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TERMINAL α(1 → 4)-LINKED *N*-ACETYLGLUCOSAMINE: A CHARACTERISTIC CONSTITUENT OF DUODENAL-GLAND MUCOUS GLYCOPROTEINS IN RAT AND PIG

A HIGH-RESOLUTION ^{1}H-NMR STUDY

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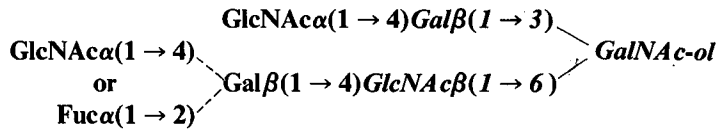
The structure of the carbohydrate chains of mucous glycoproteins from the gastro-intestinal tract was examined for species- and tissue-specificity. To this purpose, oligosaccharides were released from purified glycoprotein preparations of rat and pig gastric, duodenal-gland and small-intestinal mucus, by alkaline borohydride reductive cleavage. Based on the results of 500-MHz ^{1}H-NMR spectroscopy and of sugar analysis of the total oligosaccharide fractions, terminal GlcNAc, α(1 → 4)-linked to galactose, appears to be a characteristic constituent of duodenal-gland oligosaccharides. Similarly, NeuAc in α(2 → 3)-linkage to galactose turns out to be a typical constituent of small-intestinal mucous glycoproteins. In general, glycoproteins from gastric mucus possess larger and more-branched carbohydrate chains than those from duodenal-gland and small-intestinal mucus. Comparing rat and pig, oligosaccharide structures for corresponding tissues are less complex for the former. After fractionation, the rat duodenal-gland oligosaccharides could be characterized by application of ^{1}H-NMR spectroscopy as being branched tetra- up to hexa-saccharide chains, all sharing the italicized trisaccharide element. The chains exhibit microheterogeneity as to the termination by fucose in α(1 → 2)- or by GlcNAc in α(1 → 4)-linkage to galactose. The following structures can be proposed for the most abundant rat duodenal-gland oligosaccharides:



Introduction

The mucosa of the mammalian gastro-intestinal tract is protected against noxious external influences, e.g. those of low pH and of proteolytic enzymes, by a mucus layer that covers the epithelial lining. Mucus glycoproteins form the major structural and functional component of this layer. It is supposed that the carbohydrate chains of these glycoproteins play an essential role in its function [1,2]. Since the physiological conditions in various parts of the digestive tract differ considerably, one might wonder whether this is reflected by a differentiation of the carbohydrate structures

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of the mucous glycoproteins. For instance, the finding by Derevitskaya et al. [3] that in mucus of pig stomach α-linked GlcNAc occurs as a typical, terminal sugar might lend support to the idea that these structures exhibit a tissue-specificity. Moreover, it is well known that these carbohydrate chains may bear species- and tissue-specific antigenic determinants [4–9].

In connection with our studies on mucous glycoproteins [6,8,10–16], we focussed attention on a comparison of the *O*-glycosidically linked carbohydrate chains of mucous glycoproteins from three tissues, namely, stomach, duodenal gland (Brunner's gland) and small intestine, for two species, rat and pig. Thereby, we paid particular attention to obtaining histologically well-defined tissues for our studies, since contamination with neighbouring tissues might influence the results. High-resolution ^{1}H-NMR spectroscopy in combination with sugar analysis has proved to be a powerful method in the analysis of *O*-glycosidic carbohydrates from mucus, after release from the protein portions as oligosaccharide-alditols [6,8,16]. It has been established that mixtures of oligosaccharide-alditols can be analyzed adequately; therefore, this approach appeared invaluable in disclosing certain types of microheterogeneity [16]. Thus, it may be anticipated that in a comparative ^{1}H-NMR study of the total mucous glycoprotein oligosaccharides from three well-defined tissues for rat and pig, in particular information can be deduced with regard to terminal residues. Furthermore, the complete primary structures of some purified oligosaccharide-alditols from rat duodenal-gland glycoproteins will be reported.

Materials and Methods

Preparation of mucous glycoproteins

Gastric, duodenal-gland and small-intestinal mucous glycoproteins were isolated from the appropriate, histologically well-defined tissues of some 60 male Wistar rats (blood group A) and of three pigs (blood group unknown), and purified, as described previously [11,15].

