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Note

Human, bronchial-mucus glycoproteins: a comparison between chemical properties and affinity for lectins

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Mucus glycoproteins isolated from sputum of patients suffering from chronic bronchitis have been shown to be heterogeneous. The secretions contain neutral glycoproteins, characterized by a relatively small amount of acid groups[1–3], and acidic glycoproteins. The latter consist of a population of molecules of increasing acidity, due to an increase of the ratio of sulfate to *N*-acetylneuraminic acid. The carbohydrate chains of mucus acid-glycoproteins, obtained from patients with chronic bronchitis, showed an extreme heterogeneity in their acidic character and molecular size[4]. Mucus glycoproteins that are isolated from bronchial washings of macroscopically healthy areas of the tracheobronchial tree represent a normal bronchial secretion[5,6], and they are essentially acidic in character, with mainly sialyl groups[6].

As mucus glycoproteins have been characterized by their physical and chemical properties, and compared with glycoproteins of normal secretions[2,6], it was of interest to complete the characterization of bronchial glycoproteins by a study of their affinity for lectins that have been widely used in glycoprotein studies[7]. *Ricinus communis* lectins have already been used to purify bronchial mucus glycoproteins by affinity chromatography[8], and peroxidase-labelled *Limulus* lectin and *Ricinus* lectins to render visible the carbohydrate residues of glycoproteins in the bronchial-mucosa cells[8,9]. The aim of the present work, performed on bronchial-mucus glycoproteins obtained from five patients suffering from chronic bronchitis, and from a pool of bronchial washings, was to compare the chemical composition of the glycoproteins with their affinity for lectins.

EXPERIMENTAL

Isolation of bronchial-mucus glycoproteins. — (*a*) *From the sputum of patients*

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suffering from chronic bronchitis. Bronchial sputum was obtained from five patients suffering from chronic bronchitis; three having blood group O (No. 1, 2, and 3), and two blood group A (No. 4 and 5). The sputum was collected daily and kept frozen until use. The bronchial-mucus glycoproteins were isolated from the reduced fibrillar mucus[3] according to Lamblin *et al.*[2].

(*b*) *From washings of macroscopically healthy human-bronchiae.* Bronchial washings were obtained from five subjects (three having blood group O, one blood group A, and one blood group B) with no history of chronic bronchitis. The washings were centrifuged, and separated into a soluble fraction and a gelatinous pellet. The mucus glycoproteins contained in the pellet were isolated according to Lafitte *et al.*[5]: the fibrillar mucus was solubilized by reduction and was fractionated by ECTEOLA-cellulose chromatography into four fractions; bronchial mucus-glycoproteins FIIIb (or MGCW) were obtained from the third fraction[5] by Sepharose 4B chromatography.

Preparation of lectins. — The lectin from *Ulex europeus* (UEAI) was extracted from crude seeds by a saline solution, precipitated with ammonium sulfate[10], and purified by affinity chromatography in a column of epoxy-Sepharose–L-fucose[11]. The lectin from *Lotus tetragonolobus* was a commercial preparation (Miles Laboratories, Kankakee, IL 60901). The lectin from wheat germ (*Triticum vulgaris*, WGA) was prepared by affinity chromatography in a column of Sepharose–2-acetamido-2-deoxy-1-*N*-(6-aminohexanoyl)-β-D-glucopyranosylamine[12]. The lectin from soybean (*Glycine max*, SBA) was isolated by affinity chromatography in a column of Sepharose–1-*N*-(6-aminohexanoyl)-β-D-galactopyranosylamine[12]. The lectins from castor bean (*Ricinus communis*, RCA I and II) were obtained by the method of Tomita *et al.*[13], as modified by Lhermitte *et al.*[14]. The lectin from peanut (*Arachis hypogea*, PNA) was isolated by affinity chromatography in a column of Sepharose–1-*N*-(6-aminohexanoyl)-β-D-galactopyranosylamine[15]. The lectin from jack bean (*Concanavalia ensiformis*, Con A) was prepared according to Agrawal and Goldstein[16].

Analytical methods. — Chemical analysis of mucus glycoproteins (amino acid, carbohydrate, and sulfate content) was performed as described by Lamblin *et al.*[2]. The acidic mucus-glycoproteins of patient No. 5 were desialylated according to Cassidy *et al.*[17]. The blood-group activity of mucus glycoproteins was assayed by hemagglutination inhibition[18].

