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The Broad Diversity of Neutral and Sialylated Oligosaccharides Derived from Human Salivary Mucins[†]

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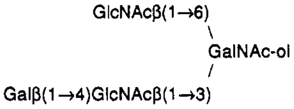
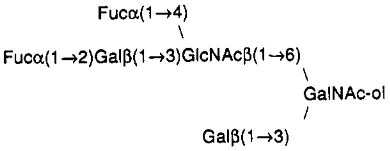
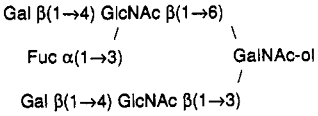
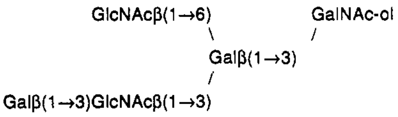
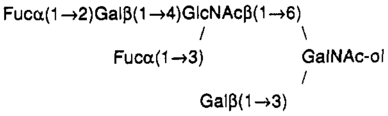
ABSTRACT: Mucin glycopeptides were prepared from the salivary mucins of 20 healthy donors with blood group O. The carbohydrate chains of the high-molecular-weight mucins were released by alkaline borohydride treatment. Neutral and monosialylated oligosaccharide-alditols were purified by ion-exchange chromatography, gel filtration, and HPLC. The structures of the oligosaccharide-alditols were determined by high-resolution ^{1}H-NMR spectroscopy in combination with fast atom bombardment mass spectrometry and methylation analysis. Thirty-seven oligosaccharide-alditols were characterized and illustrate the extreme diversity of the salivary mucins carbohydrate chains. This diversity might represent a mosaic of bacterial adhesion sites and be involved in the early events of the nonimmune defense of the oral cavity. Among these 37 oligosaccharide-alditols, 31 have not been previously described in human saliva and five of these are novel structures:











Saliva has a wide array of biological functions, including digestion, mineralization, microbial attachment, and antimicrobial activity. Human saliva is a mixture of secretions produced by different glands including the major (parotid, submandibular, sublingual) and minor (palatal and labial) salivary glands. The salivary glands comprise mucous or serous acini or both. Besides amylases, cystatins, proline-rich proteins, and statherin (Ramasubbu et al., 1991), saliva contains highly glycosylated glycoproteins known as mucins. These mucins are the main glycoproteins synthesized and secreted by glands containing mucous cells (submandibular, sublingual, labial, and palatal). Two populations of salivary mucins have been characterized so far: one with molecular weights above 10^6, and the other one with molecular weights ranging from 2×10^5 to 2.5×10^5 (Schrager et al., 1971; Loomis et al., 1987; Veerman et al., 1989; Kawagishi et al., 1990). Salivary mucins may be involved in the nonimmune protection of the oral cavity and in bacterial recognition mechanisms (Tabak et al., 1982). The carbohydrate moieties may play a role in bacterial aggregation and clearance by mucins (Cisar et al., 1986; Brack et al., 1987; Levine et al., 1978). Therefore, a knowledge of the primary structure of human salivary mucin oligosaccharide chains is important in order to elucidate mucin/bacteria recognition mechanisms.

Not much is known about the carbohydrate chains of human salivary mucins: so far, only six oligosaccharide-alditols have been identified in the low-molecular-weight salivary mucins (Reddy et al., 1985). Thus, one may wonder if the carbohydrate part of human salivary mucins is as simple as in other species or if it is as heterogeneous as the carbohydrate moiety of their bronchial (Lamblin et al., 1984; Klein et al., 1988), gastric (Slomiany et al., 1984), or colonic (Podolski et al., 1985) analogs.

In the present report, highly glycosylated regions of high-molecular-weight mucins were isolated from human total saliva and were treated by reductive β-elimination to release the oligosaccharide chains. The oligosaccharide-alditols were characterized using HPLC,[1] FAB-MS, and NMR analysis. Thirty-seven neutral and sialylated, low-molecular-mass oligosaccharide-alditols were identified. Among the 37 structures characterized, 32 have not been described previously in human salivary mucins and five of these are novel structures. This is the first demonstration of a broad diversity of carbohydrate

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[1] Abbreviations: HPLC, high-performance liquid chromatography; FAB-MS, fast atom bombardment mass spectrometry; GalNAc-ol; *N*-acetyl-D-galactosaminitol; GlcNAc, *N*-acetyl-D-glucosamine; Gal, D-galactose; Fuc, L-fucose; NAc, *N*-acetyl; NeuAc, *N*-acetylneuraminic acid.

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chains in human salivary mucins.

EXPERIMENTAL PROCEDURES

Materials. Pronase was from Calbiochem (Behring Diagnostics, La Jolla, CA); Sepharose CL-2B was from Pharmacia (Uppsala, Sweden); guanidinium chloride was from Fluka (Buchs, Switzerland); AG50WX8 (100–200 mesh) and AG1X2 (100–200 mesh) ion-exchange resins and Bio-Gel P4 (200–400 mesh) were from Bio-Rad Laboratories (Richmond, CA). Secretory IgA was a generous gift from Dr. A. Hayem (INSERM U 16, Lille, France). HPLC was performed with a Spectroflow 400 solvent-delivery system equipped with a Spectroflow 783 detector (Kratos, Ramsey, NY); the Lichrosorb-NH_2 column was from Merck (Darmstadt, FRG); the Ultrasphere ODS was from Beckman (Berkeley, CA); HPLC solvents were from Carlo Erba (Milano, Italy).

Collection of Saliva. Human total saliva (500 mL) was collected from 20 healthy donors with blood group O. It was kept frozen (−20 °C) until used. The salivary secretions were centrifuged at 3000*g* for 30 min. The pellet and supernatant were dialyzed extensively against distilled water and lyophilized. The yields were 0.815 g and 1.385 g, respectively.

Pronase Digestion of Saliva Supernatant and Fractionation by Chromatography on Sepharose CL-2B. The lyophilized supernatant (1.385 g) was digested with pronase in 0.01 M calcium acetate for 48 h at 37 °C using a final enzyme/substrate ratio of 1/40 (w/w) with a fresh addition of enzyme at 24 h. After 48 h, the mixture was centrifuged to give a pellet and a supernatant which were dialyzed extensively against distilled water and lyophilized: the yields were 0.297 g and 1.057 g, respectively. Aliquots (250 mg) of freeze-dried supernatant were submitted to gel filtration on a column of Sepharose CL-2B (2.5 × 48 cm) equilibrated and eluted with 6 M guanidinium chloride. Fractions (5 mL) were collected and analyzed for absorbance at 278 nm and for hexose by an automated orcinol assay (Demaille et al., 1965).

Purification of Oligosaccharides. Alkaline borohydride reductive degradation of salivary glycopeptides (fraction F3 from the Sepharose CL2-B column, 80 mg) was performed as described (Roussel et al., 1975) and led to a heterogeneous population of glycopeptides and reduced oligosaccharides. The mixture was applied to a column of AG50WX8, and the fraction eluted by water was further separated by ion-exchange chromatography on a Dowex AG1X2 column according to acidity. Four fractions were obtained: fraction I (neutral), fraction II (monosialylated), and fractions III and IV (sialylated and sulfated). Fractions I (22 mg) and II (6 mg) were subfractionated by gel filtation on Bio-Gel P4 into fractions Ia (4 mg), Ib (5 mg), and Ic (11 mg), and IIa (2 mg), IIb (1 mg), and IIc (1 mg), respectively. Further fractionation of neutral oligosaccharide-alditol fraction Ic was carried out by HPLC on a Lichrosorb-NH_2 column (25 × 0.46 cm internal diameter, particle size 5 μm). Elution was performed with a linear gradient of 85/15 to 60/40 (v/v) acetonitrile/water, during 60 min at room temperature and at a flow rate of 1 mL/min (Boersma et al., 1981). Oligosaccharide peaks were detected by absorbance at 206 nm. Further separation and purification was performed on an Ultrasphere ODS column (25 × 0.46 cm; particle size 5 μm) eluted isocratically with water at a flow rate of 1 mL/min (Klein et al., 1988). Fractionation of monosialylated oligosaccharide-alditol fraction IIc was carried out by HPLC on a Lichrosorb-NH_2 column (25 × 0.46 cm internal diameter; particle size 5 μm). Elution was performed with a linear gradient of 80/20 to 50/50 (v/v) acetonitrile/water containing 2.5 mM ammonium bicarbonate, during 60 min at room temperature and at a flow rate of 1 mL/min (Lamblin et al., 1983). Oligosaccharide peaks were detected by absorbance at 206 nm.

Analytical Methods. Amino acid analysis was performed as described previously (Houdret et al., 1981). Sulfate and sialic acid were measured as described (Roussel et al., 1975). Quantitative sugar analysis was carried out as described (Lamblin et al., 1984b).

