

Glycoprotein Metabolism in Inflammatory and Neoplastic Diseases of the Human Colon¹

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SUMMARY

Carbohydrate compositions of the membrane and cytoplasmic fractions of human normal and cancerous colonic mucosa were compared in patients with blood groups O and B. The total sugar content in both fractions was reduced in the cancer tissues to about one-third of that in the normal colonic mucosa. The sugars that are associated with mucinous glycoproteins such as fucose and *N*-acetylgalactosamine were reduced significantly, while sugars that are primarily associated with "serum-type" glycoproteins were relatively unchanged or reduced to a lesser extent. The activities of glycoprotein:glycosyltransferases were variable, some showing no significant change, others being significantly reduced in cancerous tissues. A polypeptidyl:*N*-acetylgalactosaminyltransferase (an enzyme that catalyzes the transfer of the first sugar to hydroxyamino acids of the protein core of mucinous glycoproteins), a sialyltransferase (involved in the addition of sialic acid to mucinous glycoproteins), and a galactosyltransferase (thought to be responsible for blood group B antigenicity) were reduced in the cancerous colonic tissue. In contrast, the activities of these glycosyltransferases were unchanged in the colonic mucosa of patients with granulomatosis or ulcerative colitis. Glycosidase activities in the normal, cancerous, and inflammatory tissues were the same. These results suggest that in colonic cancer tissues the synthesis of one type of oligosaccharide chain may be greatly affected, while another family of oligosaccharides may remain relatively unaffected.

INTRODUCTION

Mucosal cells of the gastrointestinal tract are rich in glycoproteins. Cellular glycoproteins can be broadly classified into 2 types, secretory and structural glycoproteins. An example of the former is mucin; the latter are structural components of cell surface membranes and of the membranes of various subcellular organelles.

Considerable data are available that implicate membrane glycoproteins as playing important roles in cellular phenomena that undergo alterations during cancerous transformations. These changes involve contact regulation of cell growth (1), agglutinability to lectins (7), blood group

antigenicity (9), and immunologically reactive surface components such as carcinoembryonic antigens of human colonic tumors (13). These results were obtained mainly from normal and transformed cells grown in culture. However, little information on glycoprotein metabolism in tumor tissues obtained from human subjects and experimental animals is available. There is also a paucity of knowledge about glycoproteins in the cytoplasmic fraction of normal and tumor tissues, although some tissues such as gastrointestinal mucosa and submaxillary gland tissue are rich in these glycoproteins. Recently, results from our laboratory on blood group activities and lectin-binding properties of membrane glycopeptides prepared from the colonic tissues of patients with blood type A (20) indicated that considerable changes in specific carbohydrate structures may occur in the human colonic tumor tissues.

This study reports the results of further studies on carbohydrate compositions of not only the membrane but also the cytoplasmic fractions of normal and cancerous colonic mucosa of patients with blood type O or B. Since a significant reduction in the levels of some sugars was observed in cancer tissues, we also measured the levels of glycosyltransferases involved in the synthesis of the carbohydrate portion of glycoproteins and assayed for glycosidase activities that may be required for glycoprotein degradation. For determination of whether the observed changes in the activities of some of these enzymes in the colonic cancer tissues were common to many conditions, the activities of these enzymes were measured in the colonic tissues obtained from patients with ulcerative or granulomatous colitis.

MATERIALS AND METHODS

Tissues. Normal samples were obtained from normal-appearing mucosa about 6 cm from the periphery of the cancer tissues in the same patients from whom the colonic tumors were obtained. Both normal and cancerous tissues were obtained at surgery from 6 patients having blood type O and from 2 patients with type B. The tissues were also obtained from 2 ulcerative colitis patients with blood types A and B, and from a granulomatous colitis patient with blood type O. The tissues were washed immediately with 0.9% NaCl solution and stored at -60° until used.

Preparation of Membrane and Cytoplasmic Fractions. The tissues were thawed and the mucosa was scraped. The minced tumor tissues and the normal and diseased mucosal

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scrapings were homogenized in 4 volumes of cold 0.16 M NaCl:0.01 M cacodylate:acetate buffer (pH 7.0) with a Polytron homogenizer and passed through 2 layers of cheesecloth. The homogenate was centrifuged at $120,000 \times g$ for 60 min. The resultant pellet represented membrane fractions including plasma, nuclear, mitochondrial, and microsomal membranes; the supernatant was the cytoplasmic fraction.

