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INCOMPATIBLE BLOOD-GROUP A DETERMINANTS IN TUMORAL MUCINS. ISOLATION OF OLIGOSACCHARIDES HAVING A 2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSYL GROUP AT THE NON-REDUCING END

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ABSTRACT

Two glycopeptide fractions were obtained from pseudomyxomatous mucins secreted by an ovarian cystadenocarcinoma from a female having blood-group B, and by an appendix tumor from a male having blood-group O. The carbohydrate and amino acid content of these fractions suggests the presence of numerous carbohydrate side-chains linked through *O*-glycosyl bonds to a peptide core rich in threonine and proline. The two glycopeptide fractions exhibit compatible B- and H-blood-group activities. They are reactive towards *Dolichos biflorus* lectin and human anti-A agglutinins, and so exhibit an incompatible A activity. Alkali–borohydride degradation of Pronase-digested glycopeptides gave dialyzable oligosaccharides that were purified and shown to possess 2-acetamido-2-deoxygalactitol at the terminal reducing-end. 2-Acetamido-2-deoxyglucose, galactose, fucose, and neuraminic acid were absent, or present, in variable proportions. Four oligosaccharides containing 2-acetamido-2-deoxy-D-galactose residues were reactive towards *Dolichos biflorus* lectin and human anti-A agglutinins, indicating the presence, at the nonreducing end, of a 2-acetamido-2-deoxy-α-D-galactopyranosyl group, responsible for blood-group A activity.

INTRODUCTION

Pseudomyxoma peritonei disease is characterized by mucinous implants of ovarian or appendix origin in the human peritoneal cavity. Pseudomyxoma peritonei is secondary to a mucinous cystadenoma or to a mucinous cystadenocarcinoma[1]. The paramucin stains with metachromatic dyes, such as Alcian Blue, a property that has been attributed to hyaluronic acid[2]; we were not able to confirm this result[3]. Metachromatic staining may also depend on the electronegative properties of glycoproteins. Glycoproteins containing sialic acid and fucose residues were obtained by Tomada and Kitamura[4] from the abdominal mucus of patients with pseudomyxoma peritonei. Similarities between the carbohydrate composition of the mucinous

material and that of the ovarian cyst fluids were reported by Dunstone and Morgan[5]. The ovarian cyst fluids are rich sources of glycoproteins, especially blood-group substances[6].

Previously[3,7], we have reported blood-group A activity in the glycopeptide fractions of pseudomyxomatous mucins from two patients; a patient having blood-group O and an appendix tumor, and a patient having blood-group B and an ovarian mucinous cystadenocarcinoma. We report now the characterization of the blood-group glycopeptides from these mucins.

EXPERIMENTAL

Colorimetric determinations. — Neutral sugars were determined by the orcinol–sulfuric acid[8], uronic acids by the Dische carbazole[9], hexosamines by the Elson–Morgan[10], and *N*-acetylhexosamines by the Reissig *et al.* method[11]. Sialic acid was determined by the thiobarbituric acid method[12] after hydrolysis with 50mM sulfuric acid for 1 h at 80° and after treatment with neuraminidase of *Vibrio cholerae* (Behringwerke)[13]. 2-Amino-2-deoxy-glucose, -galactose, and -galactitol were determined after hydrolysis with 4M hydrochloric acid for 4 h at 100° under reduced pressure according to Downs and Pigman[14], with an amino acid analyzer Beckman Unikrom; the column (Beckman 501 resin) was equilibrated with citrate borate buffer, pH 5.2, containing 0.35M sodium citrate–0.3M boric acid. 2-Amino-2-deoxy-D-galactitol, used as the reference compound, was prepared by reduction of 2-amino-2-deoxy-D-galactose (Sigma) with sodium borohydride[15].