FAB Mass Spectrometry. Purified oligosaccharide-alditols were methylated with methyl iodide, solid base (NaOH), and methyl sulfoxide (Ciucanu & Kerek, 1984). The methylated oligosaccharide-alditols were analyzed by FAB-MS. A Kratos concept EBEB high-resolution mass spectrometer (Kratos Analytical Instruments, Manchester, U.K.) equipped with a DS 90 (DGDG/30) data system was used in these studies. The mass spectrometer was operated at an 8-KeV accelerating potential. An Ion Tech Model B 11 NF saddle field as atom source, energized with the B 50 current regulated power supply, was scanned at 10 s/decade. The methylated oligosaccharide-alditols were dissolved in methanol containing sodium acetate (0.1%) and loaded on the copper tip with thioglycerol as the matrix.

Methylation Analysis. The methylated oligosaccharide-alditols were subjected to methanolysis and acetylation. The methyl derivatives were identified after gas–liquid chromatography by mass spectrometry (GC–MS) (Fournet et al., 1981).

^{1}H-NMR Spectroscopy. Prior to ^{1}H-NMR spectroscopic analysis, the HPLC-fractionated oligosaccharide-alditols were repeatedly treated with ^2H_2O at room temperature and pD 6. After each exchange treatment, the materials were lyophilized. Finally, each sample was redissolved in 0.4 mL of ^2H_2O (99.96 atom % ^{2}H, Aldrich). The 400-MHz ^{1}H-NMR spectroscopy was performed on a Bruker AM 400 spectrometer, operating under the control of an Aspect-3000 computer (Lille, France). The 500-MHz ^{1}H-NMR spectroscopy was performed on a Bruker AM 500 spectrometer, interfaced with an Aspect-3000 computer (Athens, GA). Experimental details have been described previously (Van Halbeek et al., 1982; Spellman et al., 1989). The probe temperature was kept at 27.0 (±0.1) °C. Chemical shifts (δ) are expressed in parts per million downfield from internal sodium 4,4-dimethyl-4-silapenthane-1-sulfonate but were actually measured by reference to internal acetone (δ = 2.225 ppm in ^2H_2O at 27 °C), with an accuracy of 0.002 ppm. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation.

RESULTS

Isolation and Purification of Human Salivary Mucin Glycopeptides. A pool of human salivas, collected from 20 donors each with blood group O, was digested by pronase. The supernatant was fractionated by gel filtration on a Sepharose CL-2B column eluted with 6 M guanidinium chloride. The elution profile permitted the distinction of four fractions (Figure 1); after dialysis and freeze-drying, the yields amounted to the following: F1 (1 mg), F2 (5 mg), F3 (80 mg), and F4 (65 mg). The chemical composition of fraction F3 is typical of mucin glycopeptides (Table I). Polyacrylamide gel electrophoresis of fraction F3 showed a pattern typical of mucin glycopeptides when stained by Schiff/periodate (data not shown). The elution volume of F3 molecules is smaller than that of secretory IgA, used as a chromatography marker, indicating an apparent molecular mass larger than 320 000.

Isolation and Purification of Oligosaccharide-alditols. Alkaline borohydride treatment of the mucin glycopeptides, followed by ion exchange chromatography (I–IV) and gel

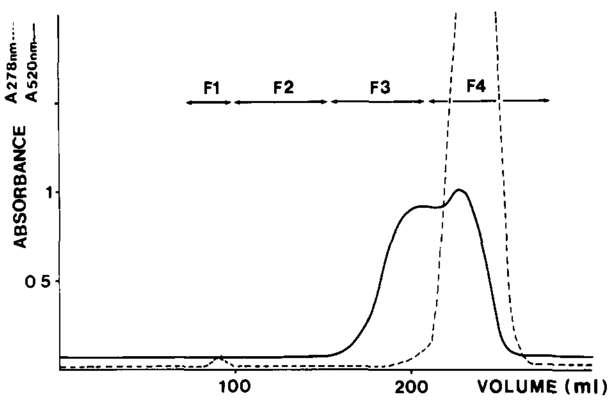

FIGURE 1: Sepharose CL-2B (2.5 × 48 cm) chromatography of pronase-treated saliva supernatant (250 mg). The eluting solution was 6 M guanidinium chloride. Representative aliquots were analyzed for neutral sugars at 520 nm (solid line) and for absorbance at 278 nm (dashed line).

Table I: Chemical Composition of Salivary Glycopeptide Fraction F3

components	amount (μmol/g)	total (% by mass)
aspartic acid	51	
threonine	408	
serine	246	
glutamic acid	83	
proline	171	
glycine	151	
alanine	159	
valine	76	
isoleucine	42	
leucine	75	
tyrosine	10	
phenylalanine	27	
histidine	36	
lysine	27	
arginine	33	
total amino acids	1595	15.6
N-acetylneuraminic acid	160	
fucose	475	
galactose	1445	
N-acetylglucosamine	1120	
N-acetylgalactosamine	510	
total carbohydrate	3710	75
sulfate	170	1.5

filtration over Bio-Gel P4 (a–c), allowed us to separate the pools of small neutral (Ic) and sialylated oligosaccharides (IIc) (Figure 2). Fraction Ic was further purified by HPLC on a normal phase column after which 23 fractions were obtained (Figure 3); subsequently, fractions 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20, 21, 22, and 23 were subfractionated on a reverse-phase column and gave fractions 7a–d, 8a, 8b, 9a, 9b, 10a–c, 11a–c, 13a–e, 14a–c, 16a–d, 17a–d, 18a–f, 19a–d, 20a–d, 21a–c, 22a–f, and 23a–c, respectively. The elution patterns of fractions 9, 10, 11, 13, 14, and 17 on this second column are shown in Figure 4.

Fractionation of monosialylated oligosaccharide-alditols (IIc) was carried out by HPLC on a normal phase column after which 10 sialylated fractions were obtained (Figure 5).

Structure Determination of Neutral Oligosaccharides

The primary structures of the oligosaccharide-alditols obtained from human salivary mucin, listed in Scheme I, were established by combining the results of methylation analysis, FAB mass spectrometry, and ^{1}H-NMR spectroscopy. The structures of the oligosaccharide-alditols were grouped into four categories on the basis of the core units. The core structure was identified from the set of ^{1}H-NMR chemical shifts of the triplet resonances of the GalNAc-ol H-2 and H-5 atoms in the $4.1 < \delta < 4.4$ region of each spectrum; the positions of these signals are known to be highly characteristic of the type of the core unit of the oligosaccharide-alditols (Van Halbeek et al., 1982; Van Halbeek, 1984).

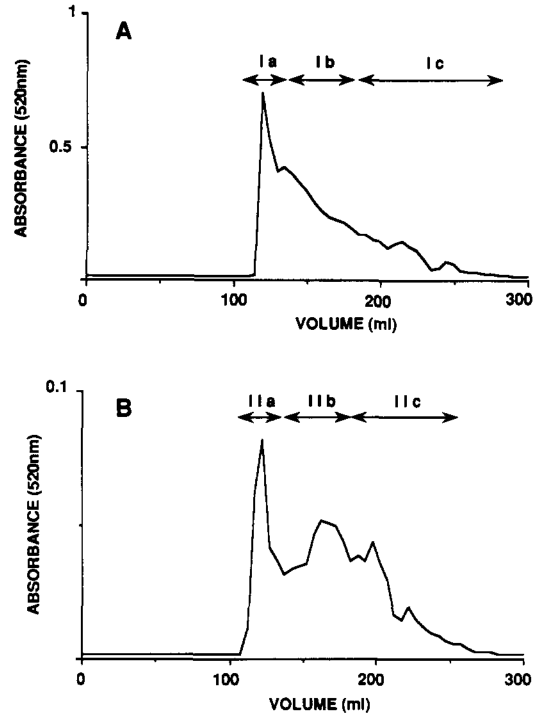

FIGURE 2: Bio-Gel P-4 (200–400 mesh) elution profile of fractions I (A) and II (B) obtained after alkaline treatment of fraction F3. The column (2 × 98 cm) was eluted with 0.1 M acetic acid. Aliquots were analyzed for neutral sugars.