Isolation and fractionation of oligosaccharides

Oligosaccharides were released from the mucous glycoproteins by alkaline-borohydride reductive degradation, performed essentially as described by Iyer and Carlson [17]. The glycoproteins were treated with 0.05 M NaOH and 1.0 M $NaBH_4$ at 50°C for 16 h. After neutralization of the reaction mixture with 1.0 M HCl and subsequent removal of borate, the residual (glyco)proteins were precipitated with ethanol (96%) in the presence of 0.02 M potassium acetate, pH 5.0, at −20°C. The precipitate was subjected to a second treatment with alkaline borohydride. The combined supernatants containing the oligosaccharides were evaporated to dryness, and partially desalted by treatment with Dowex 50W-X8 (H^+) (200–400 mesh). The crude β-elimination products were subjected to ^{1}H-NMR spectroscopy.

Subsequently, the oligosaccharides of the rat duodenal-gland mucous glycoproteins were fractionated on a Bio-Gel P2 (Bio-Rad; 200–400 mesh) column (1.5 × 90 cm), eluted with 0.1 M ammonium acetate. Fractions of 2 ml were collected. Hexose-containing fractions were pooled, desalted as described above, and lyophilized.

Analytical methods

Total hexose was assayed by colorimetry, using the orcinol/sulfuric acid reagent [18]. A mixture of galactose and fucose (ratio 2:1) was used as a standard [11,15].

Quantitative sugar analysis was performed essentially as described by Kamerling et al. [19]. Oligosaccharides were subjected to solvolysis by 1 M HCl in methanol, for 24 h at 85°C. Gas-liquid chromatography of the trimethylsilyl derivatives of the methyl-glycosides was conducted on a Varian 3700 apparatus, equipped with a WCOT CPsil5 fused-silica capillary column (25 m × 0.3 mm, i.d.) using flame-ionization detection; oven temperature was programmed from 130 to 220°C (2°/min); N_2 was used as carrier gas (flow rate 1.5 ml/min). The split ratio amounted to 1:10.

Prior to NMR spectroscopic analysis, the oligosaccharides were repeatedly treated with ^2H_2O at room temperature, with intermediate lyophilization. Finally, the samples were redissolved in 0.4 ml ^2H_2O (99.96 atom% ^{2}H, Aldrich).

The 500-MHz ^{1}H-NMR spectra were recorded on a Bruker WM-500 spectrometer (SON-NMR facility, Department of Biophysics, Nijmegen University, The Netherlands), operating in the Fourier

transform mode, and equipped with a Bruker Aspect-2000 computer. The probe temperature was kept constant at 27°C. Further experimental details were described previously [8,20,21]. Chemical shifts are expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), but were actually measured by reference to internal acetone ($\delta = 2.225$ ppm) with an accuracy of 0.002 ppm.

Results

Comparison of the carbohydrate moieties from gastric, duodenal-gland and small-intestinal mucous glycoproteins for rat and pig

Alkaline borohydride treatment of the mucous glycoproteins of stomach, duodenal gland and small intestine yielded for rat 65, 96 and 75% of the total sugar moieties, respectively. For pig, the corresponding yields were 60, 92 and 69%. A second β-eliminative cleavage of the residual glycoproteins from stomach and small intestine resulted in the release of another 20 and 15% sugar for rat, and of 25 and 15% for pig, respectively.

TABLE I

MOLAR CARBOHYDRATE COMPOSITION OF RAT AND PIG MUCOUS GLYCOPROTEINS FROM STOMACH, DUODENAL GLAND AND SMALL INTESTINE, AND OF THEIR ALKALINE BOROHYDRIDE CLEAVAGE PRODUCTS

The molar sugar composition of the intact glycoproteins was calculated on the basis of 1 mol of galactose; that of the oligosaccharide fractions was based on 1 mol of GalNAc-ol. To facilitate comparison, the latter results were also recalculated for 1 mol of galactose (—, less than 0.1).

Animal	Tissue	Material	Monosaccharide (mol/mol)					
			Fuc	Gal	GlcNAc	GalNAc	GalNAc-ol	NeuAc
Rat	stomach	glycoprotein	0.6	1.0	0.9	0.3	—	—
		total oligosaccharide	0.6	1.0	0.9	0.1	0.2	—
			2.7	4.9	4.2	0.7	1.0	—
Rat	duodenal	glycoprotein	0.3	1.0	1.5	0.6	—	—
	gland	total oligosaccharide	0.3	1.0	1.6	0.1	0.5	—
			0.6	2.1	3.4	0.3	1.0	—
		Bio-Gel fraction *I*	0.6	2.2	2.7	—	1.0	—
		II	0.3	1.2	1.7	—	1.0	—
		III	0.2	0.9	1.6	—	1.0	—
Rat	small	glycoprotein	0.6	1.0	0.9	0.6	—	0.4
	intestine	total oligosaccharide	0.7	1.0	0.8	0.1	0.5	0.3
			1.4	2.0	1.4	0.3	1.0	0.6
Pig	stomach	glycoprotein	0.7	1.0	0.8	0.2	—	—
		total oligosaccharide	0.7	1.0	0.9	0.1	0.1	—
			6.5	9.0	8.7	1.1	1.0	—
Pig	duodenal	glycoprotein	0.8	1.0	0.6	1.0	—	—
	gland	total oligosaccharide	0.8	1.0	0.7	0.2	0.9	—
			0.9	1.1	0.7	0.2	1.0	—
Pig	small	glycoprotein	0.4	1.0	0.8	1.2	—	0.3
	intestine	total oligosaccharide	0.4	1.0	0.9	0.2	0.9	0.3
			0.4	1.1	1.0	0.2	1.0	0.3

