin was prepared from bovine submaxillary glands as described [6]. Asialomucin was prepared from the major mucin by mild acid hydrolysis (0.05 M H_2SO_4, 80°C, 1 h).

2.3. *Preparation of enzyme source*

A portion of each tumor tissue was minced and homogenized with a Potter-Elvehjem type homogenizer in 0.1 M imidazole/HCl buffer, pH 7.2. Triton X-100 was added to the homogenate to 1%, and proteins were extracted with constant stirring for 1 h at 0°C. The solution was centrifuged at 10000 × *g* for 20 min. The supernatant obtained was used as an enzyme source.

2.4. *Assay of GalNAc transferase and characterization of the reaction products*

Incubation mixtures contained the following components in a final volume of 800 μl: enzyme proteins (~3 mg protein), 4 μmol BAL (2,3-dimercapto-1-propanol), 0.08 μmol ATP, 40 μmol imidazole/HCl buffer, pH 7.2, 8 μmol $MnCl_2$, 3 mg Triton X-100, acceptor proteins (6 mg mucin or 4 mg asialomucin), and 120 nmol UDP-[1-^{14}C]GalNAc (10000 dpm/nmol).

After incubation of the reaction mixture at 37°C for 2 h with a few drops of toluene, the reaction was terminated by adding 100 μl of 0.5 M EDTA. Insoluble materials formed during the incubation were removed by centrifugation at 105400 × *g* for 30 min. The supernatant was concentrated to a small volume by a rotary evaporator and then applied to a column of Sephadex G-50 (fine, 1.0 × 94 cm) developed with 0.2 M NaCl. Radioactive products in the void fraction that contained acceptor proteins were separated from the radioactive low-M_r materials derived from ^{14}C-labeled sugar nucleotides and their degradation products. The void fraction was dialyzed against water and lyophilized. The lyophilized materials were subjected to alkaline borohydride degradation as in [4], and the released oligosaccharides were applied to a column of Sephadex G-25 (superfine, 1.8 × 180 cm) developed with 0.05 M pyridine/acetic acid buffer, pH 5.0.

2.5. *Analytical methods*

Protein was determined by the method of Lowry et al. [7] with bovine serum albumin as the standard. High-voltage paper electrophoresis was performed on Toyo no.51A paper using 0.06 M borate buffer, pH 9.5, at 35 V/cm for 4 h [8]. Sugar standards were localized on paper with alkaline silver nitrate reagent after treatment with periodate. Radioactivity was determined by liquid scintillation counting. The digestion of oligosaccharides with α-*N*-acetylgalactosaminidase was carried out as in [5].

3. RESULTS AND DISCUSSION

We recently reported the structures of the major oligosaccharides isolated from a human rectal adenocarcinoma, including a novel trisaccharide, Siaα2→6(GalNAcα1→3)GalNAcol [5]. In addition, isolation of oligosaccharide chains containing the GalNAcα1→3GalNAcol structure has been reported from a few laboratories [9–12]. To characterize the GalNAc transferase involved in the biosynthesis of this unique structure, 6 human cancerous tissues have been examined for transferase activities. For the incubation, it was essential to use BAL and ATP as inhibitors of nucleotide pyrophosphatase which degrades sugar nucleotides [13].

The acceptors to which [1-^{14}C]GalNAc was transferred were recovered by gel filtration on Sephadex G-50, and successively applied to a column of Sephadex G-25 after reductive cleavage of *O*-glycosidically linked sugar chains. Five out of 6 cancerous tissues showed the same elution profile on Sephadex G-25 chromatography (not shown). The only discernible radioactive peaks appeared at the elution position corresponding to that of GalNAcol. These facts suggested the presence of GalNAc transferase in these tissues that catalyzed the transfer of GalNAc from UDP-GalNAc to serine or threonine residues of the polypeptide chain.

One of the tissues gave a quite different elution profile on the same gel filtration (fig.1). In addition to a peak which corresponded to GalNAcol, several peaks could be seen when mucin or

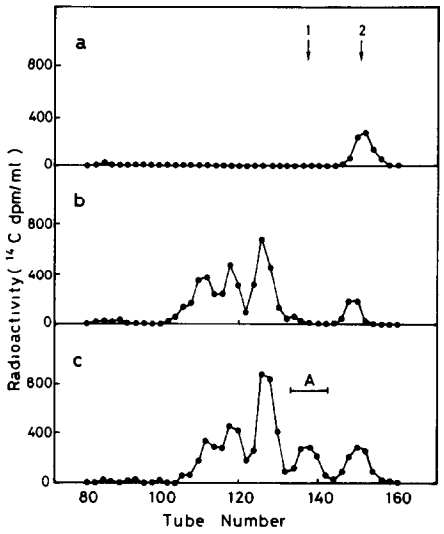

Fig.1. Sephadex G-25 column chromatography of the oligosaccharides of the reaction products of one of the 6 tissues. Arrows indicate the positions of the standard sugars: (1) GalNAcα1→3GalNAcol, (2) GalNAcol. (a) No acceptor added, (b) mucin added as an acceptor, (c) asialomucin added as an acceptor.