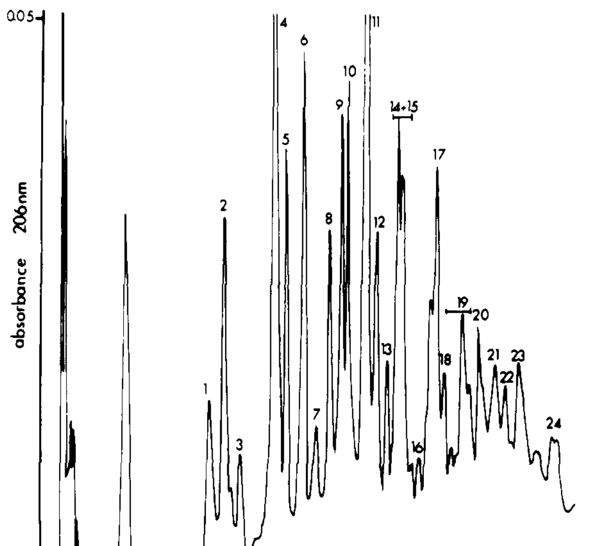

FIGURE 3: HPLC elution profile of salivary oligosaccharide-alditols of fraction Ic on a 5-μm Lichrosorb-NH$_2$ column, eluted with an acetonitrile/water gradient (85/15 to 60/40 v/v). Oligosaccharide peaks were detected by absorbance at 206 nm.

The structures in category i possess a C3-monosubstituted GalNAc-ol residue; the substituent monosaccharide is β-linked Gal. The spectra of these oligosaccharide alditols have in common the presence of the GalNAc-ol H-2/H-5 signals at

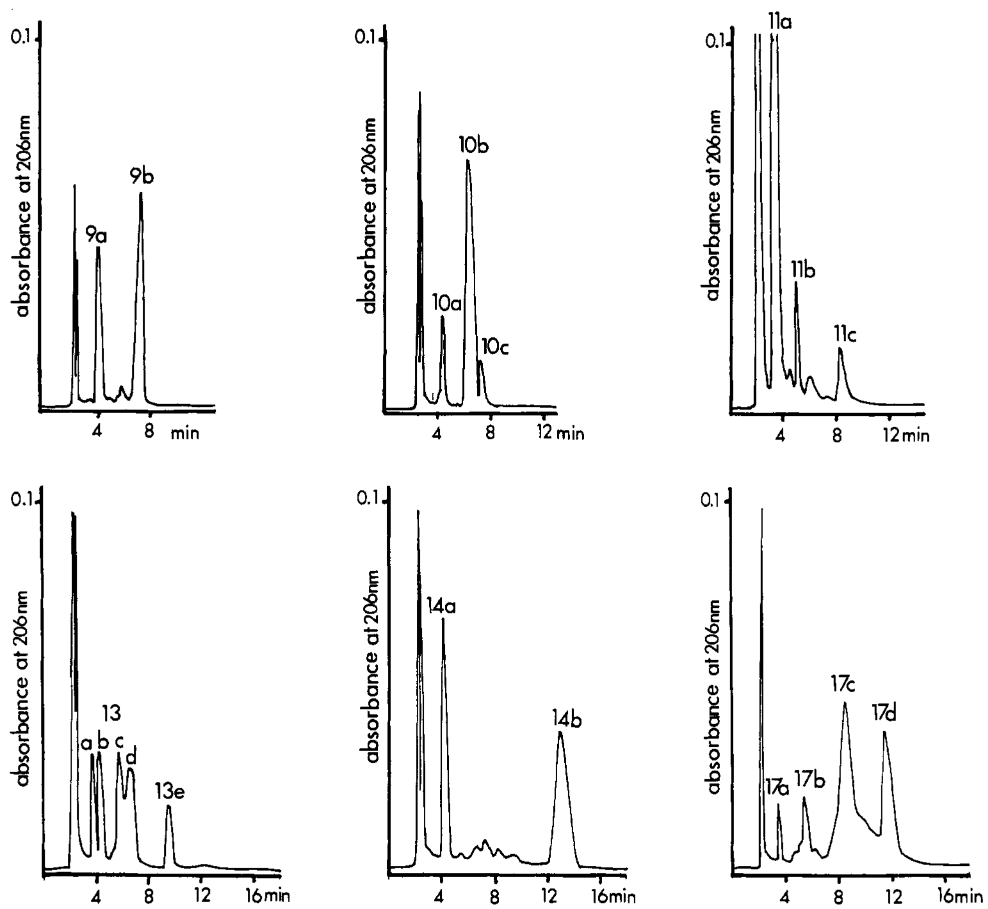

FIGURE 4: Reverse-phase HPLC elution profiles of fractions **9**, **10**, **11**, **13**, **14**, and **17** from pool Ic on an Ultrasphere ODS column, eluted isocratically with water. Oligosaccharide peaks were detected by absorbance at 206 nm.

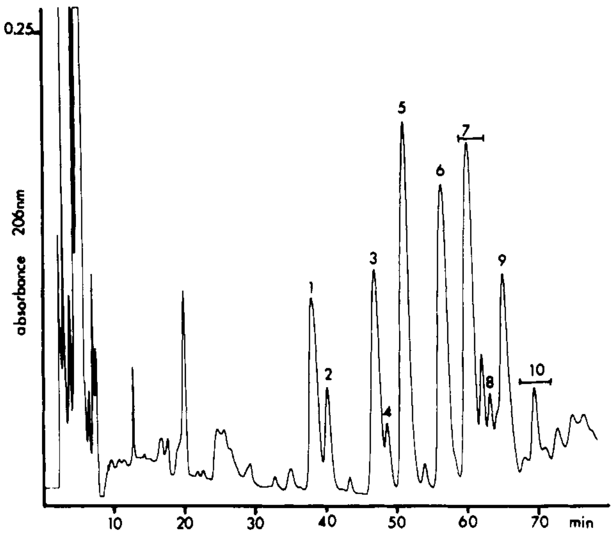

FIGURE 5: HPLC elution profile of salivary oligosaccharide-alditols of fraction IIc on a 5-μm Lichrosorb-NH_2 column, eluted with an acetonitrile/water gradient (80/20 to 50/50 v/v), containing 2.5 mM ammonium bicarbonate. Oligosaccharide peaks were detected by absorbance at 206 nm.

δ 4.39/4.18, respectively. Category ii involves structures of the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol core type. Typical chemical shifts for GalNAc-ol H-2 and H-5 atoms for compounds in this category are δ 4.39 and δ 4.26, respectively. Group iii of structures is of the GlcNAcβ(1→3)GalNAc-ol core type: the GalNAc-ol H-2 and H-5 signals in the corresponding NMR spectra are found at δ 4.28 and δ 4.13, respectively. Set iv of oligosaccharide-alditols has the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol core structural element; the GalNAc-ol H-2 and H-5 chemical shifts are δ 4.27 and δ 4.22. According to this classification, the chemical shifts of the structural-reporter groups for the oligosaccharide-alditols obtained from human salivary mucins have been compiled in Tables II–V, respectively.

(i) Structures of the Galβ(1→3)GalNAc-ol Core Type

Structures **2**, **3**, **9a**, **10a**, *and* **13a** were identified solely on the basis of their NMR features, summarized in Table II. The deduction of the primary structure of these oligosaccharide-alditols has been described previously, the compounds being obtained from different sources [namely, compounds **2**, **3**, **10B**, and **14** in Lamblin et al., (1984a) and **5** and **10** in Mutsaers et al. (1986)].

Structure **13d**. The GC-MS analysis of the methyl glycosides indicated terminal galactose and terminal glucosamine, glucosamine substituted at C3, galactose disubstituted at C3 and C6, and galactosaminitol substituted at C3. The ^{1}H-NMR spectrum indicates the presence of a pentasaccharide-alditol containing Gal, GlcNAc, and GalNAc-ol in a molar ratio of 2/2/1. This compound represents the only structure in this series where the core Gal serves as a branch point in the structure for two GlcNAc residues. The core itself is GalNAc-ol monosubstituted at C3 with β-Gal as indicated by the chemical shifts of H-2 and H-5 of GalNAc-ol at δ 4.389 and δ 4.16, respectively. The chemical shift of Gal^3 H-1 is δ 4.469. The two GlcNAc residues substituting the core galactose at C3 and C6 have their H-1 at δ 4.65 and δ 4.583, respectively.[2]