Gas–liquid chromatography. — Analyses were performed with a Girdel model 75 E 1 gas chromatograph. For neutral sugar determination, hydrolysis with trifluoroacetic acid was used according to Arakawa *et al.*[15]. Neutral sugars were determined, after trifluoroacetylation of alditols in the presence of *myo*-inositol as an internal standard, with a glass column (0.3 × 300 cm) packed with 5% OV 210 on a support of Chromasorb, as described by Zanetta *et al.*[16].

Electrophoresis, paper chromatography, and high-pressure liquid chromatography. — Zone electrophoresis was performed on Cellogel strips Sebia at 150 V for 90 min, in 0.1M pyridine formate (pH 3.0) and 0.15M zinc acetate (pH 6.0) buffers. The experiments were performed in duplicate, and the strips stained with 0.5% Alcian Blue and with the periodic acid–Schiff reagent. Sensitivity to hyaluronidase[17] and chondroitinases[18] ABC and AC, was tested by zone electrophoresis.

The following solvent systems were used for descending paper-chromatography: (*A*) 35:39:26 1-butanol–pyridine–water, (*B*) 6:4:3: 1-butanol–pyridine–water, and (*C*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, all v/v. Whatman papers No. 3 MM and No. 1 were used for preparative and analytical purposes, respectively. For the separation of monosaccharides and oligosaccharides, the chromatograms were irrigated for one day and 3–4 days, respectively. Carbohydrates were made visible with the periodate–benzidine reagent[19].

High-pressure liquid chromatography was performed at room temperature

with a Waters Associates Instrument equipped with a model M-6 000 A solvent-delivery system, model U 6K manual injector, model R 401 differential refractometer, and two columns (300 × 3.9 cm) of μ Bondapack (carbohydrates) at a flow rate of 0.5 mL/min, and a mobile phase composed of 2:1:2 (v/v) acetonitrile–water–methanol.

Preparation of glycopeptides by Pronase digestion. — The mucinous secretions of an ovarian tumor from a female patient (G) having blood-group B, and of an appendix tumor from a male patient (P) having blood-group O were collected during surgery and stored as the acetone powder after elimination of blood-cell material (yields: 100 g for G and 70 g for P). The glycopeptides were isolated after Pronase digestion of the acetone powder as previously described[20]. The acid-soluble material obtained by proteolytic digestion was fractionated by successive precipitation with quaternary ammonium salt, cetylpyridinium chloride, and ethanol.

Preparation of perchloric acid extracts. — Perchloric acid extracts of pseudo-myxomatous mucin powder were obtained according to the procedure of Von Kleist[21].

Isolation of reduced oligosaccharides. — The glycopeptide fractions (designated G and P) obtained after Pronase digestion, were treated under the conditions of Iyer and Carlson[22] in the presence of sodium borotritide, including desalting the samples by treatment with Dowex 50 (H^+), followed by evaporation of boric acid as the methyl ester. The carbohydrate-containing fractions were dialyzed against distilled water (10 vol.). Dial. I was a pool of five successive dialyzates, each obtained after 30 min, Dial. II was a pool of four successive dialyzates, three obtained after 24 h, and the fourth after 48 h of dialysis. Dial. I and Dial. II were fractionated on a Bio-Gel P-2 (<400 mesh) column (100 × 2 cm) calibrated with Blue Dextran, lactose, and D-galactose. Fractions of 2 mL were collected, and aliquots were analyzed for radioactivity and carbohydrates. The fractions containing reduced oligosaccharides were submitted to analytical and preparative paper-chromatography, the latter being repeated until a homogeneous material was obtained. H.p.l.c. was used at the end of the fractionation procedure as an additional criterion of purity.

Treatment with N-*acetyl-α-*D-*galactosaminidase.* — Oligosaccharides were incubated with *N*-acetyl-α-D-galactosaminidase of *Charonia lampas* (EC 3.2.1.49, Seikagaku Fine Biochemicals, Tokyo) (0.01 enzyme unit per mg of oligosaccharide) in 50mM citrate phosphate buffer, pH 4.0, for 18 h at 37°, before and after treatment with neuraminidase. The released 2-acetamido-2-deoxy-D-galactose was separated and quantitatively determined by a colorimetric method after gel filtration of the samples on a Bio-Gel P-2 column (15 × 0.9 cm).