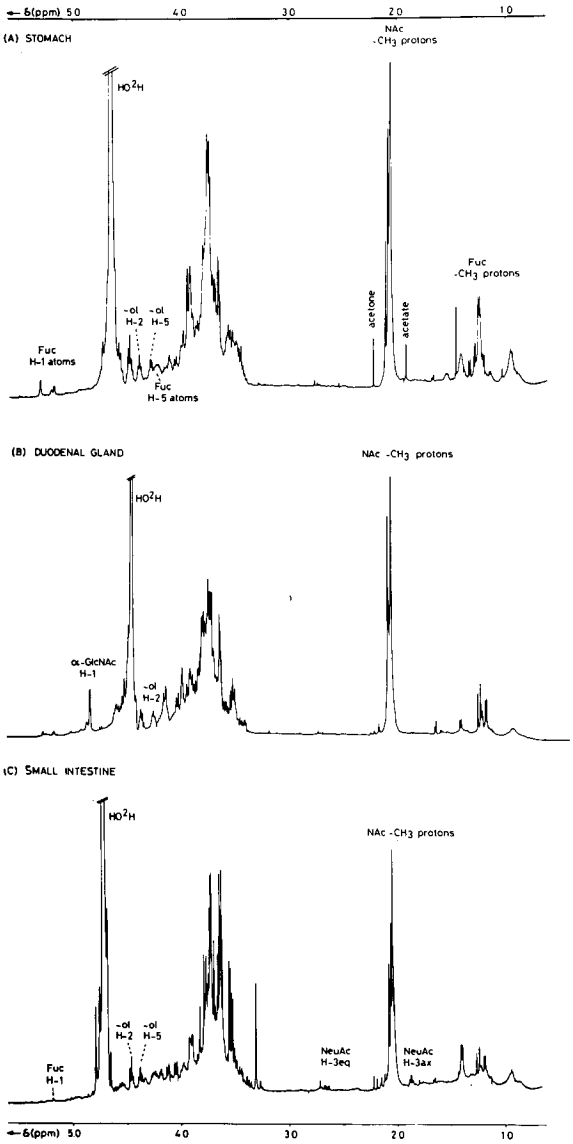


Fig. 1. Overall 500-MHz ^{1}H-NMR spectra of total oligosaccharide fractions released from pig gastric (A), duodenal-gland (B) and small-intestinal (C) mucous glycoproteins. The spectra were recorded for ^2H_2O solutions at 27°C. The resonance position of the HO^2H line is sensitive to the salt concentration of the sample; here, for HO^2H, $\delta \simeq 4.5$-4.6 ppm is found, probably due to a rather high amount of CsCl present [11,15].

These recoveries (80–96%) of oligosaccharides, calculated relative to the carbohydrate content of purified glycoprotein preparations (cf. Refs. 11 and 15), were judged to be sufficient to perform comparative structural studies.

The molar carbohydrate compositions of the rat and pig mucous glycoproteins from the three tissues, together with those of the total oligosaccharide fractions released, are summarized in Table I. It should be noted that, during methanolysis of the oligosaccharide-alditols, GalNAc-ol is partly converted into its 1,4-anhydro- (12%), its 3,6-anhydro- (17%) and its 1,4-3,6-di(anhydro)- (6%) analogues [22]. The trimethylsilyl derivatives of these anhydro-GalNAc-ol products could be traced in the gas chromatogram (retention times 0.96, 0.90 and 0.51, respectively, as compared to mannitol). Assuming the molar adjustment factor for the trimethylsilyl anhydro derivatives to be equal to that of trimethylsilyl GalNAc-ol itself (R_{mannitol} 1.24; 65%, molar adjustment factor 1.0), the molar ratios of the total oligosaccharide fractions could be calculated relative to 1 mol of GalNAc-ol (see Table I).