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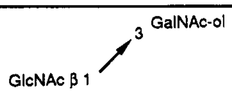
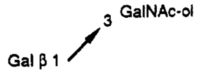
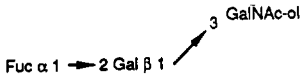
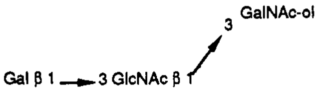
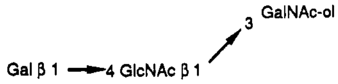
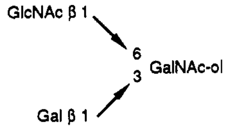
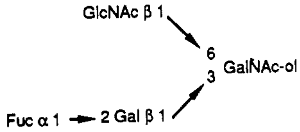
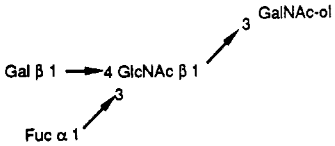
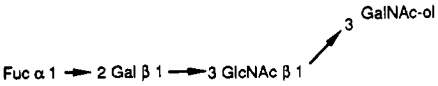
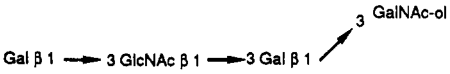
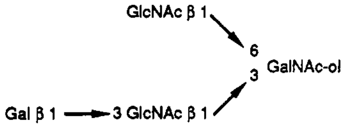
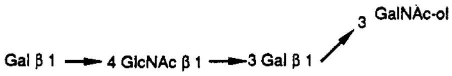
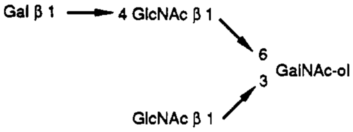
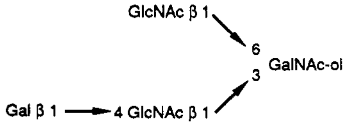
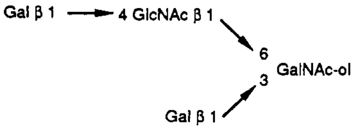
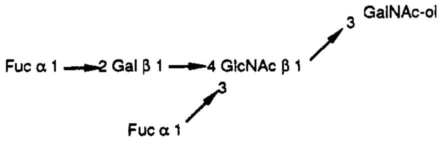
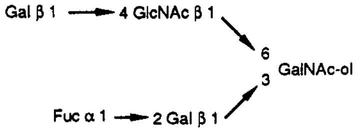
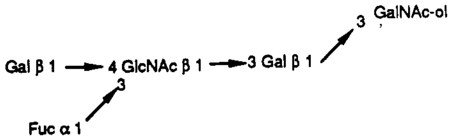
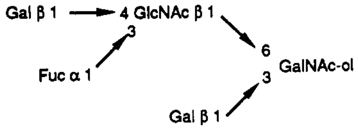
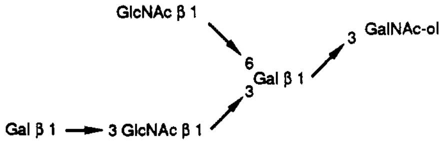
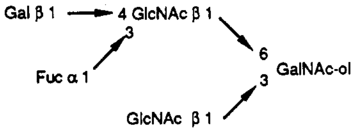
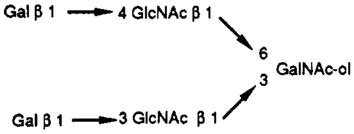
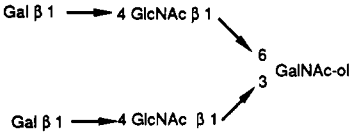
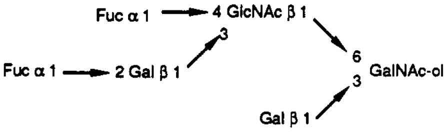
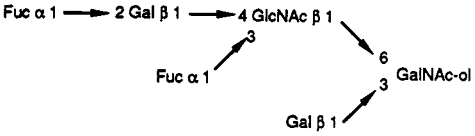
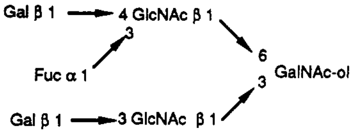
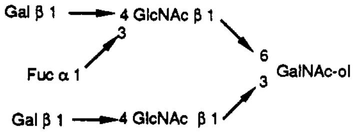
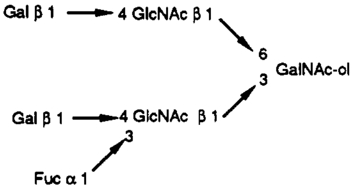
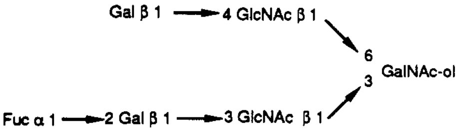
[2] A superscript at the name of a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. Two superscripts map out the pathway from the residue toward the GalNAc-ol residue.

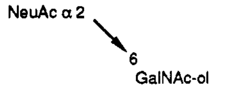
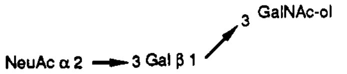
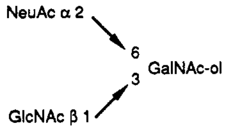
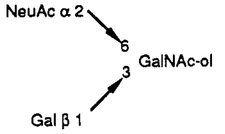
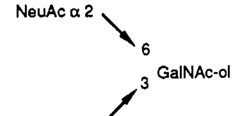
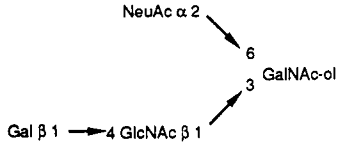
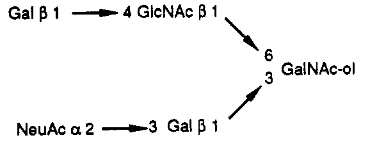
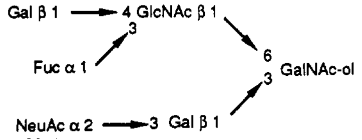
Scheme I: Structures of 37 Low-Molecular-Mass Neutral and Monosialylated Oligosaccharide-alditols Isolated from the Saliva of 20 Healthy Donors with Blood Group O[a]

compound	CCSD numbers	FCI	
1	153–160, 3944	0003eb6b	
2	64–77, 2332, 2333, 3029, 3030, 3152, 3945, 3946, 3975–3977, 4753	000287cd	
3	239–243, 3947–3948, 4902–4905	00591dc2	
4	207, 338–341, 3950	000d4193	
5	283–289, 3042, 3951, 4045	000f18b7	
6	401–408, 2440, 3953, 4075–4076, 4816–4819	000afeb7	
7d	573, 574, 4912	0063e8b0	
8a	675, 676	008599a8	
8b	443, 444, 3955	009c7d6a	
9a	511, 4845	001697bb	
9b	8223	00180c81	
10a	471–473, 2516, 3054, 3954, 4087, 4855	00193827	
10b	596–598, 3957	001c5519	
10c	novel		
11a	577–579, 581–590, 2532–2534, 3682, 3683, 3956, 4132, 4846–4850	001af17b	
11b	904	014ed883	

Scheme I (Continued)

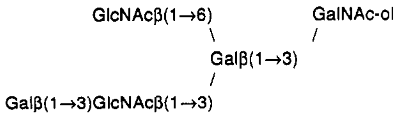
compound	CCSD numbers	FCI	
12	915, 916, 2638, 3719, 3962	007ca604	
13a	847–850, 3065, 4956	00dcd41a	
13c	944–946, 3074	00de6d6e	
13d	novel		
14b	954, 955	00dfd10c	
14c1	3959, 4207, 4871	0030c9c5	
14c2	907, 908, 910, 3707, 3960	0032a0f9	
17a	novel		
17b	novel		
17c1	8227	013de194	
17c2	novel		
17c3	8228	00b30fca	
17d	1031, 2964, 3790	00ca338c	

Scheme I (Continued)

compound	CCSD numbers	FCI	
S1	186–194, 196, 2338, 3185	066ec379	
S3	219–230, 232, 2400, 3035, 3036, 3371, 4036, 4037, 5119–5126	0bedd939	
S3	393–399, 3569, 5073	06d59267	
S4	411–413, 415–420, 425, 2468, 3468, 3469, 5063, 5064, 5068	06d42ec9	
S6.1	3647	0741d527	
S6.2	618–620, 622, 623, 3648	0743ac46	
S7	867–875, 2629, 3740, 4204, 5135–5137	0c116176	
S9	1237–1241, 2741	0d1e793a	

[a] The eight-digit/letter combination is the full complexity index (FCI) of the structure in the CCSD (complex carbohydrate structure database) (Doublet et al., 1989); it is a unique identifier for the given structure. A particular structure has more than one CCSD accession number when the structure has been determined independently more than once, as reported in different publication.

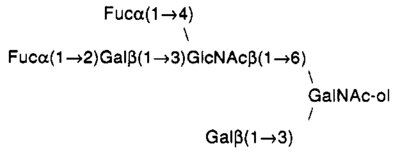
The terminal Gal is attached to GlcNAc³ and has its H-1 at δ 4.45. These assignments are readily verified by comparison to those for structure **2B** (Mutsaers et al., 1986) and structures **9a** and **20** (Lhermitte et al., 1991). The structure of compound **13d** is therefore



(ii) Structures of the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol Core Type

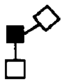
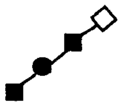
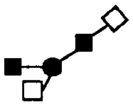
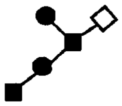
Structures **6**, **7d**, **11a**, **12**, *and* **14b**. The NMR data of these compounds (Table III) match those of the oligosaccharides-alditols **7**, **8d**, **11a**, **12**, and **16a** described previously (Klein et al., 1988).