Affinity assays. — (*a*) *Precipitation analysis by diffusion in gel.* The double-diffusion method according to Ouchterlony[19] was performed, on microscope slides, in a 1% agarose gel in Veronal buffer (pH 8.2). The center wells were filled with 20 μL of a solution of lectin (4 mg/mL for RCA I and II, and 2 mg/mL for the other lectins), and the outer wells were filled with 10 μL of a solution of mucus glycoproteins (2 mg/mL). The slides were incubated in a humid chamber for 18 h, and then washed, dried, and stained with Coomassie Blue[20].

(*b*) *Hemagglutination assays.* The titration and inhibition assays were performed, according to the method described previously[21], with erythrocytes freshly obtained from donors. Human blood-group O cells were used for ulex and lotus lectins, human blood-group A cells for wheat germ agglutinin, human blood-group B

TABLE I

CHEMICAL COMPOSITION OF MUCUS GLYCOPROTEINS OF FIVE PATIENTS AND OF BRONCHIAL WASHINGS

Glycoprotein fractions	*Yield (%)*	*Components (μmol/g)*						
		Amino acids	*Fuc*	*Gal*	*GalNAc*	*GlcNAc*	*NeuAc*	*SO_4*
Patient No. 1								
MGA	1.63	1517	540	1440	430	1076	40	100
MGB	1.79	1908	450	1240	640	1020	140	50
MGC1	1.18	1676	490	1350	650	990	170	70
MGC2	0.48	1519	640	1490	570	1040	120	210
MGC3	0.23	1306	804	1460	420	990	100	300
Patient No. 2								
MGA	0.05	1868	700	1180	630	1080	20	0
MGB	0.41	2207	1090	1040	480	930	110	90
MGC1	0.75	1811	670	1100	710	1030	180	50
MGC2	0.52	1952	850	1360	600	1190	100	250
Patient No. 3								
MGA	0.38	1508	820	1230	410	950	90	40
MGB	1.07	1565	1080	1130	420	820	210	70
MGC1	1.00	1579	670	1340	800	670	420	60
MGC2	1.74	1514	760	1380	570	930	290	90
Patient No. 4								
MGA	7.10	1001	1230	1460	930	990	0	0
MGB	2.68	1275	1160	1220	810	970	140	50
MGC1	0.34	1802	880	1380	740	790	270	170
MGC2	0.72	1312	900	1510	520	920	180	300
Patient No. 5								
MGA	4.30	1530	1010	1220	980	1000	0	0
MGB	1.79	1869	1030	1280	740	790	110	0
MGC1	0.23	1604	830	1410	840	830	110	110
MGC2	0.60	1485	920	1420	690	940	60	350
Bronchial washings								
MGCW	3.06	1440	591	955	597	561	336	0

cells for *Ricinus communis* agglutinins, desialylated human blood-group O cells for peanut agglutinin[15], and trypsinized, rabbit-blood cells for soybean agglutinin[22].

RESULTS

Isolation and chemical composition of bronchial glycoproteins from patients suffering from chronic bronchitis. — Mucus glycoproteins from patients with chronic bronchitis were purified from reduced, fibrillar mucus[2]. The proportions of secreted

TABLE II

BLOOD-GROUP ACTIVITY OF BRONCHIAL-MUCUS GLYCOPROTEINS

Patient no.	*Blood group activity*[a]	*Mucus glycoproteins from chronic bronchitis*				
		MGA	*MGB*	*MGC1*	*MGC2*	*MGC3*
1	A	0	0	0	0	0
(Blood group O)	B	0	0	0	0	0
	H	0	0	0	0	0
2	A	0	0	0	0	
(Blood group O)	B	0	0	0	0	
	H	+++	+	+	0	
3	A	0	0	0	0	
(Blood group O)	B	0	0	0	0	
	H	+++	+++	+	+	
4	A	+++++	+++	+	0	
(Blood group A)	B	0	0	0	0	
	H	+++	+++	+	0	
5	A	+++++	+++	+	0	
(Blood group A)	B	0	0	0	0	
	H	+++	+++	+	+	
				MGCW		
Bronchial washings from subjects with blood group A, B, and O activity	A			+		
	B			(+)		
	H			+++		

[a]Determination of the blood-group activity of each glycoprotein was performed by the hemagglutination-inhibition test. A solution (100 μL) of bronchial-mucus glycoproteins was added to anti-A, -B, or -H serum (100 μL) containing 2 hemagglutining doses. The mixture was incubated for 15 min at room temperature. After the addition of human-blood cells (100 μL), the mixture was shaken and incubated for 2 h at room temperature, and the agglutination was read. The hemagglutination inhibition is given as: +++++, very strong; +++, strong; +, weak; (+), very weak; and 0, no inhibition.

mucus-glycoproteins (MGA, MGB, MGC1, MGC2, and MGC3) for each patient are shown in Table I. They correspond to glycoproteins of increasing acidity (Table I) and had a high proportion of hydroxyamino acids ranging from 37.14 to 51.30% (data not shown). Mucus glycoproteins of patient No. 1 showed no A, B, or H blood-group activity. Those of the other patients (No. 2, 3, 4, and 5) showed blood-group H or A activities decreasing with the acidity of the glycoproteins (Table II).