Chemical Analysis. The membrane and cytoplasmic fractions were treated with chloroform:methanol (2:1, v/v) for 20 hr at 25° to remove glycolipids, and the glycoprotein fraction was hydrolyzed as described previously (24). Neutral sugar analysis was carried out on a Perkin-Elmer gas-liquid chromatograph, using inositol as an internal standard (19). The content of glucosamine and of galactosamine were determined by the method of Bella and Kim (3) with a Beckman Model 120-C amino acid analyzer. Sialic acid was measured according to the method of Aminoff (2).

Glycosyltransferase Assays. The following 10 glycosyltransferases were assayed in the homogenates essentially as described previously (4, 21-23); 4 sialyltransferases, 3 galactosyltransferases, 2 fucosyltransferases, and 1 polypeptidyl:*N*-acetylgalactosaminyltransferase. The following glycoprotein, protein, and oligosaccharide acceptors were used for the glycosyltransferase assays: galactosyltransferase I, OSM² minus sialic acid; galactosyltransferase II, α_1 -acid glycoprotein minus sialic acid minus galactose; fucosyltransferase I, fucosyllactose; fucosyltransferase II, lactose; and polypeptidyl:*N*-acetylgalactosaminyltransferase, the A1 basic protein from myelin. The myelin basic protein has been shown to act as an acceptor for the same glycosyltransferase that adds GalNAc (15, 16). For the assay of 4 sialyltransferases the following acceptors were used: α_1 -acid glycoprotein minus sialic acid; fetuin minus sialic acid; OSM minus sialic acid; or tumor glycopeptides minus sialic acid. Basic myelin protein was a gift from Dr. Edwin H. Eylar, and tumor glycopeptides were prepared from Novikoff ascites tumor cells according to the method of Walborg *et al.* (30). The rest of the acceptors were prepared as described previously (4, 21-23). All enzyme assays were performed under conditions in which the reaction was linear with respect to enzyme concentration and time of incubation.

Mixing Experiment. For determination of the presence of activators or inhibitors, the normal and cancerous tissue homogenates were mixed and assayed for glycosyltransferase activities. For the respective assays, 70 μ g of normal tissue homogenate protein and 100 μ g of cancer tissue homogenate protein were used. For the mixing experiments, 70 μ g of normal tissue homogenate protein were added to 100 μ g of cancer tissue homogenate protein. The conditions for the assay of the 2 galactosyltransferases, II and III, were the same as those described previously (21, 23).

Glycosidase Assays. The following 4-methylumbelliferone derivatives of 5 sugars (Koch-Light Laboratories, Ltd. Colnbrook, Buckinghamshire, England) were used for the determination of glycosidase activities: α -D-galactopyrano-

side, β -D-galactopyranoside, 2-acetamido-2-deoxy- β -D-galactopyranoside, 2-acetamido-2-deoxy- β -D-glucopyranoside, and α -D-mannoside. The assay conditions for each glycosidase were described previously (20). All the enzyme reactions were carried out under conditions in which linearity was obtained with respect to time and the amount of enzyme added.

RESULTS

Carbohydrate Composition of Membrane and Cytoplasmic Fractions. As shown in Table 1, a distinct reduction in the sugar content was observed with the membrane fraction of the cancer tissues compared to that of the adjacent normal colonic tissues. The levels of *N*-acetylneuraminic acid, fucose, GalNAc, and *N*-acetylglucosamine were significantly reduced in the cancer tissues. Mannose and galactose were not significantly different in cancerous and normal tissues. The extent of the reduction in each sugar varied, as evidenced by the molar ratios of each sugar compared to mannose. The molar ratios of *N*-acetylneuraminic acid, GalNAc, and fucose were reduced the most. A similar reduction in sugar content occurred in the cytoplasmic fraction of tumor tissues. Fucose, *N*-acetylneuraminic acid, GalNAc, *N*-acetylglucosamine, and galactose were reduced significantly. The amount of mannose was again unchanged. However, as was observed with the membrane fractions, all of the sugars were not reduced to the same extent in tumor tissues.