Structure **17a** consists of GalNAc-ol, Gal, GlcNAc, and Fuc in a ratio of 1/2/1/2 as indicated by ion (M + Na)⁺ at *m*/*z* 1332 in the FAB-MS analysis of the permethylated fraction **17a**. The ¹H-NMR spectrum indicates the presence of core type 2 with the H-2 and H-5 signals of GalNAc-ol at δ 4.398 and δ 4.271, respectively. The core Gal is terminal as indicated by its H-1 resonating at δ 4.464 (compare **6**, **11a**, and **14b** in Table III). The oligosaccharide **17a** contains a Lewis^b determinant Fucα(1→2)Galβ(1→3)[Fucα(1→4)]GlcNAcβ-(1→6) with the Fuc⁴ H-1 resonating at δ 5.026 and the Fuc² H-1 and CH_3 at δ 5.155 and δ 1.243, respectively (Klein et al., 1991). For compound **17a**, these results provide the structure

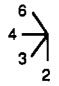


Structure **17b** consists of GalNAc-ol, Gal, GlcNAc, and Fucose in a ratio of 1/2/1/2 as indicated by pseudomolecular ion (M + Na)⁺ at *m*/*z* 1332 in the FAB-MS analysis of the permethylated fraction **17b**. The ¹H-NMR spectrum indicates the presence of one compound that can be conceived as an extension of structure **14b**; the core type 2 is defined by the H-2 and H-5 signals of GalNAc-ol resonating at δ 4.391 and δ 4.267, respectively. The Gal³ is terminal as indicated by its H-1 signal at 4.463. The GalNAc-ol residue is 6-substituted by the Y determinant as deduced from comparison with oli-

Table II: ^{1}H Chemical Shifts of Structural Reporter Groups of Monosaccharides for the HPLC-Fractionated, Neutral Human Salivary Mucin Oligosaccharide-alditols Possessing the Galβ(1→3)GalNAc-ol Core Unit[a]

		chemical shift in compound					
residue	reporter group	**2** 	**3** 	**9a** 	**10a** 	**13a** 	**13d** 
GalNAc-ol	H-2	4.391	4.396	4.394	4.396	4.39	4.389
	H-3	4.064	4.090	4.049	nd	nd	nd
	H-4	3.508	3.521	3.498	nd	nd	nd
	H-5	4.192	4.160	4.180	4.184	4.18	4.16
	NAc	2.050	2.046	2.047	2.048	2.046	2.047
Gal^3	H-1	4.477	4.582	4.465	4.463	4.464	4.469
	H-4	3.901	3.926	4.126	4.125	4.13	nd
GlcNAc^6	H-1						4.583
	H-6						nd
	NAc						2.056
Gal^4	H-1				4.480	4.460	
GlcNAc^3	H-1			4.711	4.688	nd*	4.65
	H-6			3.897	nd	nd	nd
	NAc			2.035	2.042	2.031	2.033
Gal^{3,3}	H-1			4.448			4.45
	H-4			3.915			nd
Fuc^2	H-1		5.255				
	H-5		4.275				
	CH_3		1.244				
Fuc^3	H-1					5.139	
	H-5					4.83	
	CH_3					1.177	

[a] A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. An asterisk indicates that the chemical shift was obscured by the residual HOD in the solvent; nd, not determined. Compounds **2**, **3**, and **9a** were analyzed at 400 MHz; compounds **10a**, **13a**, and **13d** were analyzed at 500 MHz. In the column headings, the structures are represented by short-hand symbolic notation: ◇ = GalNAc-ol, ■ = Gal, ● = GlcNAc, □ = Fuc. The linkage position is specified by the direction of the connecting bars as follows:



gosaccharide-alditol **20** (Klein et al., 1988). Thus, the structure proposed for **17b** is

Fucα(1→2)Galβ(1→4)GlcNAcβ(1→6)
/ \
Fucα(1→3) GalNAc-ol
/
Galβ(1→3)

(iii) Structures of the GlcNAcβ(1→3)GalNAc-ol Core Type

Structures **1**, **4**, **5**, **8a**, **8b**, *and* **11b**. The chemical shifts of the structural-reporter groups of these compounds (Table IV) are identical to those previously observed for bronchial mucus oligosaccharide-alditols **1**, **5**, **6**, **N1**, **9**, and **11b** (Breg et al., 1988), respectively.

(iv) Structures of the GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol Core Type

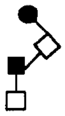
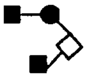
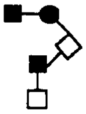
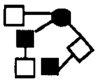
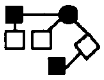
Structures **9b**, **10b**, **13c**, **14c1**, **14c2**, *and* **17d**. The NMR characteristics of these compounds (Table V) match those of oligosaccharide-alditols **9Bb** (Lhermitte et al., 1991), **10b**, **3a$_2$**, **15b** (Breg et al., 1988), compound **15.1B** (Lamblin et al., 1984), and compound **19** (Breg et al., 1988), respectively. As an example, the 400-MHz ^{1}H-NMR spectrum of fraction 9b is shown in Figure 6.

Structure **10c**. FAB-MS analysis of permethylated fraction **10c** showed the presence of only one pseudomolecular ion (M + Na)$^+$ at m/z 1025, indicating a tetrasaccharide constituted of GalNAc-ol, Gal, GlcNAc in a ratio of 1/1/2. The GC-MS analysis of the methyl glycosides indicated terminal galactose and terminal glucosamine, glucosamine substituted at C4, and galactosaminitol substituted at C3 and C6, confirmed by the presence of trimethylanhydrogalactosaminitol (Wieruszeski et al., 1987). The ^{1}H-NMR analysis revealed that structure **10c** is an isomer of structure **9b** with terminal galactose linked β1→4 to the core GlcNAc^3. The core is defined by the GalNAc-ol signals at 4.284 for H-2 and δ 4.236 for H-5. The H-1 doublet at δ 4.546 is attributed to the GlcNAc^6 residue in the terminal position (compare with **9b**). The chemical shifts of the anomeric proton and NAc signals of GlcNAc^3 at δ 4.628 and δ 2.079 and the terminal Gal H-1 signal at δ 4.456 point to the occurrence of a type 1 disaccharide attached to the C3 of GalNAc-ol and compare favorably to structure **14c1**. Structure **10c** is therefore

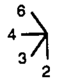
GlcNAcβ(1→6)
\
GalNAc-ol
/
Galβ(1→4)GlcNAcβ(1→3)

Structures **17c1**, **17c2**, *and* **17c3**. FAB-MS analysis of the permethylated fraction **17c** showed the presence of a single pseudomolecular ion (M + Na)$^+$ at m/z 1403, indicating one or more hexasaccharides with GalNAc-ol, Gal, GlcNAc, and Fuc in a ratio of 1/2/2/1. The GC-MS analysis of the methyl glycosides indicated terminal galactose and terminal fucose. Glucosamine was found 3,4-disubstituted, 3-monosubstituted, and 4-monosubstituted; galactosaminitol was found disubstituted at C3 and C6 (confirmed by the presence of trimethylanhydrogalactosaminitol). The ^{1}H-NMR spectrum of fraction **17c** (Figure 7) shows that this fraction contains several hexasaccharide-alditols all having in common the core element GlcNAcβ(1→6)[GlcNAcβ(1→3)]GalNAc-ol. The spectrum is reminiscent of that of fraction **19** from nonsecretory respiratory mucin oligosaccharides (Lhermitte et al., 1991). The

Table III: ^{1}H Chemical Shifts of Structural Reporter Groups of Monosaccharides for the HPLC-Fractionated, Neutral Human Salivary Mucin Oligosaccharide-alditols Possessing the GlcNAcβ(1→6)[Galβ(1→3)]GalNAc-ol Core Unit[a]

residue	reporter group	chemical shift in compound						
		6	**7d**	**11a**	**12**	**14b**	**17a**	**17b**
								
GalNAc-ol	H-2	4.391	4.401	4.391	4.402	4.390	4.398	4.391
	H-3	4.059	nd	4.060	4.084	4.058	nd	nd
	H-4	4.470	nd	3.467	3.498	3.455	nd	nd
	H-5	4.276	4.25	4.279	4.254	4.267	4.271	4.267
	NAc	2.066	2.055	2.066	2.055	2.068	2.048	2.066
Gal^3	H-1	4.465	4.574	4.464	4.573	4.463	4.464	4.463
	H-4	3.901	nd	3.901	3.926	3.899	nd	nd
GlcNAc^6	H-1	4.538	4.553	4.560	4.573	4.561	4.585	4.546
	H-6	3.932	nd	3.997	3.999	4.010	nd	nd
	NAc	2.066	2.058	2.063	2.055	2.055	2.069	2.057
Gal^4	H-1			4.469	4.472	4.447		4.498
	H-4			3.926	3.926	3.923		nd
Gal^{3,6}	H-1						4.448	
	H-4						nd	
Fuc^2	H-1		5.224		5.222		5.155	5.279
	H-5		4.27		4.275		4.353	4.270
	CH_3		1.246		1.246		1.250	1.234
Fuc^3	H-1					5.107		5.098
	H-5					4.826		4.85
	CH_3					1.174		1.166
Fuc^4	H-1						5.026	
	H-5						4.82	
	CH_3						1.243	