Assay for blood-group activity by inhibition of hemagglutination. — A, B, H Blood-group activity was determined on solutions in 0.9% sodium chloride. To serial dilutions (200 μL; dilution factor 1:2) of the glycopeptide solution to be tested were added appropriately diluted antisera (200 μL; human anti-A serum, anti-A_1 of *Dolichos biflorus* lectin, human anti-B serum, or human anti-H serum CNTS-Paris), and the suspension was kept for 4 h at room temperature. A 1%

suspension of red cells in 0.9% sodium chloride (400 μL) was added to each tube (the red cells were washed three times with 0.9% sodium chloride prior to use). After being kept for 18 h at 4°, the suspensions were centrifuged at 2 000 r.p.m. for 2 min, and the sediment was resuspended in order to estimate the degree of agglutination. The glycopeptide concentrations reported in Table III correspond to a total absence of agglutination. Blood-group activity was also determined by hemagglutination inhibition with a Takatsy microtitrator using 25-μL loops and a 1% suspension of erythrocytes*.

CEA assays. — CEA activity was identified** by the Ouchterlony double-diffusion method[23], and CEA levels were measured*** by radioimmunoassays[24] (Prof. Burtin's laboratory, IRSC Villejuif).

RESULTS

The glycopeptide fractions were obtained from mucin acetone powders by Pronase digestion, in 26 and 35% yield for G and P, respectively. Cellulose acetate electropherograms of both fractions gave only one band staining with Alcian Blue and the PAS reagent. The patterns were the same when the electrophoresis was performed at various pH values: 3.0, 6.0, and 9 in borate buffer. Fractions G and P were not degraded by hyaluronidase and chondroitinases ABC and AC, and the colorimetric assay for uronic acids was negative.

The composition in carbohydrates and amino acids is listed in Tables I and II. Fraction G contained 73% carbohydrate and 5% sulfate groups, and Fraction P 74% carbohydrate and 3% sulfate groups. Both fractions had the same qualitative carbohydrate composition, the molar ratio of galactosamine to glucosamine being 1

TABLE I

CARBOHYDRATE COMPOSITION OF GLYCOPEPTIDE FRACTIONS G AND P

Component (%)	*Fraction*	
	G	*P*
Hexosamines	32	38.6
Galactose	25.5	19.3
Fucose	9.5	6.2
N-Acetyl-neuraminic acid	6	10
Molar ratios		
GalN:GlcN:Gal:Fuc:NeuAc	1:1:1.6:0.6:0.4	1:0.66:0.8:0.26:0.43

*Experiment performed by Dr. Lopez.
**Experiment performed by Dr. Hirsch-Marie.
***Experiment performed by Dr. Troupel.

TABLE II

AMINO ACID AND AMINO SUGAR COMPOSITIONS OF GLYCOPEPTIDE FRACTIONS G AND P, BEFORE AND AFTER ALKALI–BOROHYDRIDE DEGRADATION

Amino acids and amino sugars (mol/100 mol)	*Fraction*			
	G		*P*	
	Before	*After*	*Before*	*After*
Aspartic acid	3	3	1	1
Threonine	40	16	46	20
Serine	13	3	6	1
Glutamic acid	4	7	5	5
Proline	17	19	28	34
Glycine	8	6	4	4
Alanine	6	11	1	6
α-Aminobutyric acid		22		20
Valine	3	4	4	4
Isoleucine	2	4	4	4
Leucine	1	2	1	4
Phenylalanine				
Lysine	3	4		
Histidine				
Arginine				
Galactosamine	58	22	53	25
Glucosamine	60	56	27	35
2-Amino-2-deoxygalactitol		30		34