Glycosyltransferase Activities. Table 2 shows the levels of 7 glycosyltransferase activities in normal, cancerous, and diseased mucosa. A varying degree of alteration in the level of each glycosyltransferase in the tumor tissue was observed. There was a statistically significant reduction in the activities of the galactosyltransferase II, 2 fucosyltransferases, and the polypeptidyl:*N*-acetylgalactosaminyltransferase in the tumor tissues. Although the level of a galactosyltransferase III was quite low in the tumor tissues, *i.e.*, about one-fifth of that in the normal tissues, statistics could not be applied since this enzyme is believed to be responsible for formation of the B determinant, and only 2 patients had blood type B. The activities of a sialyltransferase and the galactosyltransferase I were not significantly different in normal and cancerous tissues. The activities of all the glycosyltransferases were unchanged in the colonic mucosa of patients with inflammatory bowel diseases.

Since there are several sialyltransferases in mammalian tissues, we prepared various acceptors and compared these sialyltransferase activities with 4 different acceptors in the normal and cancerous tissues of the colon. As shown in Table 3, sialyltransferase activity was essentially the same with 3 of the acceptors. However, when desialyzed OSM was the acceptor, the sialyltransferase was nearly 4 times higher in the normal tissues than in the cancer tissues.

When the homogenates of normal and cancerous tissues were mixed, the activity of 2 galactosyltransferases of the mixture was the same as the additive value of each fraction assayed separately.

Glycosidase Activities. Of 5 glycosidases examined, none

²The abbreviations used are: OSM, ovine submaxillary mucin; GalNAc, *N*-acetylgalactosamine.

Table 1
Carbohydrate composition of the membrane and cytoplasmic fractions of normal and cancerous colonic mucosa

	Carbohydrate composition (nmoles/mg protein)					
	Pellet			Cell sap		
	Normal	Cancer	<i>p</i> ^a	Normal	Cancer	<i>p</i>
Fucose	40.5 ± 18.1 ^b (1.27) ^c	16.8 ± 7.4 (0.61)	<0.05	62.4 ± 28.8 (2.38)	18.7 ± 5.1 (0.93)	<0.01
Mannose	32.0 ± 16.4 (1)	27.7 ± 8.7 (1)	N.S.	26.2 ± 9.5 (1)	20.1 ± 5.4 (1)	N.S.
Galactose	79.6 ± 36.8 (2.49)	42.5 ± 19.2 (1.53)	N.S.	66.9 ± 17.9 (2.55)	26.4 ± 10.6 (1.31)	<0.005
GlcNAc ^d	86.2 ± 35.1 (2.69)	43.3 ± 11.1 (1.56)	<0.05	83.7 ± 15.4 (3.19)	43.1 ± 13.4 (2.14)	<0.005
GalNAc	59.7 ± 29.0 (1.87)	18.9 ± 6.9 (0.68)	<0.01	57.4 ± 11.2 (2.19)	23.8 ± 5.8 (1.18)	<0.001
NANA	70.3 ± 21.1 (2.20)	21.4 ± 10.8 (0.77)	<0.005	62.7 ± 19.3 (2.39)	28.6 ± 6.8 (1.42)	<0.01

^a Student's *t* test; N.S., *p* > 0.05.

^b Mean values from 5 patients ± S.D.

^c Numbers in parentheses, molar ratio of each sugar when the value for mannose is taken as 1.

^d GlcNAc, *N*-acetylglucosamine; NANA, *N*-acetylneuraminic acid.

Table 2
Glycosyltransferases of normal and cancerous mucosa

Glycosyltransferase	Reaction	cpm/mg protein/hr (× 10 ⁻³)		
		Normal (8) ^a	Cancer (8)	Inflammatory bowel diseases (3)
Sialyltransferase	NANA →Gal—	39.1 ± 18.7 ^b	35.8 ± 10.8	36.4 ± 12.4
Galactosyltransferase I	Gal →GalNAc—	8.7 ± 4.6	5.4 ± 2.3	9.8 ± 2.7
Galactosyltransferase II	Gal →GlcNAc—	79.3 ± 19.5	43.7 ± 12.8 ^c	72.1 ± 28.0
Galactosyltransferase III ^d	Gal →Gal-Glc	78.7 ± 28.2	15.8 ± 1.4	
Fucosyltransferase I	Fuc Fuc →Gal-Glc	75.7 ± 11.2	32.9 ± 15.5 ^e	70.0 ± 19.3
Fucosyltransferase II	Fuc Fuc →Gal-Glc	59.2 ± 28.8	21.9 ± 9.5 ^f	42.9 ± 15.0
Polypeptidyl: <i>N</i> -acetylgalactosaminyltransferase	GalNAc →protein	3.2 ± 1.6	1.2 ± 0.3 ^g	3.0 ± 1.1

^a Number in parentheses, number of subjects studied.