[a] A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. Compounds **6**, **11a**, **12**, and **14b**, were analyzed at 400 MHz; compounds **7d**, **17a**, and **17b** were analyzed at 500 MHz. In the column headings, the structures are represented by short-hand symbolic notation: ◇ = GalNAc-ol, ■ = Gal, ● = GlcNAc, □ = Fuc. The linkage position is specified by the direction of the connecting bars as follows:



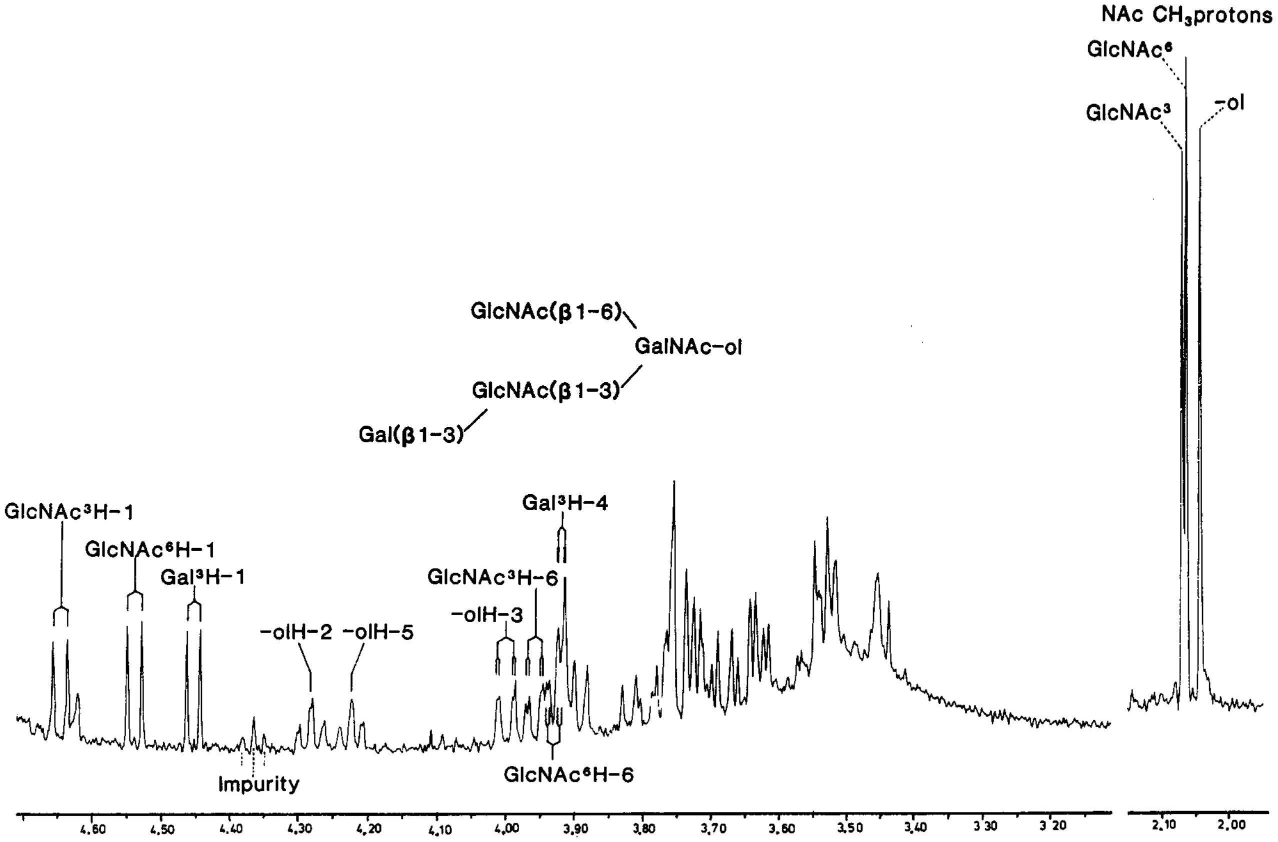
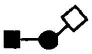
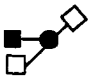
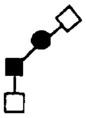
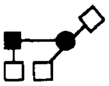
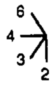


FIGURE 6: A 400-MHz ^{1}H-NMR spectrum (^2H_2O, p^{2}H 7, 27 °C) of neutral salivary oligosaccharide-alditol fraction **9b**.

Table IV: ^{1}H CHemical Shifts of Structural Reporter Groups of Monosaccharides for the HPLC-Fractionated, Neutral Human Salivary Mucin Oligosaccharide-alditols Possessing the GlcNAcβ(1→3)GalNAc-ol Core Unit[a]

		chemical shift compound					
		1	4	5	8a	8b	11b
residue	reporter group						
GalNAc-ol	H-2	4.285	4.287	4.286	4.272	4.262	4.270
	H-3	3.994	4.011	4.000	3.998	3.981	nd
	H-4	3.547	3.562	3.552	3.510	nd	nd
	H-5	4.138	4.134	4.139	4.124	4.110	4.132
	NAc	2.037	2.034	2.038	2.031	2.037	2.030
GlcNAc^{3}	H-1	4.603	4.653	4.630	4.644	4.654	4.63
	H-6	3.949	3.953	4.020	4.036	3.951	nd
	NAc	2.084	2.073	2.083	2.074	2.113	2.075
Gal^{3}	H-1		4.460			4.577	
	H-4		3.919			3.893	
Gal^{4}	H-1			4.454	4.430		4.484
	H-4			3.926	3.898		nd
Fuc^{2}	H-1					5.209	5.285
	H-5					4.268	4.270
	CH_3					1.231	1.28
Fuc^{3}	H-1				5.139		5.129
	H-5				4.810		4.85
	CH_3				1.178		1.24

[a] A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. Compounds **1**, **4**, **5**, **8a**, and **8b** were analyzed at 400 MHz; compound **11b** was analyzed at 500 MHz. In the column headings, the structures are represented by short-hand symbolic notation: ◇ = GalNAc-ol, ■ = Gal, ● = GlcNAc, □ = Fuc. The linkage position is specified by the direction of the connecting bars as follows:



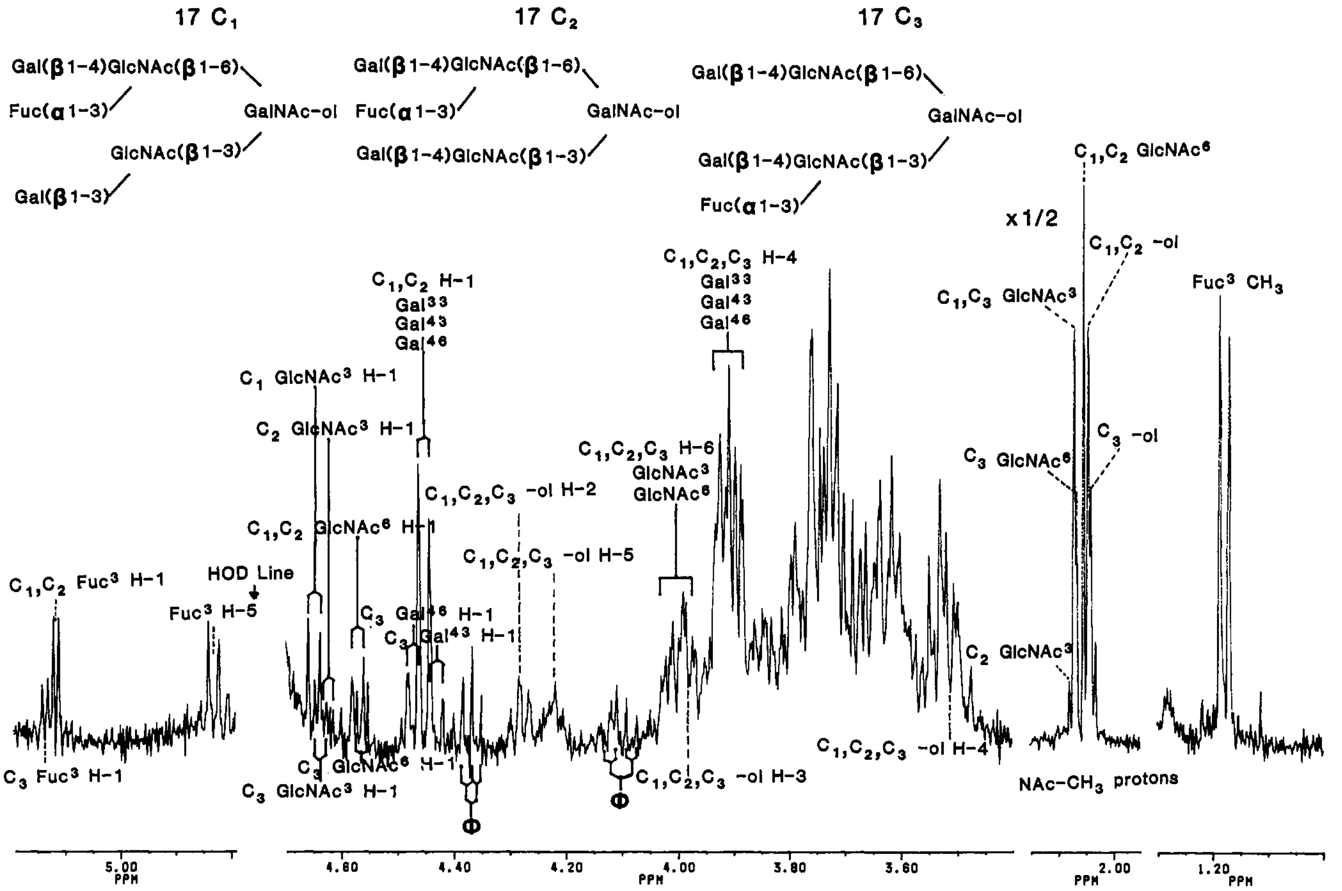
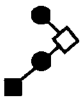
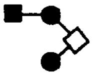
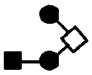
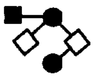
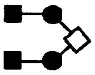
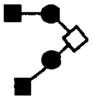
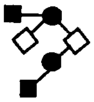
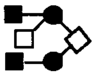
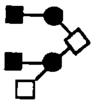
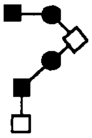
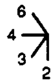


FIGURE 7: A 400-MHz ^{1}H-NMR spectrum ($^{2}H_2O$, p^{2}H 7, 27 °C) of neutral salivary oligosaccharide-alditol fraction **17c**. Resonances marked by Φ stem from non-carbohydrate contaminants.

Table V: ^{1}H Chemical Shifts of Structural Reporter Groups of Monosaccharides for the HPLC-Fractionated, Neutral Human Salivary Mucin Oligosaccharide-alditols Possessing the GlcNAcβ(1→6)[GlcNAcβ(1→3)] GalNAc-ol Core Unit[a]

		chemical shift in compound									
		9b	10b	10c	13c	14c1	14c2	17c1	17c2	17c3	17d
residue	reporter group										
GalNAc-ol	H-2	4.283	4.279	4.284	4.27	4.280	4.280	4.282	4.282	4.282	4.265
	H-3	4.000	3.985	nd	nd	3.987	3.987	3.982	3.982	3.982	nd
	H-4	3.530	3.516	nd	nd	3.523	3.523	3.512	3.512	3.512	3.560
	H-5	4.223	4.234	4.236	4.23	4.229	4.229	4.220	4.220	4.220	4.212
	NAc	2.044	2.045	2.045	2.047	2.044	2.044	2.044	2.044	2.040	2.043
GlcNAc^3	H-1	4.648	4.597	4.628	4.57	4.623	4.648	4.646	4.626	4.636	4.653
	H-6	3.958	3.950	nd	nd	4.024	3.952	3.954	4.019	4.030	3.953
	NAc	2.070	2.082	2.079	2.081	2.079	2.069	2.069	2.079	2.069	2.108
GlcNAc^6	H-1	4.540	4.564	4.546	4.57	4.562	4.562	4.568	4.568	4.562	4.562
	H-6	3.928	3.998	nd	nd	3.997	3.997	4.009	4.009	3.998	3.994
	NAc	2.064	2.061	2.064	2.054	2.062	2.062	2.053	2.053	2.065	2.060
Gal^3	H-1	4.454					4.453	4.452			4.570
	H-4	3.920					3.917	3.914			3.892
Gal^{4,3}	H-1			4.456		4.453			4.452	4.428	
	H-4			nd		3.927			3.928	3.890	
Gal^{4,6}	H-1		4.472		4.453	4.473	4.473	4.452	4.452	4.471	4.470
	H-4		3.928		nd	3.927	3.927	3.914	3.914	3.928	3.926
Fuc^2	H-1										5.211
	H-5										4.270
	CH$_3$										1.233
Fuc^{3,3}	H-1									5.135	
	H-5									4.829	
	CH$_3$									1.178	
Fuc^{3,6}	H-1				5.120			5.115	5.115		
	H-5				4.83			4.829	4.829		
	CH$_3$				1.179			1.178	1.178		

[a] A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosically linked. Compounds **9b**, **10b**, **14c1**, **14c2**, **17c1**, **17c2**, **17c3**, and **17d** were analyzed at 400 MHz; compounds **10c** and **13c** were analyzed at 500 MHz. In the table headings, the structures are represented by short-hand symbolic notation: ◇ = GalNAc-ol, ■ = Gal, ● = GlcNAc, □ = Fuc. The linkage position is specified by the direction of the connecting bars as follows:



assignments of the structural reporter group protons of the three structures **17c1** and **17c3** match those of **19a** and **19b**, respectively (see Table V). The remainder of the signals arise from a third compound (**17c2**), that differs from **17c1** in the presence of a type 2 unit at the GalNAc-ol C3 rather than a type 1. This is evidenced by the H-1 signal of Gal^4 at 4.452 being three times as intense as the other H-1 signals in the spectrum. From all these data, the structures of three oligosaccharide-alditols present in fraction **17c** were deduced to be the following:

Gal β(1→4) GlcNAc β(1→6)
/ \
17c1 Fuc α(1→3) GalNAc-ol
/
Gal β(1→3) GlcNAc β(1→3)

Gal β(1→4) GlcNAc β(1→6)
/ \
17c2 Fuc α(1→3) GalNAc-ol
/
Gal β(1→4) GlcNAc β(1→3)

Gal β(1→4) GlcNAc β(1→6)
\
17c3 GalNAc-ol
/
Gal β(1→4) GlcNAc β(1→3)
/
Fuc α(1→3)

Structure Determination of Sialylated Oligosaccharides

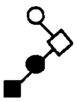
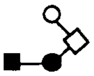
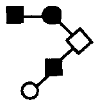
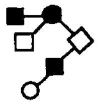
The primary structures of the monosialylated oligosaccharide-alditols obtained from human salivary mucin were established by ^{1}H-NMR spectroscopy. The chemical shifts of H-3ax and of H-3eq atoms of NeuAc α2→6 linked to GalNAc-ol are significantly different from those of NeuAc α2→3 linked to Gal (Van Halbeek et al., 1988).

NMR characteristics of compounds **S1**, **S2**, **S3**, **S4**, **S6.2**, **S7**, **S9**, and **S6.1** (Table VI) match those described for oligosaccharide-alditols **1**, **2**, **3**, **4**, **6a1**, **7**, and **9a1** in Van Halbeek et al. (1988) and **F-II-1-6** in Capon et al. (1989), respectively.