Isolation and chemical composition of bronchial-mucus glycoproteins from a pool of bronchial washings. — Bronchial-mucus glycoproteins MGCW were isolated from bronchial washings. They contained 42.24% of hydroxyamino acids. Their chemical composition (Table I) shows that they correspond to acidic glycoproteins. They showed blood-group A, B, and H activity (Table II).

TABLE III

AFFINITY OF BRONCHIAL-MUCUS GLYCOPROTEINS FOR LECTINS BY DOUBLE DIFFUSION ASSAYS

Patient No.[a]	*Bronchial mucus glycoproteins*	*Lectins*[b]							
		UE I	*Lotus*	*WGA*	*SBA*	*RCA I*	*RCA II*	*PNA*	*Con A*
1 (O)	MGA	0[c]	+[c]	++[c]	0	++	+[d]	[d]	0
	MGB	0	+	++	0	++	++	[d]	0
	MGC1	0	+	+	0	+	++	[d]	0
	MGC2	0	+	0	0	++	++	[d]	0
	MGC3	0	+	0	0	++	+	[d]	0
2 (O)	MGA	++	+	++	++	++	++	+	0
	MGB	++	++	++	++	++	++	+	0
	MGC1	+	++	+	+	++	++	0	0
	MGC2	+	0	+	0	++	+	0	0
3 (O)	MGA	++	++	++	+	+	+	+	0
	MGB	++	++	++	++	++	+	+	0
	MGC1	+	++	++	++	++	++	0	0
	MGC2	+	0	+	0	++	++	0	0
4 (A)	MGA	++	+	++	++	++	+	+[d]	0
	MGB	++	++	++	++	+	++	++	0
	MGC1	++	++	++	+	+	++	+	0
	MGC2	++	++	0	0	++	++	0	0
5 (A)	MGA	++	+	++	++	++	++	+	0
	MGB	++	++	++	+	++	++	++	0
	MGC1	+	++	++	+	++	++	+	0
	De-sialylated MGC1	++	++	++	+	++	++	++	0
	MGC2	++	+	+	0	++	+	0	0
	De-sialylated MGC2	++	++	+	+	++	++	++	0

[a]Blood group given in parentheses. [b]Abbreviations: UEA *I*, *U. europeus* phytohemagglutinin; WGA, wheat germ agglutinin; SBA, soybean agglutinin; RCA *I* and RCA *II*, *Ricinus communis* agglutinins; PNA, peanut agglutinin; and Con A, concanavalin. [c]The precipitation line is indicated as: ++, strong; +, weak; and 0, no line. [d]Not determined.

Affinity of bronchial-mucus glycoproteins for lectins. — All glycoproteins from patients with chronic bronchitis were tested by affino-diffusion with each lectin (except the glycoproteins of patient No. 1, which were obtained in too small quantities). The results show (Table III) no affinity of the glycoproteins for Con A, whereas all glycoproteins reacted with RCA I and II. The reaction of the other lectins was variable, generally according to the acidity of the glycoproteins. However, no glycoprotein from patient No. 1 reacted with ulex and soybean agglutinins. Table III shows that the glycoproteins MGC1 and MGC2 formed more precipitate with peanut and soybean agglutinins after removal of sialic acid than before. The affinity of bronchial-mucus glycoproteins from patients Nos. 2, 3, 4, and 5, and of glycoproteins MGCW from the pool of bronchial washings was quantitatively determined by heamagglutination inhibition (Table IV). This test was not performed with Con A,