^b Mean ± S.D.

^c *p* < 0.01.

^d The enzyme activity was present in 2 patients with blood type B but was absent in 6 patients with blood type O. Therefore, the value given is the mean value obtained from 2 patients.

^e *p* < 0.001.

^f *p* < 0.025.

showed an appreciable difference among normal, cancerous, and diseased tissues. These results are shown in Table 4. In all of the tissues, the activities of the α-galactosidase and the α-mannosidase were much lower than those of the β-glycosidases.

DISCUSSION

Our previous study (20) indicated that the membrane glycopeptides of human colonic adenocarcinoma from blood type A patients showed a loss of glycoprotein-associated blood group A activity. The data presented in this study have demonstrated that considerable alterations

in glycoprotein compositions occur not only in the membrane fractions but also in the cytoplasmic fractions of colonic neoplasia in patients with blood types O and B. In the cytoplasmic and membrane fractions of the tumor tissues, the total sugar content was reduced to about one-third of that in the normal colonic mucosa. When the molar ratios of each sugar in the cytoplasmic and membrane fractions were compared in normal and tumor tissues there was a differential reduction in these sugars, suggesting that there were several types of oligosaccharide chains present in these fractions. However, we do not know at this time whether these oligosaccharides are present on the same or different glycoproteins. In both fractions the sugars that are associated with mucinous glycoproteins such as fucose

Table 3
Sialyltransferases of normal and cancerous mucosa

Acceptors	cpm/mg protein/hr ($\times 10^{-3}$)		
	Normal (6) ^a	Cancer (6)	Inflammatory bowel diseases (3)
α_1 -Acid glycoprotein	34.2 \pm 16.1 ^b	33.4 \pm 11.2	36.4 \pm 12.4
Fetuin	41.4 \pm 16.9	44.1 \pm 18.8	40.7 \pm 14.6
OSM	14.2 \pm 9.6	3.7 \pm 1.7 ^c	17.6 \pm 4.9
Tumor glycopeptides	4.9 \pm 1.8	5.0 \pm 1.6	5.1 \pm 1.5

^a Number in parentheses, number of subjects studied.

^b Mean \pm S.D.

^c $p < 0.025$.

Table 4
Glycosidase activities in normal and cancerous colonic mucosa

Glycosidases	nmoles/mg protein/hr		
	Normal (8) ^a	Cancer (8)	Inflammatory bowel diseases (3)
β -Galactosidase	128.1 \pm 22.3 ^b	117.4 \pm 49.2	123.8 \pm 23.9
α -Galactosidase	10.2 \pm 3.9	9.4 \pm 2.9	8.8 \pm 3.1
β -N-Acetylgalactosaminidase	119.6 \pm 49.5	91.3 \pm 12.8	87.9 \pm 77.2
β -N-Acetylglucosaminidase	235.4 \pm 47.1	197.2 \pm 38.7	196.3 \pm 31.2
α -Mannosidase	25.6 \pm 8.9	25.8 \pm 12.1	20.7 \pm 8.2

^a Number in parentheses, number of subjects studied.

^b Mean \pm S.D.

and GalNAc were reduced significantly, while sugars that are primarily associated with serum-type glycoproteins were relatively unchanged or reduced to a lesser extent.