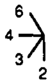
DISCUSSION

The chemical composition of the glycopeptides isolated after pronase treatment of the salivary glycoproteins is typical of mucin with a high content in carbohydrate (75%) and with serine and threonine accounting for 40% of the amino acids. The composition is similar to those described for high-molecular-weight human salivary mucins (Loomis et al., 1987; Veerman et al., 1989; Kawagashi et al., 1990). One of the differences is that the molar ratio of *N*-acetylgalactosamine to the total hydroxy amino acids for our human salivary mucin glycopeptides is larger than one. This ratio suggests that some *N*-acetylgalactosamine residues are not involved in carbohydrate–peptide linkages. Since the 20 donors were of blood

Table VI: ^{1}H Chemical Shifts of Structural Reporter Groups of Monosaccharides for the HPLC-Fractionated, Human Salivary Mucin Sialylated Oligosaccharide-alditols[a]

		chemical shift in compound							
		1	2	3	4	6.1	6.2	7	9
residue	reporter group								
GalNAc-ol	H-2	4.246	4.387	4.259	4.377	4.261	4.261	4.386	4.385
	H-3	nd	4.075	3.984	4.057	nd	nd	4.068	4.068
	H-4	3.413	3.496	nd	3.536	3.519	3.519	3.437	3.425
	H-5	4.020	4.184	4.183	4.243	4.180	4.180	4.268	4.262
	H-6′	3.533	nd	3.490	3.489	3.490	3.490	nd	nd
	NAc	2.056	2.045	2.033	2.047	2.035	2.035	2.065	2.066
Gal^{3}	H-1		4.545		4.473			4.530	4.532
	H-3		4.120		nd			4.114	4.113
	H-4		3.929		3.896			3.930	3.928
GlcNAc^{3}	H-1			4.608		4.658	4.634		
	H-6			3.940		3.971	4.005		
	NAc			2.078		2.066	2.076		
Gal^{3,3}	H-1					4.454			
	H-4					3.920			
Gal^{4,3}	H-1						4.465		
	H-4						3.926		
GlcNAc^{6}	H-1							4.558	4.560
	H-6							3.994	4.005
	NAc							2.065	2.056
Gal^{4,6}	H-1							4.468	4.446
	H-4							3.922	3.891
Fuc^{3}	H-1								5.104
	H-5								4.829
	CH_3								1.174
NeuAc^{3}	H-3ax		1.800					1.800	1.800
	H-3eq		2.773					2.773	2.773
	NAc		2.033					2.033	2.033
NeuAc^{6}	H-3ax	1.701		1.695	1.693	1.697	1.697		
	H-3eq	2.730		2.732	2.728	2.734	2.734		
	NAc	2.033		2.030	2.033	2.031	2.029		

[a] A superscript at a monosaccharide residue indicates to which position of the adjacent monosaccharide it is glycosidically linked. All compounds were analyzed at 400 MHz. In the column headings, the structures are represented by short-hand symbolic notation: ◇ = GalNAc-ol, ■ = Gal, ● = GlcNAc, □ = Fuc, ○ = NeuAc. The linkage position is specified by the direction of the connecting bars as follows:



group O, *N*-acetylgalactosamine residues in excess might be either part of Cad/Sd^{a} antigens, like in monkey cervical mucins (Nasir-Ud-Din et al., 1986), or part of the core sequence of GalNAcα(1→3)GalNAc-ol found in oligosaccharides from meconium glycoproteins (Hounsell et al., 1985) or from avian salivary mucins (Wieruszeski et al., 1987). Such structures have not been found among the human salivary oligosaccharides purified so far, but Sd^{a} blood group activity has been detected in human saliva before (Morton et al., 1970).

Alkaline borohydride treatment of salivary mucin glycopeptides, which were isolated from total saliva of 20 donors with blood group O, produced a mixture of glycopeptides and oligosaccharide-alditols. Fractionation by ion-exchange chromatography and gel filtration yielded a pool of relatively small neutral oligosaccharide-alditols, designated Ic, and a pool of small monosialylated oligosaccharide-alditols, designated IIc. Fraction Ic was fractionated by HPLC using two columns sequentially, one containing an alkylamine-bonded phase, which led to 23 fractions, and another containing an octadecylsilane-bonded phase, which allowed the subfractionation of 15 of the 23 fractions into 64 subfractions; fraction IIc was separated into 10 fractions after HPLC. ^{1}H-NMR spectroscopy provided the primary structure of 37 mucin-type oligosaccharide-alditols (Scheme I).

The amounts of carbohydrates in fractions **7a**, **7b**, **7c**, **11c**, **13b**, **13e**, **14a**, **16a**, **16b**, **16c**, **16d**, **S8**, and **S10** were too low to determine their structures; this was also the case of fraction **S5**, which gave a high absorption at 206 nm but did not contain much carbohydrate. The occurrence of these minor fractions and also of the more acidic fractions, III and IV, which have not been studied so far, indicates that the heterogeneity of oligosaccharide-alditols in salivary glycoproteins is even larger than detailed in this study.

The 37 oligosaccharide structures which have been determined ranged from di- to hexasaccharide-alditols. Their cores belonged to one of the four classical types of core: type 1 (Galβ(1→3)GalNAc-ol), type 2 (Galβ(1→3)[GlcNAcβ(1→6)]-GalNAc-ol), type 3 (GlcNAcβ(1→3)GalNAc-ol), or type 4 (GlcNAcβ(1→3)[GlcNAcβ(1→6)]GalNAc-ol) (Van Halbeek, 1984; Brockhausen et al., 1985). Their "backbones" contain either type 1 (Galβ(1→3)GlcNAc) or type 2 (Galβ-(1→4)GlcNAc) disaccharide units. Fucose and sialic acid residues define the "periphery" of the carbohydrate chains: fucose residues give rise to different determinants such as H, X (Gooi et al., 1981), Y (Abe et al., 1983), or Lewis^{b} antigens (Lloyd et al., 1968).

The carbohydrate chains derived from porcine (Carlson et al., 1968; Baig et al., 1972; Van Halbeek et al., 1981), bovine

(Savage et al., 1986; Tsuji et al., 1986), goat (Dutta et al., 1982), rat (Slomiany et al., 1978), canine (Lombart et al., 1974), ovine (Gottschalk et al., 1972), and avian salivary mucins (Wieruszeski et al., 1987) have been studied in great detail. The carbohydrate moieties of salivary mucins can be very simple, ie., made up of single *N*-acetylgalactosamine residues or *N*-acetylneuraminyl$\alpha(2\rightarrow6)$*N*-acetylgalactosamine as in ovine submaxillary mucins (Graham et al., 1960). In porcine submaxillary mucins, four different trisaccharides containing *N*-acetyl- or *N*-glycolyl-neuraminic acid were found (Savage et al., 1986), and in rat sublingual glycoprotein, five sialyloligosaccharides composed of 9–15 sugar residues have been isolated (Slomiany et al., 1978). The carbohydrate chains from these animal sources do not seem to be very diverse.

In contrast, the 37 oligosaccharide-alditols characterized in the present study illustrate the wide heterogeneity of the low-molecular-weight neutral and sialylated carbohydrate chains isolated from the highly glycosylated regions of high-molecular-mass human salivary mucins. Among these 37 carbohydrate chains, six oligosaccharide-alditols were also partially characterized from the low-molecular-weight salivary mucins (Reddy et al., 1985); five of them correspond to oligosaccharides **2**, **3**, **17b**, **S2**, and **S9** of this study.

The origin of this heterogeneity is probably not related to degradation by glycosidases produced by the bacteria of the oral cavity since the saliva was immediately frozen after sampling. Such a heterogeneity might be due to the superposition of the different glycosylation phenotypes of the 20 salivas that form this pool: however, we tried to reduce this type of heterogeneity by collecting only saliva from patients with blood group O. Part of the heterogeneity of the salivary carbohydrate chains might also be related to the different origins of the salivary secretions, with submandibular, sublingual, and minor glands producing slightly different mucins as suggested by lectin histochemical studies (Mc Mahon et al., 1989; Laden et al., 1984; Cohen et al., 1990, 1991).

In the case of human exocrine secretions, a wide heterogeneity of carbohydrate chains has been observed in gastric (Slomiany et al., 1984) and colonic (Podolsky et al., 1985) mucins. For human bronchial mucin oligosaccharides, a similar heterogeneity was found in secretions from a single patient as in the pooled secretions of different patients suffering from cystic fibrosis or chronic bronchitis (Lamblin et al., 1984a,b; Klein et al., 1988).

This diversity of carbohydrate chains raises the question of its function. It has been suggested that the wide diversity of carbohydrate chains of human respiratory mucins might represent multiple recognition sites for bacterial adhesins, allowing the adhesion and the removal of inhaled bacteria (Lamblin et al., 1991). Therefore, the diversity of carbohydrate chains from human salivary mucins might have a protective role. Several bacteria isolated from the oral cavity, or from the tracheobronchial tree in pathological conditions, possess adhesins able to recognize carbohydrate structures similar to some of the chains that we have isolated: e.g., Gal$\beta(1\rightarrow3)$GalNAc for *Actinomyces naeslundii* (Brennan et al., 1987); NeuAc$\alpha(2\rightarrow3)$Gal$\beta(1\rightarrow3)$GalNAc for *Streptococcus sanguis*, *Streptococcus mitis* (Murray et al., 1982), and *Escherichia coli* (Leffler et al., 1986); *N*-acetylneuraminic acid for *Pseudomonas aeruginosa* strains isolated from the oral cavity (Komiyama et al., 1987); type 1 (Gal$\beta(1\rightarrow3)$GlcNAc) or type 2 (Gal$\beta(1\rightarrow4)$GlcNAc) disaccharide units for other strains of *Pseudomonas aeruginosa* (Ramphal et al., 1991) and for *Streptococcus pneumoniae* (Andersson et al., 1983).

Thus, the carbohydrate heterogeneity of the human salivary mucins may represent a mosaic of bacterial adhesion sites and be involved in the early events of the nonimmune defense of the oral cavity.

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