for Fraction G and 1.5 for Fraction P. The percentage of amino acids was about 22% for Fraction G, and 23% for Fraction P, as estimated from the amino acid composition. The main amino acids present were threonine and proline, which constituted 2/3 of the amino acid content, and to a lesser extent serine. Threonine and serine constituted 50% of the amino acid content. Cysteine and aromatic acids were lacking. Alkali–borohydride degraded 34 mol of hydroxyamino acids of Fraction G to give 30 mol of 2-acetamido-2-deoxy-D-galactitol. Identical treatment of Fraction P degraded 31 mol of threonine and serine to give 34 mol of 2-acetamido-2-deoxy-D-galactitol. These results (Table II) indicate that one out of three amino acids is linked by an *O*-glycosyl linkage between threonine, or serine, and 2-acetamido-2-deoxy-D-galactose in Fractions G and P.

The blood-group activities of both Fractions G and P, before and after removal of sialic acid residues, are shown in Table III. With human agglutinins, Fraction G (from the blood-group B patient) exhibited blood-group B and H activity, and Fraction P (from the blood-group O patient) H activity. The two fractions inhibit human anti-A agglutinins and *Dolichos biflorus* agglutinin. The blood-group A activity of fractions G and P was about ten times higher with *Dolichos biflorus* agglutinin, as a source of anti-A_1, than with human anti-A agglutinins. The removal

TABLE III

HEMAGGLUTINATION-INHIBITION ASSAYS OF GLYCOPEPTIDE FRACTIONS G AND P FOR A, B, H BLOOD-GROUP ACTIVITIES BEFORE AND AFTER REMOVAL OF SIALIC ACID[a]

Fraction	*A_1 Red cells–*		*B Red cells–human anti-B*	*O Red cells–human anti-H*
	Human anti-A	*Dolichos anti-A_1*		
G				
before	$2 \cdot 10^{-1}$	$2.4 \cdot 10^{-2}$	$2.4 \cdot 10^{-3}$	$5 \cdot 10^{-4}$
after	$2 \cdot 10^{-1}$	$2.4 \cdot 10^{-2}$	$2.4 \cdot 10^{-3}$	$1.2 \cdot 10^{-4}$
P				
before	$1 \cdot 10^{-1}$	$1.2 \cdot 10^{-2}$		$8 \cdot 10^{-2}$
after	$2 \cdot 10^{-1}$	$0.6 \cdot 10^{-2}$	3.2	$4 \cdot 10^{-2}$
2-Acetamido-2-deoxy-D-galactose as reference (blood-group A activity)		6		

[a]Values are μmol of hexosamine content per 0.2 mL of suspension, indicating the minimum concentration of substance that gives complete inhibition of hemagglutination.

TABLE IV

AMINO SUGAR COMPOSITION AFTER ALKALI–BOROHYDRIDE DEGRADATION OF VARIOUS FRACTIONS

Fraction	*Amino sugar*[a]			
	GalN	*GlcN*	*GalNol*	*GalN + GlcN/GalNol*
G	0.74	1.85	1	2.59
Dial I[b]	1.46	3.66	9	0.57
	(0.16)	(0.41)	(1)	
Dial II[b]	13.0	33.6	23.4	1.98
	(0.55)	(1.43)	(1)	
P	0.94	1.20	1	2.14
Dial I[b]	3.0	3.5	24.1	0.26
	(0.13)	(0.14)	(1)	
Dial II[b]	13	20	40	0.82
	(0.32)	(0.5)	(1)	

[a]Abbreviation: GalNol, 2-amino-2-deoxy-D-galactitol. [b]Recovery of amino sugars after alkali–borohydride degradation expressed in μmol of amino sugars in the fractions. In parentheses, relative proportions of amino sugars after alkali–borohydride degradation expressed in mol hexosamine/GalNol.

of sialic acid residues did not enhance the blood-group activities of Fractions G or P.