That the observed alteration may be mainly due to decreased synthesis is suggested by the observation that the activities of some glycosyltransferases were low in cancerous tissues. Glycosidase activities assayed with synthetic substrates showed no appreciable differences between normal and cancerous tissues, although glycoprotein substrates were not used. The decrease in glycosyltransferase activity was not due to inhibitors or activators as was indicated by the results of the mixing experiment. The reduction in the levels of glycosyltransferases appeared to be quite selective. There are several sialyltransferases in mammalian tissues, the activities of which can be measured with different acceptors. When desialyzed α_1 -acid glycoprotein is used as an acceptor, an enzyme catalyzes the addition of sialic acid to terminal galactose residues of the oligosaccharides. With fetuin as an acceptor, it is likely that to a large extent the same enzyme is being measured. However, with desialyzed OSM as an acceptor, the enzyme catalyzing the transfer of sialic acid:*N*-acetylgalactosamine is measured. Recently, Buck *et al.* (6) have observed that virally transformed cells had a higher level of sialyltransferase than the normal cells did when membrane glycopeptides obtained from transformed fibroblasts and from Novikoff ascites tumor cells were used as acceptors. However, the structure of these tumor glycopeptides has not yet been established. In this study, when desialyzed fetuin, α_1 -acid glycoprotein, or tumor glycopeptides were used as acceptors both normal and tumor tissues had similar sialyltransferase activities.

When desialyzed OSM was used as an acceptor, the enzyme activity was found to be reduced considerably in the cancer tissues. The activity of the galactosyltransferase III, thought to be responsible for blood group B activity, was also markedly reduced in colon cancer tissues of both patients with blood type B. Thus 2 glycosyltransferases responsible for the human blood group activities, *i.e.*, an *N*-acetylgalactosaminyltransferase (20) and a galactosyltransferase, described in this study have been shown to be markedly reduced in colon cancer tissues. The observed reduction in the activities of glycosyltransferases in the cancerous tissues does not appear to be a common phenomenon in the diseased colonic mucosa, since the enzyme activities were unchanged in the colonic tissues of ulcerative or granulomatous colitis. Recently, LaMont *et al.* (25) reported that, in rats given injections of dimethylhydrazine, the colon tumor cells showed a marked decrease in cell surface glycosyltransferase activity compared to normal colon epithelial cells. Although the precise role of the cell surface glycosyltransferases and the proportion of total cellular activity that they constitute is not clear, our results of the total cellular glycosyltransferase activity in human colonic cancer tissues show a similar trend.

Although little is known about the cytoplasmic glycoproteins, a considerable amount of data are available on the membrane-associated glycoproteins of cells transformed by RNA or DNA oncogenic viruses (5, 6, 11, 12, 14, 26, 28, 31). A marked decrease in most neutral and aminosugar contents of membrane carbohydrates has been reported to occur in SV40 virus-transformed 3T3 mouse fibroblasts (14, 26), while others (28) have found an increase in sialic acid

content. When sialyltransferase activity was measured in these cells using desialyzed bovine submaxillary mucin and fetuin as acceptors, the results were contradictory (5, 14). Recent studies on glycopeptides released from membrane glycoproteins by Pronase digestion indicated that the major change after transformation was an increase in glycopeptides of apparently higher molecular weight (12, 31). These changes were observed not only for surface membranes but also for membranes of other subcellular fractions. This study indicates that alteration in glycoprotein biosynthesis may occur in both cytoplasmic and membrane fractions of cancerous tissues.

Considerable alterations in glycolipid composition and in the levels of activities of glycolipid:glycosyltransferases have been reported to occur in cultured fibroblasts transformed by tumorigenic viruses, chemical carcinogen as well as in spontaneous human cancer (17, 29). Although the results vary, most investigators believe that "incomplete" synthesis of the carbohydrate chains occurs in the transformed cells (8, 10, 17, 27, 29). Kijimoto and Hakomori (18) reported that the activity of a UDP-galactose:lactosylceramide α -galactosyltransferase in polyoma transformants was 10 to 50% of the activity in normal cells while the activity of a UDP-galactose:glucosylceramide β -galactosyltransferase, an enzyme that is involved in the transfer of more internal galactose residue, was unaffected by transformation. Their data provide some of the evidence for the incompleteness theory.

In this study, a polypeptidyl:*N*-acetylgalactosaminyltransferase, an enzyme that catalyzes the transfer of the 1st sugar to the hydroxyamino acids of the protein core of mucinous glycoproteins, was greatly reduced in tumor tissues. Furthermore, only that sialyltransferase involved in the addition of sialic acid to mucinous glycoprotein was reduced in the colonic cancer tissues, while that involved in the addition of sialic acid to serum-type glycoproteins remained unchanged. These data together with the observation that the sugars that are present in mucinous glycoproteins were mainly reduced in the cancer tissues suggest that the synthesis of one type of oligosaccharide chain may be greatly affected in colonic neoplasia, while another family of oligosaccharides may remain relatively unaffected.

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