TABLE IV

INHIBITORY EFFECT OF BRONCHIAL-MUCUS GLYCOPROTEINS ON HEMAGGLUTINATING ACTIVITY OF LECTINS

Patient No.	*Bronchial mucus glycoproteins*	*Lectins*[b]						
		UE I[a]	*Lotus*	*WGA*[a]	*SBA*[a]	*RCA I*[a]	*RCA II*[a]	*PNA*[a]
2	MGA	225	225	1 000	500	650	375	4 500
	MGB	250	225	1 250	1 000	650	375	5 350
	MGC1	300	275	1 500	1 250	700	400	>6 000
	MGC2	300	>325	2 000	>3 000	700	400	>6 000
3	MGA	200	225	1 250	1 250	700	375	5 250
	MGB	225	225	1 500	1 250	700	400	5 500
	MGC1	275	250	1 500	1 500	800	400	>6 000
	MGC2	300	>325	2 500	>3 000	750	425	>6 000
4	MGA	250	250	1 250	500	600	375	4 750
	MGB	250	250	1 250	750	650	350	4 750
	MGC1	250	225	2 000	1 250	650	400	5 250
	MGC2	275	325	>3 000	>3 000	700	450	>6 000
5	MGA	200	250	1 000	500	650	350	4 500
	MGB	200	250	1 000	500	700	350	5 250
	MGC1	250	275	1 750	1 000	750	400	5 250
	Desialylated MGC1	225	275	1 500	775	700	400	5 000
	MGC2	275	325	2 250	>3 000	750	400	>6 000
	Desialylated MGC2	225	300	1 750	1 250	700	375	5 250
Bronchial washings	MGCW	250	500	1 250	1 000	775	500	>6 000

[a]The results are expressed in μg/mL able to inhibit hemagglutination. [b]For abbreviations, see footnote to Table III.

as no bronchial-mucus glycoproteins are precipitated by this lectin. Inhibition of WGA, SBA, and PNA by glycoproteins MGC1, and especially MGC2, increased after neuraminidase treatment (Table IV). It was not possible to study, by this test, the affinity of all glycoproteins of patient No. 1 because of lack of material, but it was established that, at a concentration of 3 mg/mL, the glycoproteins MGA had no affinity for ulex phytohemagglutin, and the glycoproteins MGA and MGB had no affinity for SBA. Glycoproteins MGCW from bronchial washings had no affinity for PNA, but reacted with the other lectins.

DISCUSSION

Bronchial-mucus glycoproteins were obtained from the reduced, fibrillar mucus of five patients suffering from chronic bronchitis. The proportion of secreted glycoproteins has been shown to vary with each patient, and it may be related to the evolution of the bronchial affection[1]. These glycoproteins are rich in threonine and

TABLE V

ANALYTICAL DATA FOR BRONCHIAL-MUCUS GLYCOPROTEINS OF PATIENTS WITH CHRONIC BRONCHITIS

Blood group	*Mucus glycoproteins glycoproteins*	*Number of acid residues*[a]	*Ratio NeuAc/SO*$_4$	*Average length*[b]
O	MGA	20–140	0–2.2	5.7–8.5
	MGB	190–280	1.2–3	5–9
	MGC1	230–480	2.4–7	4.9–5.6
	MGC1-2	340–400	0.3–3.2	6.8–9.6
A	MGA	0	0	
	MGB	110–190	0–2.8	
	MGC1	220–240	1–1.6	
	MGC2	410–480	0.2–0.6	

[a]Number of acid residues = NeuAc + SO_4 (from Table I). [b]Average carbohydrate-chain length of bronchial-mucus glycoproteins[2].

serine, and the chemical composition shows the heterogeneity of the carbohydrate chains.

By assuming that all the 2-acetamido-2-deoxy-D-galactose residues of the glycoproteins obtained from blood-group O patients are involved in linkage to hydroxyamino acids[23], the average length of carbohydrate chains may be estimated from the chemical composition[6], and compared with the acidic properties of these glycoproteins (Table V). The purified mucus glycoproteins consist of "neutral bronchial" and "acidic" glycoproteins, which represent two overlapping groups of molecules. The less acidic contain mainly sialyl, and the more acidic, mainly sulfate groups. The latter glycoproteins have a longer average chain-length than the former. Because of blood-group A activity, the average length of the carbohydrate chains of the glycoproteins obtained from the other patients could not be calculated. The mucus glycoproteins, isolated from a pool of the bronchial washings of macroscopically healthy areas of the bronchial tree, contain sialyl groups (Table I). These molecules are similar to the sialyl-containing glycoproteins obtained from chronic-bronchitic sputum[6].

Some details of the structure of bronchial mucus glycoproteins may be established more precisely by a study of lectin affinity. No glycoproteins showed affinity for concanavalin A (Table III and IV), which binds specifically to α-D-mannopyranosyl residues[24,25].