The alkali–borohydride degradation of both glycopeptides was performed under the conditions of Iyer and Carlson[22]. Table IV shows the amino sugar composition of the various oligosaccharide fractions (Dial. I and II) obtained after dialysis.

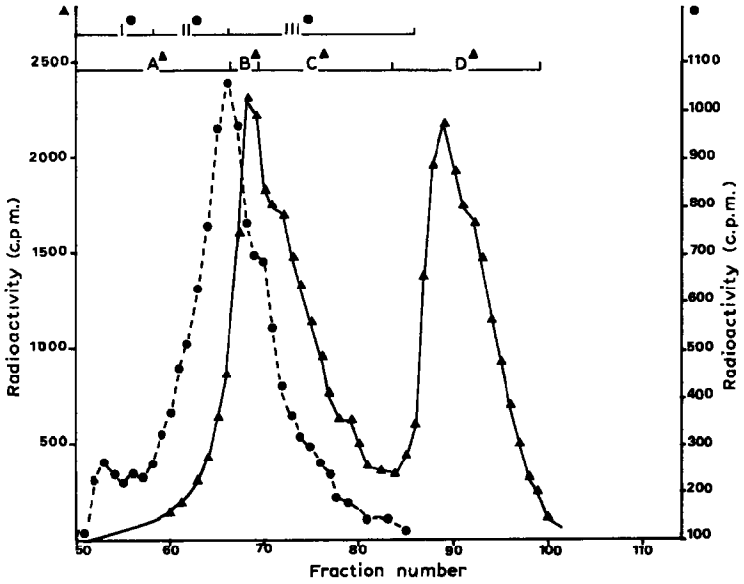

Fig. 1. Elution patterns, from Bio-gel P-2, of Dial. I (▲) (fractions A, B, C, and D were pooled) and Dial. II (●) (fractions I, II, and III were pooled) of glycopeptide fraction P.

These results suggest that not all 2-acetamido-2-deoxy-D-galactose residues are linked to protein, as characterized by isolation of 2-acetamido-2-deoxy-D-galactitol. Dial. I, obtained from fractions G and P, 13 and 11 %, respectively, of the 2-acetamido-2-deoxy-D-galactose residues were not involved in the glycoside linkage. For Dial. II, 35 and 24%, respectively, of these residues were not involved. The ratio (GalN + GlcN)/GalNol shows, in the various fractions, heterogeneity in the size of oligosaccharides, and in the GalNol, GlcN, and GalN content. This suggests that the reduced oligosaccharides originated from a heterogenous population of chains directly attached by *O*-glycosyl linkages to the polypeptide backbone.

The elution profile, from a Bio-Gel P-2 column, of the Dial. I and II products of glycopeptide Fraction P, is shown in Fig. 1. A similar elution profile was obtained for Dial. I and Dial. II of Fraction G. Dial. I was separated into four fractions and Dial. II into three fractions, based upon the radioactivity of the labelled, reduced oligosaccharides. Each fraction was lyophilyzed, and then separated by preparative, descending paper-chromatography in solvent *C*. The oligosaccharides were eluted from the paper, rechromatographied in solvents *A* and *B*, pooled when they were indistinguishable by paper chromatography, and rechromatographed on Bio-Gel P-2. The purity of the compounds obtained was verified, after removal of sialic acid, by elution as a single peak in h.p.l.c.

