Structure of two sulphated oligosaccharides from respiratory mucins of a patient suffering from cystic fibrosis

A fast-atom-bombardment m.s. and ^1H -n.m.r. spectroscopic study

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Human respiratory-mucin glycopeptides were isolated from the sputum of a patient suffering from cystic fibrosis. They were subjected to treatment with alkaline borohydride. Application of ion-exchange chromatography afforded carbohydrate fractions containing sulphate. One of these fractions containing a mixture of sulphated oligosaccharides was subsequently submitted to gel-filtration chromatography and h.p.l.c. Two sulphated oligosaccharide-alditols, A and B, were prepared; their structure was determined by means of 400 MHz ^1H -n.m.r. spectroscopy and fast-atom-bombardment m.s. They had a core type 2 and the sulphate was 3-linked to a terminal galactose residue:

INTRODUCTION

Cystic fibrosis (CF) is the most common fatal autosomal recessive disease in Caucasian population, with an incidence of 1 in 2000 births. It is characterized by abnormal electrolyte transport in the apical membrane of several epithelia (Quinton, 1989). In the epithelium of the large airways failure to secrete Cl^- and excessive reabsorption of Na^+ (Knowles *et al.*, 1986) lead to mucus dehydration, abnormal mucociliary clearance and mucus obstruction.

But this abnormal electrolyte transport alone cannot explain the persistent infection of CF patients by selective microbial pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, bacteria that destroy the lung tissue (Hoiby, 1988). Chronic infections by these micro-organisms do not occur so rapidly in patients suffering from other genetic diseases with failure of mucociliary clearance, such as the dysmature cilia syndrome (Eliason *et al.*, 1977).

Identification of the gene defect that causes CF (Riordan *et al.*, 1989) suggests that the protein responsible for CF, or cystic fibrosis transmembrane regulator, belongs to the superfamily of membrane-associated transport proteins, P-glycoproteins (Juranka *et al.*, 1989). The cellular localization and the precise function of this protein are still unknown.

Previous studies by our group and others (Roussel *et al.*, 1975; Boat *et al.*, 1977; Lamblin *et al.*, 1977a) have shown that respiratory mucins secreted by CF patients were highly sulphated. More recently it has been reported that 'mucins' synthesized by

cultured CF nasal epithelial cells were more sulphated than 'mucins' produced by normal cells (Cheng *et al.*, 1989). Sulphate uptake has also been shown to increase in skin fibroblasts from CF patients (Elgavish & Meezan, 1988).

In order to investigate further the sulphation of respiratory mucins in CF, we have started a structural comparison of sulphated oligosaccharides from respiratory mucins secreted by CF patients and by patients suffering from other chronic bronchial diseases. In the present paper we describe the isolation, purification and structure of two sulphated oligosaccharides isolated from respiratory mucins of a patient suffering from CF.

MATERIALS AND METHODS

Sampling of respiratory mucus

Human sputum from a patient (Lab...) with blood group O suffering from CF was collected every day and kept frozen until used. The bronchial secretions were then thawed at 4 °C, diluted 1 to 12 with deionized water and stirred overnight at 4 °C (Slayter *et al.*, 1984). The diluted mucus was then centrifuged at 3000 *g* for 30 min, dialysed and freeze-dried.

Preparation of respiratory-mucin glycopeptides

The freeze-dried supernatant was digested at 37 °C with Pronase (Pronase R proteinase; Calbiochem, San Diego, CA, U.S.A.) in 0.01 M-calcium acetate buffer, pH 7.0, at an enzyme/substrate ratio of 1:40 and with a fresh addition of enzyme at

Abbreviations used: CF, cystic fibrosis; f.a.b.-m.s., fast-atom-bombardment; COSY, correlation spectroscopy.

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24 h. After 48 h, the mixture was centrifuged and the supernatant was dialysed and freeze-dried.