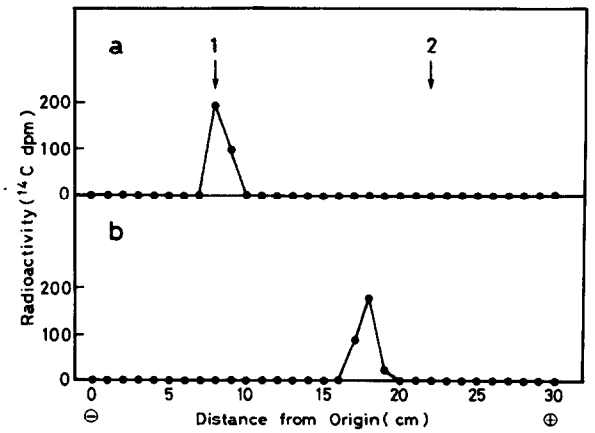

Fig.2. High-voltage paper electrophoresis of fraction A before and after α-*N*-acetylgalactosaminidase digestion. Electrophoresis was carried out as described in section 2. Migration was toward the anode (left to right). The paper was cut into 1.0 cm wide segments that were extracted with 0.6 ml distilled water and radioactivities of the extracts were determined. Arrows indicate the positions of the standard sugars: (1) GalNAc, (2) GalNAcol. (a) α-*N*-Acetylgalactosaminidase digest of fraction A, (b) intact fraction A.

asialomucin was used as an acceptor. The peaks at tube no.110–130 appeared to be produced by the transfer of GalNAc to complex carbohydrate chains of acceptor glycoproteins. The occurrence of such complex carbohydrate chains besides the major disaccharide, NeuAcα2→6GalNAc, in bovine submaxillary gland mucin has been reported by Herp et al. [14] inter alia. This also raised the possibility that multiple GalNAc transferase activities were enhanced in this tissue. Of these oligosaccharide chains, fraction A eluted at the same position as GalNAcα1→3GalNAcol, was produced only when asialomucin was used as an acceptor.

To investigate the carbohydrate structure of fraction A (its homogeneity was checked by high-voltage paper electrophoresis as shown in fig.2), its aliquot was digested with α-*N*-acetylgalactosaminidase and the digest was applied to the same Sephadex G-25 column. The elution position of the radioactivity shifted to that corresponding to that of GalNAc or GalNAcol, suggesting the release of GalNAc upon the enzymatic digestion. The release of GalNAc was further confirmed by high-voltage paper electrophoresis that is capable of separating GalNAc and GalNAcol from each other (fig.2). After digestion, the position of the radioactive peak shifted to that of GalNAc, confirming the release of α-linked GalNAc on α-*N*-acetylgalactosaminidase digestion. Thus, fraction A was identified as GalNAcα→GalNAcol. It is therefore apparent that in this tissue GalNAc transferase is present which transfers GalNAc in the α-configuration to GalNAc attached to the polypeptide chain.

This enzyme is distinct from UDP-GalNAc:polypeptide GalNAc transferase since 5 out of 6 tissues that have the latter enzyme activity cannot produce the GalNAcα→GalNAc sequence from asialomucin used as an acceptor. This is also consistent with the results of specificity studies on the purified UDP-GalNAc:polypeptide GalNAc transferase [6]. Other α-GalNAc transferases which have been investigated in detail are UDP-GalNAc:globoside α-GalNAc transferase and A blood group specific GalNAc transferase [15,16]. The former enzyme, that produces Forssman antigen, was purified from canine spleen [15]. We prepared a Triton X-100 extract of canine spleen, but the extract did not generate the disaccharide (GalNAcα→GalNAcol). Thus, this enzyme is not

responsible for the biosynthesis of GalNAcα→GalNAc→polypeptide. The activity of A blood group specific GalNAc transferase, assayed using lact-*N*-fucopentaose I as an acceptor, was detected in the tissue extract in which the present new GalNAc transferase activity was detected. However, the GalNAc transfer to lact-*N*-fucopentaose I was not inhibited by asialomucin indicating that these two transferases are different.

Although the assignment of the position of GalNAc attachment to GalNAcol was not carried out owing to the limited amounts of the material, it is possible that this disaccharide may be a precursor of the trisaccharide, Siaα2→6(GalNAcα1→3)GalNAcol.

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