The chemical composition of the oligosaccharides (Table V) indicates that compound I_G is an octasaccharide that contains 2-acetamido-2-deoxy-D-galactose and shows blood-group A activity: 9 μg was necessary to inhibit 0.2 mL of anti-A serum diluted to 1/64 (agglutination titer 1/64), and 18 μg to inhibit 0.2 mL of anti-A

TABLE V

CARBOHYDRATE COMPOSITION OF REDUCED OLIGOSACCHARIDES OBTAINED FROM G FRACTIONS AND P[a]

Fraction	*Oligo-saccharide*	R_{Lac}	*Sugar residue*[b]				
			GalN	*GlcN*	*Gal*	*Fuc*	*NeuAc*
G	I_G	0.10	0.98	2.2	2.18	0.98	1.15
	II_G	0.25		1.8	2.09	1.13	0.89
	III_G	0.43	0.96	0.91	0.89		0.92
	IV_G	0.67	1.12	1.84	4.0	1.88	
	V_G	1.34			0.85		
P	I_P	0.36	0.86	0.78	0.77		1.90
	II_P	0.39		0.88	0.83		0.98
	III_P	2.20					

[a]The chromatograms were developed in solvent *C* for three days for oligosaccharides having $R_{Lac} < 1$ and for one day for oligosaccharides having $R_{Lac} > 1$. [b]Molar ratio relative to 2-acetamido-2-deoxy-D-galactitol (1.0).

Dolichos lectin diluted to 1/64 (agglutination titer 1/64). Compound III_G is a pentasaccharide having one 2-acetamido-2-deoxy-D-galactose residue: 18 μg was necessary to inhibit 0.2 mL of anti-A serum diluted to 1/64, and 0.2 mL of anti-A *Dolichos* lectin diluted to 1/64. Compound IV_G seems to be a decasaccharide having one 2-acetamido-2-deoxy-D-galactose residue; the hemagglutination inhibition assay could not be performed because of insufficient material. Compound V_G is a disaccharide composed of one residue of galactose per residue of 2-acetamido-2-deoxy-D-galactose. Compound I_P is a hexasaccharide showing a blood-group A activity: 5 μg was necessary to inhibit 0.2 mL of anti-A serum diluted to 1/64, and 10 μg to inhibit 0.2 mL of anti-A *Dolichos* lectin diluted to 1/64. Compound II_P is a tetrasaccharide, and Compound III_P a monosaccharide.

To confirm the presence of unsubstituted 2-acetamido-2-deoxy-D-galactose residues in compounds I_G, III_G, and I_P, and to establish the anomeric configuration of these residues, the oligosaccharides were treated with *N*-acetyl-α-D-galactosaminidase. This enzyme released 68, 85, and 90%, respectively, of the total 2-acetamido-2-deoxy-D-galactose residues, and removal of sialic acid did not enhance the enzymic liberation of the 2-acetamido-2-deoxy-D-galactose residues.

DISCUSSION

The two pseudomyxomatous mucins each gave an acidic glycopeptide fraction having a chemical composition characteristic for a gastrointestinal glycoprotein of the mucin type. The presence of some sulfate groups in glycopeptide fractions has been reported for cancerous gastric[25] and colonic human mucosa[26]. The carbohydrate and amino acid compositions, before and after alkaline degradation, show that the

glycopeptide fractions G and P consist of a peptide core having a large number of oligosaccharide side-chains.

The content in hexosamine and amino acid is within the ranges usually observed for human blood-group substances[27]. Blood-group assay demonstrated the presence of blood-group A determinants in both glycopeptide fractions derived from patients having blood-group B or O. The appearance of incompatible blood-group antigens, foreign to the host, has been reported for some human tumors. Häkkinen[25] described the presence of A-like antigen in stomach cancers from patients with blood-group O or B. The active fraction was attributed to a sulfoglycoprotein. Hakomori *et al.*[28,29] demonstrated the presence of Forssman antigen in tumors from O,F^-, or B,F^- individuals, and its possible identity with "A-like antigen" in human cancer.