Portions (200 mg) of freeze-dried Pronase-digested supernatant were submitted to gel filtration on a Sepharose CL-4B (Pharmacia Uppsala, Sweden) column (2.5 cm × 48 cm) equilibrated and eluted with 0.2 M-NaCl/0.1 M-Tris/HCl buffer, pH 8.0. Fractions (10 ml) were collected and analysed for absorbance at 278 nm and for hexose by an automated orcinol assay (Demaille *et al.*, 1965). Three fractions, F₁, F₂ and F₃, were separated, dialysed and freeze-dried.

Isolation of oligosaccharide-alditols

O-Linked oligosaccharides were released from fraction F₂ by alkaline-borohydride treatment in 2 M-NaBH₄/0.05 M-NaOH at 45 °C for 16 h (Roussel *et al.*, 1975). The solution was applied to a column (2.5 cm × 54 cm) of AG 50W X8 (Bio-Rad Laboratories, Richmond, CA, U.S.A.) in order to remove salts, and then the mixture was fractionated by ion-exchange chromatography (AG 1 X2) and gel-filtration chromatography (Bio-Gel P-4) according to acidity and molecular size respectively as previously described (Lamblin *et al.*, 1977b). By this procedure, four pools of oligosaccharide-alditols were obtained: Ic (neutral), IIc (sialylated) and IIIc and IVc (sulphated).

The sulphated oligosaccharide fraction IIIc was subsequently applied to a column (1 cm × 150 cm) of Bio-Gel P-2 (200–400 mesh; Bio-Rad Laboratories) with 0.1 M-ammonium acetate as eluent, resulting in the separation of six oligosaccharide fractions (IIIc₁–IIIc₆).

Fractionation of acidic oligosaccharide-alditols of fraction IIIc₆ was carried out by reverse-phase h.p.l.c. on an Ultrasphere IP (particle size 5 µm; Beckman, Palo Alto, CA, U.S.A.) column (25 cm × 4.6 mm) run isocratically with water as eluent at a flow rate of 0.5 ml/min at room temperature. Oligosaccharide-alditols were detected by measuring the absorbance at 206 nm.

Analytical methods

Amino acid analyses were performed as previously described (Lamblin *et al.*, 1977b).

Sugar analysis was carried out by g.l.c. of trimethylsilyl derivatives of methyl glycosides formed by methanolysis in 1.5 M-HCl in methanol at 80 °C for 24 h (Lamblin *et al.*, 1984a). *N*-Acetylneuraminic acid and sulphate were measured as previously described (Roussel *et al.*, 1975).

Fast-atom-bombardment m.s.

F.a.b.-m.s. of native oligosaccharide-alditols was performed with a Kratos MS-50 mass spectrometer. The samples (1 µg) were applied to the target in aqueous solution, and glycerol was used as matrix. The target was bombarded with Xe atoms having a kinetic energy equivalent to 9 keV. The spectra were recorded in negative-ion mode at 7 keV acceleration voltage, in a mass-controlled linear scan at a resolution of 300 p.p.m.

400 MHz ¹H-n.m.r. spectroscopy

Oligosaccharide-alditols were repeatedly exchanged in ²H₂O (99.96 atom% ²H; Aldrich Chemical Co., Milwaukee, WI, U.S.A.) with intermediate freeze-drying, and were analysed with a Bruker AM-400 WB spectrometer operating at 400 MHz in the Fourier-transform mode at a probe temperature of 300 K. Chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulphonate, but were actually measured indirectly to acetone in ²H₂O (γ = 2.225 p.p.m.).

The two-dimensional homonuclear COSY 45 experiments were performed by use of the standard Bruker pulse program COSY.

RESULTS

The carbohydrate composition of bronchial mucin glycopeptides from patient Lab... suffering from CF (fraction 2 in Fig. 1) is given in Table 1.

An 890 mg amount of glycopeptides F₂ was obtained from 650 ml of sputum. Fraction F₂ was submitted to alkaline-borohydride degradation and fractionated by ion-exchange

Fig. 1. Chromatography on a Sepharose CL-4B column (4 cm × 50 cm) of mucus (Lab...) supernatant after Pronase digestion (210 mg)

The column was eluted with 0.2 M-NaCl/0.1 M-Tris/HCl buffer, pH 8.0. Fractions (10 ml) were collected and analysed for absorbance at 278 nm (○) and for hexose (—). The fractions indicated by horizontal bars were dialysed and freeze-dried.