Ulex europeus phytohemagglutinin (UEAI), which is specific for L-fucose residues[26], reacted with glycoproteins having blood-group H activity, but not with glycoproteins devoid of blood-group H activity (patient No. 1, Tables III and IV). The acidic glycoproteins of patients Nos. 2 and 3 showed an affinity weaker than that of the acidic glycoproteins of patient No. 4 (Table IV). Removal of *N*-acetylneuraminic acid (Table IV) shows the role of this residue, since the hemagglutinating inhibition activity of glycoproteins MGC1 and MGC2 increased after neuraminidase treatment.

Lotus tetragonolobus agglutinin, which is specific for L-fucose residues[27,28], showed an affinity for all the glycoproteins, except for the more acidic MGC2 obtained from blood-group O patients. Cleavage of *N*-acetylneuraminic acid increased the hemagglutinating-inhibitory activity of the acidic glycoproteins MGC2 (Table IV). As compared with the results obtained for ulex-phytohemagglutinin affinity, the results obtained with the lotus agglutinin show that glycoproteins devoid of blood-group activity contain carbohydrate structures having an L-fucose residue, perhaps as part of a structure analogous to that of the L antigen[29]. These results also suggest the simultaneous presence, in the glycoproteins of blood-group A and H activity, of several L-fucose-containing structures, some having H blood-group activity, others devoid of it.

As oligosaccharides with blood-group B activity[30,31] reacted poorly with *L. tetragonolobus* agglutinin, the weak inhibitory power of the pool of glycoproteins MGCW might be explained by the presence of such B structures.

Wheat-germ agglutinin[32] reacted with all glycoproteins, except the sulfated glycoproteins of patients Nos. 1 and 4 (Tables III and IV). Its affinity decreased with the increase in acidity of the glycoproteins, but cleavage of *N*-acetylneuramic acid increased the hemagglutinating-inhibitory activity of the glycoprotein molecules (Table IV). This observation is not in agreement with the results of Bhavanandan and Katlic[33].

A positive reaction with castor-bean agglutinins (RCA I and RCA II) demonstrated the presence of D-galactose or 2-acetamido-2-deoxy-D-galactose (or both) residues[34] in all glycoproteins (Tables III and IV).

Soybean agglutinin (SBA), specific for 2-acetamido-2-deoxy-D-galactose and D-galactose residues[35,36], had no affinity for the glycoproteins devoid of blood-group activity. Its affinity decreased with the acidity of molecules. However, cleavage of *N*-acetylneuraminic acid increased the hemagglutinating inhibitory power of the glycoproteins containing sialyl groups (Table IV). The observation that glycoproteins having blood-group H activity have an affinity for SBA is not in agreement with the results obtained by Pereira *et al.*[37], who had demonstrated that SBA precipitates glycoproteins possessing blood-group A, but not H, activity, except when these glycoproteins had been subjected to mild periodate oxidation[38]. As human-bronchial glycoproteins having blood group A or H activity were precipitated by SBA, whereas glycoproteins devoid of blood-group A, B, and H activity did not react, other carbohydrate structures may be present in the blood-group H active glycoproteins, possibly incomplete chains for which SBA has affinity.

Study of the affinity of RCA I and II, and SBA showed that the hemagglutinating inhibitory powers of bronchial glycoproteins were different for each lectin. Glycoproteins devoid of blood-group activity and the most acidic glycoproteins had no affinity for SBA; a repulsion effect depending on the acidity of SBA[22] may exist for the latter glycoproteins.

Peanut agglutinin (PNA), specific[15] for D-galactose residues and antigen T, reacted with the less acidic, but not with the more acidic, glycoproteins (Table III

and IV). Absence of reaction of these glycoproteins may be explained by the number of acidic (especially *N*-acetylneuraminic acid) residues, since glycoproteins MGC1 showed, after desialylation, a higher hemagglutinating inhibitory activity than native glycoproteins, and glycoproteins MGC2 acquired an inhibitory activity (Table IV). These results are in agreement with those of Lotan *et al.*[15], who have shown that the desialylation of glycophorin increased its affinity for PNA.

The heterogeneity of carbohydrate chains has been demonstrated for mucus glycoproteins isolated from patients suffering from chronic bronchitis[1,2,4] or cystic fibrosis[3,9], and the results obtained with affinity reactions support the same conclusions. Some specific carbohydrate residues of glycoproteins in the human bronchial mucosa cells have previously been located with lectins[8,9]. The present results obtained with mucins secreted under normal and pathological conditions suggest that lectins be used to render them visible in the bronchial cells.

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