Previous studies of the antigenic determinants of the carcinoembryonic antigen (CEA) in human gastrointestinal tumors have emphasized the presence of blood-group antigens; in particular, Turner *et al.*[30], and Gold and Gold[31] reported that their CEA preparation bears an A-like antigenic determinant. Consequently, we investigated the relationship between CEA activity and blood-group A antigen in our mucins. CEA activity was present in perchloric extracts of both mucins, in addition to blood-group antigens. No CEA activity was detected in glycopeptide fractions G and P, which carry blood-group specificities and are *O*-glycosylated[32]. This is in agreement with the observation that the immunologically active glycopeptides of human gastrointestinal tumors that contain the CEA determinant are *N*-glycosylated[33]. Furthermore, the antigenic sites for CEA and blood-group antigens in tumor extracts were found to be located on different molecules[34,35].

The heterogeneity of glycopeptide fractions G and P that exhibited compatible blood-group antigenicities and an incompatible blood-group A specificity had been examined by chromatography on Sepharose–*Dolichos* lectin columns[7]. These assays monitored by hemagglutination-inhibition tests gave no positive results. The A blood-group specificities were always associated with the B and H blood-group specificities, which suggested that the A, B, and H blood-group determinants are carried by oligosaccharide side-chains linked to the same peptidic core.

Alkaline degradation of glycopeptide fractions G and P gave oligosaccharides having a 2-acetamido-2-deoxy-D-galactitol residue at the (potential) terminal reducing-end. Amongst the dialyzable oligosaccharides obtained from fraction G, the smaller is a disaccharide (V_G) that contains one galactosyl residue per residue of 2-acetamido-2-deoxy-D-galactitol. The *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-galactose chain has been found linked to a protein core in many glycoproteins, although it is usually masked by an *N*-acetylneuraminic acid residue[36-38]. This structure, which is always present in the more complex oligosaccharide side-chains[6], is an integral part of the T antigen, which has been reported to be a characteristic of cancerous mammary tissue[39].

The smallest dialyzable saccharide obtained from Fraction P is a single residue of 2-acetamido-2-deoxy-D-galactose directly linked to the protein backbone. The same structure has been reported previously for a human ovarian cyst[6]. The presence

of this monosaccharide in Fraction P could explain the greater reactivity of this fraction towards *Dolichos biflorus* agglutinin.

The oligosaccharides I_G, III_G, IV_G, and I_P isolated from both fractions, G and P, possess two 2-acetamido-2-deoxy-D-galactose residues, one, as 2-acetamido-2-deoxy-D-galactitol, at the terminal (potential) reducing-end. Compounds I_G, III_G, and I_P were reactive towards *Dolichos biflorus* agglutinin, which recognizes specifically terminal, nonreducing 2-acetamido-2-deoxy-α-D-galactopyranosyl groups[40]. The results of the treatment with *N*-acetyl-α-D-galactosaminidase agree with the reactivity towards *Dolichos biflorus* lectin and demonstrate the presence, at the terminal, nonreducing end, of these groups, which confer the blood-group A specificity. Oligosaccharides I_G, III_G, and I_P seem more reactive towards human anti-A agglutinins than the other oligosaccharides isolated. The structure that is most reactive towards human anti-A agglutinins and is involved in all blood-group determinants is the trisaccharide residue[40] α-L-Fuc*p*-(1→2)-[α-D-Gal*p*NAc-(1→3)]-D-Gal. The chemical and immunochemical data of compound I_G agree with this structure. Some urinary oligosaccharides[41] having the structure α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→4)-D-Glc were able to inhibit the hemagglutination with human anti-A agglutinins. The oligosaccharides I_P and III_G, which do not possess an L-fucose residue, could bear the disaccharide residue α-D-(Gal*p*NAc)-(1→3)-D-Gal at the nonreducing end.

These results suggest that the glycoproteins of pseudomyxomatous mucins originating from ovarian and intestinal tumors result from an aberration in the synthesis of glycoproteins possessing blood-group activities. The quantitative expression of "A antigen", foreign to the host in the examined tumors, must depend on the action of a specific gene for synthesis of an *N*-acetyl-α-D-galactosaminyltransferase that is necessary to convert the H substance into the blood-group A antigen. In B and O individuals, this enzyme is absent or repressed, and present or derepressed in tumors.

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