Table 1. Chemical composition of mucus glycopeptides (F₂) from CF sputum after Pronase treatment, and Sepharose 4B chromatography

Component	Amount (mmol/g)	Total (% by mass)
Amino acids		
Aspartic acid	26	
Threonine	330	
Serine	140	
Glutamic acid	39	
Proline	119	
Glycine	63	
Alanine	97	
Valine	39	
Methionine	6	
Isoleucine	22	
Leucine	43	
Tyrosine	2	
Phenylalanine	10	
Histidine	21	
Lysine	19	
Arginine	23	
Total	999	9.8
Carbohydrates		
<i>N</i> -Acetylneuraminic acid	240	
Fucose	1210	
Galactose	1180	
<i>N</i> -Acetylglucosamine	1030	
<i>N</i> -Acetylgalactosamine	510	
Total	4170	82.7
Sulphate	235

Fig. 2. Fractionation of fraction III obtained after AG 1X 2 column chromatography by chromatography on a Bio-Gel P-4 column (2 cm × 98 cm) eluted with 0.1 M-ammonium acetate

Fractions (10 ml) were collected and analysed for hexose (—). Fractions a, b and c indicated by horizontal bars were freeze-dried.

Fig. 3. Fractionation of fraction IIIc by chromatography on a Bio-Gel P-2 column (1 cm × 150 cm) eluted with 0.1 M-ammonium acetate

Fractions (2 ml) were collected and analysed for hexose (—). Fractions indicated by horizontal bars were freeze-dried.

chromatography into four fractions (Lamblin *et al.*, 1977b). Fraction III, eluted from the Dowex AG 1 X2 with 0.025 M- H_2SO_4 , was subfractionated into fractions IIIa, IIIb and IIIc by chromatography on Bio-Gel P-4 (Fig. 2) (Lamblin *et al.*, 1977b). Fraction IIIc (18 mg) corresponded to a mixture of sulphated and sialylated oligosaccharide-alditols, as shown by its carbohydrate composition (Table 2).

In order to separate the different oligosaccharide-alditols present in fraction IIIc, this fraction was subsequently applied to a Bio-Gel P-2 column eluted with 0.1 M-ammonium acetate (Fig. 3). Five main fractions were obtained and pooled as indicated: fractions IIIc₂ (1 mg), IIIc₃ (1.5 mg), IIIc₄ (2.9 mg), IIIc₅ (2.2 mg) and IIIc₆ (1.5 mg) (Fig. 3). The carbohydrate compositions of the subfractions from pool IIIc are compiled in Table 3. All fractions contain *N*-acetylgalactosaminitol, *N*-acetylglucosamine, galactose and fucose. Fractions IIIc₂ and IIIc₃ contain both sialic acid and sulphate, whereas fractions IIIc₄, IIIc₅ and IIIc₆ contain sulphate but no sialic acid. On the assumption of one residue of *N*-acetylgalactosaminitol per oligosaccharide-alditol molecule, the components obtained have an average carbohydrate chain

length from 4.8 sugar residues (fraction IIIc₆) to 10 sugar residues (fraction IIIc₂).

Oligosaccharide-alditols were analysed by f.a.b.-m.s. in the negative-ion mode. Only the spectrum of fraction IIIc₆ was interpretable, the others being too complex and corresponding to mixtures of large numbers of oligosaccharide-alditols.

The f.a.b.-m.s. spectrum of fraction IIIc₆ (Fig. 4) shows the presence of three major ions at *m/z* 975, 829 and 667. A minor ion is also observed at *m/z* 1121, in low amount (the signal is increased 10-fold).

The *m/z* 975 ion may correspond to a sulphated pentasaccharide-alditol with sulphate, fucose, galactose, *N*-acetylhexosamine and *N*-acetylhexosaminitol in the molar proportions 1:1:2:1:1. The *m/z* 829 ion may correspond either to a fragment ion formed by loss of fucose from the *m/z* 975 ion or to the molecular ion of a sulphated tetrasaccharide-alditol with sulphate, galactose, *N*-acetylhexosamine and *N*-acetylhexosaminitol in the molar proportions 1:2:1:1. Equally, the *m/z* 667 ion may correspond either to the molecular ion of a sulphated trisaccharide-alditol or to a fragment ion formed by the loss of fucose and galactose from the *m/z* 975 ion. N.m.r. data and further fractionation of fraction IIIc₆ by h.p.l.c. could give the answer.

Table 2. Carbohydrate and sulphate composition of fractions IIIa, IIIb and IIIc

The molar ratio of the oligosaccharide-alditols was calculated on the basis of one residue of *N*-acetylgalactosaminitol (or *N*-acetylgalactosaminitol + *N*-acetylgalactosamine) per molecule. The carbohydrate chain length was calculated as the ratio of total carbohydrate residues to the sum of *N*-acetylgalactosamine and *N*-acetylgalactosaminitol.

	Fraction IIIa		Fraction IIIb		Fraction IIIc	
	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)
Carbohydrates						
<i>N</i> -Acetylneuraminic acid	480	1.2	400	0.7	810	1
Fucose	855	2.2	725	1.4	585	0.7
Galactose	1170	3	1150	2.1	1070	1.3
<i>N</i> -Acetylglucosamine	760	1.9	805	1.5	680	0.8
<i>N</i> -Acetylgalactosamine	295		245		180	
<i>N</i> -Acetylgalactosaminitol	95	1	290	1	660	1
Sulphate	185	0.5	295	0.6	720	0.9
Carbohydrate chain length		9.3		6.7		4.8

Table 3. Carbohydrate and sulphate composition of fractions eluted from the Bio-Gel P-2 column (IIIc₂, IIIc₃, IIIc₄, IIIc₅ and IIIc₆)

The molar ratio of the oligosaccharide-alditols was calculated on the basis of one residue of *N*-acetylglactosaminitol (or *N*-acetylglactosamine and *N*-acetylglactosaminitol) per molecule. The carbohydrate chain length was calculated as the ratio of total carbohydrate residues to the sum of *N*-acetylglactosamine and *N*-acetylglactosaminitol.

	Fraction IIIc ₂		Fraction IIIc ₃		Fraction IIIc ₄		Fraction IIIc ₅		Fraction IIIc ₆	
	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)	(μ mol/g)	(molar proportions)
Carbohydrates										
<i>N</i> -Acetylneuraminic acid	150	0.4	440	0.8	0		0		0	
Fucose	1790	5.0	1640	2.9	1150	1.9	840	1.6	420	0.7
Galactose	920	2.6	1300	2.4	1155	1.9	1150	2.0	1270	2.1
<i>N</i> -Acetylglucosamine	355	1	390	0.7	590	1.0	615	0.9	590	1.0
<i>N</i> -Acetylglactosamine	270		120		0			0		0
<i>N</i> -Acetylglactosaminitol	90	1	430	1	605	1	705	1	610	1
Sulphate	925	2.5	370	0.7	495	0.8	850	1.2	690	1.1
Carbohydrate chain length		10		7.8		5.8		5.5		4.8

The one-dimensional ¹H-n.m.r. spectrum of fraction IIIc₆ is depicted in Fig. 5. The chemical shifts of the H-2 and H-5 resonances of *N*-acetylglactosaminitol (at δ 4.40 and 4.27 p.p.m. respectively) are characteristic of the core Gal β 1-3(GlcNAc β 1-6)-GalNAc-ol (Vliegthart *et al.*, 1981).

On the COSY spectrum (Fig. 6), the H-1 resonance observed at δ 4.587 p.p.m. is connected to the H-2 (δ 3.670 p.p.m.), H-3 (δ 4.337 p.p.m.) and H-4 (δ 4.292 p.p.m.) resonances of the sugar unit. In particular, the low coupling constant $J_{4,5}$ indicates the sugar to be a galactose residue. Its downfield-shift H-3 resonance is attributable to the C-3 substitution by a sulphate residue.

The fucose residue is α -1,2-linked to a Gal³, as shown by the typical H-1, H-5 and H-6 resonances of Fuc and H-1 resonance of Gal³, observed at 4.572 p.p.m., as in reference compounds 11 and 12 (Lamblin *et al.*, 1984b). The H-1 resonance of GlcNAc⁶ (at δ 4.572 p.p.m.) of the Gal β 1-4GlcNAc unit is characteristic of the β -1,4-substituted GlcNAc⁶ residue.

On the basis of these observations, the following structure was attributed to the main oligosaccharide-alditol of fraction IIIc₆:

The second compound, devoid of fucose, possess an H-1 Gal resonance at 4.464 p.p.m., characteristic of the terminal non-reducing, Gal³ residue, and consequently its structure was identified as:

The two oligosaccharides A and B contained in fraction IIIc₆ were in the ratio 7:3. These structures correspond to the major ions at m/z 975 and 829 observed by f.a.b.-m.s. These oligosaccharides were further separated by h.p.l.c. (as described in the Materials and methods section), and the n.m.r. spectra obtained from the two major peaks 4 and 13 (Fig. 7) confirmed that they corresponded to the structures determined in fraction IIIc₆ (Figure 8): peak 4 is the oligosaccharide B and peak 13 is the oligosaccharide A. No peak corresponding to a sulphated trisaccharide could be obtained; therefore the ion at m/z 667 is not a molecular ion and represents an $[(S_n + H) - H]^+$ even-electron fragment ion (Kamerling *et al.*, 1983).

DISCUSSION

Human respiratory mucins have been shown to be very heterogeneous with regard to acidity, molecular size and structure of their carbohydrate chains (Roussel *et al.*, 1975; Lamblin *et al.*, 1979, 1984a,b; Breg *et al.*, 1987, 1988; Klein *et al.*, 1988; Van Halbeek *et al.*, 1988). Sulphated chains are abundant, especially in the case of mucins from patients with CF. However, our knowledge of the structures of sulphated chains has been so far limited owing to the predominance of sulphate ester on long-chain oligosaccharides (Roussel *et al.*, 1975; Lamblin *et al.*, 1979), which makes it very hard to isolate them in a pure state necessary for further structure determinations.

In the present work, ¹H-n.m.r. spectroscopy in combination with f.a.b.-m.s. in the negative-ion mode were used for the investigation of two new structures of sulphated oligosaccharides with core 2 and sulphate 3-linked to galactose.

The n.m.r. data presented in Table 4 illustrate the possibility

Fig. 6. Two-dimensional homonuclear COSY spectrum of fraction IIIc₆.

Fig. 7. Fractionation of fraction IIIc₆ by h.p.l.c.

Elution was performed isocratically with water on an Ultrasphere C₁₈ IP (particle size 5 μ m) column. Oligosaccharide peaks were detected by absorbance at 206 nm. Peaks 4 and 13 were collected and freeze-dried.

Fig. 8. 400 MHz ¹H-n.m.r. spectrum of compounds A and B obtained after fractionation of fraction IIIc₆ by h.p.l.c.

Table 4. Chemical shifts of structural reporter-group protons of the constituent monosaccharides for sulphated oligosaccharide-alditols derived from respiratory mucins of a CF patient and for reference compounds 11 and 12 (Lamblin *et al.*, 1984*a,b*)

Key: ◇, GalNac-ol; ■, Gal; ●, GlcNAc; □, Fuc. A superscript at the name of the sugar indicates to which position of the adjacent monosaccharide it is glycosidically linked.

		Chemical shift (p.p.m.)			
		Compound 11	Compound B	Compound 12	Compound A

Residue	Reporter Group				
GalNac-ol	H-2	4.399	4.391	4.405	4.400
	H-3	4.061	4.060	4.085	4.085
	H-4	3.460	3.468	3.499	3.502
	H-5	4.285	4.271	4.269	4.265
	NAc	2.067	2.067	2.055	2.056
Gal ³	H-1	4.463	4.464	4.573	4.572
	H-4	3.898	3.901	3.923	3.923
GlcNAc ⁶	H-1	4.557	4.560	4.570	4.572
	H-6	3.996	4.006	3.998	4.006
	NAc	2.066	2.064	2.055	2.056
Gal ⁴	H-1	4.469	4.587	4.469	4.587
	H-2	—	3.670	—	3.670
	H-3	—	4.337	—	4.337
	H-4	3.923	4.292	3.923	4.292
Fuc ²	H-1	—	—	5.221	5.225
	H-5	—	—	4.278	4.288
	CH ₃	—	—	1.244	1.245

Other sulphated sugars have been identified in different mucins. The presence of galactose 6-sulphate has been already suggested in human respiratory (Roussel *et al.*, 1975; Mawhinney *et al.*, 1987) and rat gastric mucins (Liau & Horowitz, 1982), whereas *N*-acetylglucosamine 6-sulphate has been identified in ovomucin (Strecker *et al.*, 1989) and rat salivary (Green & Embery, 1987) and gastric mucins (Carter *et al.*, 1988). *N*-Acetylglucosamine 4-sulphate has been found in dog (Lombart & Winzler, 1974), rat (Liau & Horowitz, 1982) and monkey (Tabak & Levine, 1981) salivary mucins.

In the present work, f.a.b.-m.s. of the four pools of oligosaccharides contained in the other fractions eluted from the Bio-Gel P-2 column (IIc₂, IIc₃, IIc₄ and IIc₅) has shown that they corresponded to very complex mixtures of sulphated and sulphated/sialylated oligosaccharides (results not shown). Using a different fractionation procedure, Mawhinney *et al.* (1987) have already described small linear sulphated oligosaccharides from respiratory mucins with core 1 or 3 and sulphate 6-linked to galactose in human respiratory mucin from CF patients.

Therefore, in CF respiratory mucins, sulphate may be located, at least, at the C-6 (Mawhinney *et al.*, 1987) or at the C-3 of terminal galactose residues, and it is highly probable that several sulphotransferases are responsible for the sulphation of those respiratory mucins.

In the future it will be of interest to set up methods allowing the isolation of the different sulphated oligosaccharides, to compare their distribution (6-sulphated versus 3-sulphated) in mucins from patients suffering from CF and from other bronchial disorders and to define their role in the selection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the airways of CF patients.

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REFERENCES

- Boat, T. F., Cheng, P. W. & Wood, R. E. (1977) *Mod. Probl. Paediatr.* **19**, 141–152
- Breg, J., Van Halbeek, H., Vliegthart, J. F. G., Lamblin, G., Houvenaghel, M. C. & Roussel, P. (1987) *Eur. J. Biochem.* **168**, 57–68
- Breg, J., Van Halbeek, H., Vliegthart, J. F. G., Klein, A., Lamblin, G. & Roussel, P. (1988) *Eur. J. Biochem.* **171**, 643–654
- Capon, C., Leroy, E., Wieruszski, J. M., Picart, G., Strecker, G., Montreuil, J. & Fournet, B. (1989) *Eur. J. Biochem.* **182**, 139–152
- Carter, S. R., Slomiany, A., Gwozdinski, K., Liau, Y. H. & Slomiany, B. L. (1988) *J. Biol. Chem.* **263**, 11977–11984
- Cheng, P. W., Boat, T. F., Cranfill, K., Yankaskas, J. R. & Boucher, R. C. (1989) *J. Clin. Invest.* **84**, 68–72
- Contreras, R. R., Kamerling, J. P., Breg, J. & Vliegthart, J. F. G. (1988) *Carbohydr. Res.* **179**, 411–418
- Demaille, J., Dautrevaux, M., Havez, R. & Biserte, G. (1965) *Bull. Soc. Chim. Fr.* 3506–3511
- Elgavish, A. & Meezan, E. (1988) *Biochem. Biophys. Res. Commun.* **152**, 99–106
- Eliason, R., Mossberg, B., Camner, P. & Afzelius, B. A. (1977) *N. Engl. J. Med.* **297**, 1–6
- Green, D. R. J. & Embery, G. (1987) *Arch. Oral Biol.* **32**, 392–399
- Hoiby, N. (1988) *Chest* **94** (Suppl.), 97s–103s
- Juranka, P. F., Zastawny, R. L. & Ling, V. (1989) *FASEB J.* **3**, 2583–2592
- Kamerling, J. P., Heerma, W., Vliegthart, J. F. G., Green, B. N., Lewis, I. A. S., Strecker, G. & Spik, G. (1983) *Biomed. Mass Spectrom.* **10**, 420–425
- Kato, Y. & Spiro, R. G. (1989) *J. Biol. Chem.* **264**, 3364–3371
- Klein, A., Lamblin, G., Lhermitte, M., Roussel, P., Breg, J., Van Halbeek, H. & Vliegthart, J. F. G. (1988) *Eur. J. Biochem.* **171**, 631–642
- Knowles, M. R., Stutts, M. J., Yankaskas, J. R., Gatz, J. T. & Boucher, R. C. (1986) *Clin. Chest Med.* **7**, 285–297
- Lamblin, G., Lafitte, J. J., Lhermitte, M., Degand, P. & Roussel, P. (1977a) *Mod. Probl. Paediatr.* **19**, 153–164

- Lamblin, G., Humbert, P., Lhermitte, M., Degand, P. & Roussel, P. (1977b) *Clin. Chim. Acta* **77**, 425–436
- Lamblin, G., Lhermitte, M., Degand, P., Roussel, P. & Slayter, H. S. (1979) *Biochimie* **61**, 23–43
- Lamblin, G., Boersma, A., Klein, A., Roussel, P., Van Halbeek, H. & Vliegthart, J. F. G. (1984a) *J. Biol. Chem.* **259**, 9051–9058
- Lamblin, G., Boersma, A., Lhermitte, M., Roussel, P., Mutsaers, J. H. G. M., Van Halbeek, H. & Vliegthart, J. F. G. (1984b) *Eur. J. Biochem.* **143**, 227–236
- Liau, Y. H. & Horowitz, M. I. (1982) *J. Biol. Chem.* **257**, 4709–4718
- Lombart, C. G. & Winzler, R. J. (1974) *Eur. J. Biochem.* **49**, 77–86
- Mawhinney, T. P., Adelstein, E., Morris, D. A. Mawhinney, A. M. & Barbero, G. J. (1987) *J. Biol. Chem.* **262**, 2994–3001
- Quinton, P. M. (1989) *Clin. Chem.* **35**, 726–730
- Riordan, J. R., Rommens, J. M., Kerem, B. S., Alon, N., Rozmahel, R., Grzelckzak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J. L., Drumm, M. C., Lannuzzy, M. C., Collins, F. C. & Tsui, L. C. (1989) *Science* **245**, 1066–1073
- Roussel, P., Lamblin, G., Degand, P., Walker-Nasir, E. & Jeanloz, R. W. (1975) *J. Biol. Chem.* **250**, 2214–2222
- Slayter, H. S., Lamblin, G., Le Treut, A., Galabert, C., Houdret, N., Degand, P. & Roussel, P. (1984) *Eur. J. Biochem.* **142**, 209–218
- Spiro, R. G. & Bhoyroo, V. D. (1988) *J. Biol. Chem.* **263**, 14351–14358
- Strecker, G., Wieruszski, J. M., Martel, C. & Montreuil, J. (1989) *Carbohydr. Res.*, **185**, 1–13
- Tabak, L. A. & Levine, M. J. (1981) *Arch. Oral Biol.* **26**, 315–327
- Van Halbeek, H., Breg, J., Vliegthart, J. F. G., Klein, A., Lamblin, G. & Roussel, P. (1988) *Eur. J. Biochem.* **177**, 443–460
- Vliegthart, J. F. G., Van Halbeek, H. & Dorland, L. (1981) *Pure Appl. Chem.* **53**, 45–77

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