From comparison of the data for the glycoproteins with those for the corresponding, liberated oligosaccharides it is obvious that alkaline borohydride treatment led to the formation of GalNAc-ol; concomitantly, a proportional decrease of the GalNAc content is observed. No other reduced monosaccharides were detected, indicating the absence of oligosaccharide degradation due to peeling [23]. One of the striking features of the carbohydrate analyses is that sialic acid appears to be present in small intestinal mucus. This finding is in accordance with literature data [24–26]. However, the absence of sialic

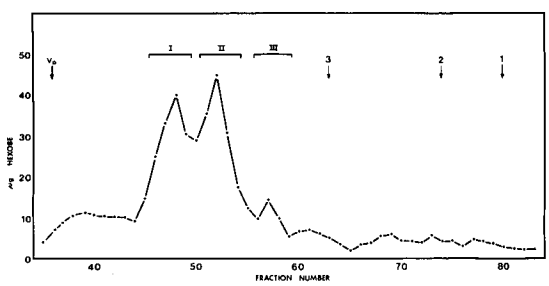
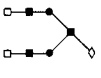


Fig. 2. Elution profile of the fractionation of rat duodenal-gland total oligosaccharides (1 mg) on a Bio-Gel P2 column (1.5 × 90 cm; eluted with 0.1 M ammonium acetate; 2-ml fractions were collected). The fractions indicated by bars were pooled, desalted, lyophilized and used for structural investigations. Elution positions of standards are indicated: 1, galactose; 2, sucrose; 3, stachyose; V_0, void volume.

TABLE II

^{1}H CHEMICAL SHIFTS OF PERTINENT STRUCTURAL-REPORTER GROUPS FOR TWO OLIGOSACCHARIDE-ALDITOL FRACTIONS (*I* AND *III*) FROM RAT DUODENAL-GLAND MUCOUS GLYCOPROTEINS, TOGETHER WITH THOSE FOR SOME REFERENCE COMPOUNDS (*A*–*F*)

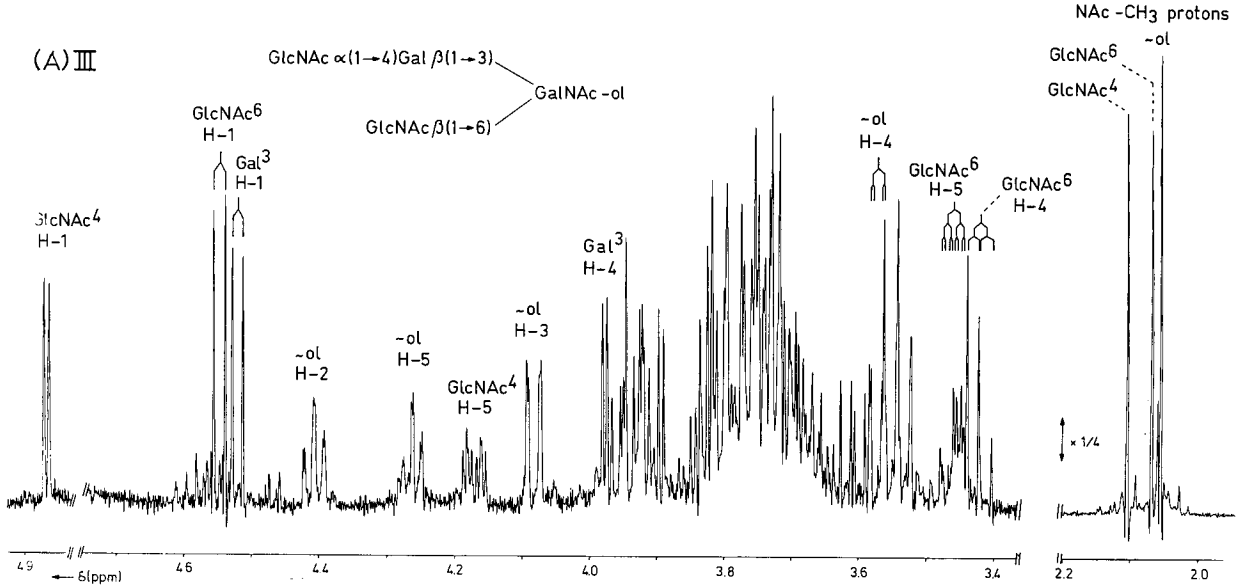
A superscript at the name of a sugar residue indicates to which position of the adjacent monosaccharide it is glycosidically linked; for example, Gal[3] means Gal $\beta(1 \rightarrow 3)$-linked (in this case, to GalNAc-ol) (cf. Refs. 8, 16, 28). Chemical shifts are in ppm relative to internal DSS in ^2H_2O at 27°C, acquired at 500 MHz. In the table heading, structures are represented by short-hand symbolic notation (cf. Refs. 8, 16); ◇ = GalNAc-ol; ■ = galactose; ● = β-GlcNAc; ○ = α-GlcNAc; □ = fucose; ⧈ = α-GlcNAc or fucose.

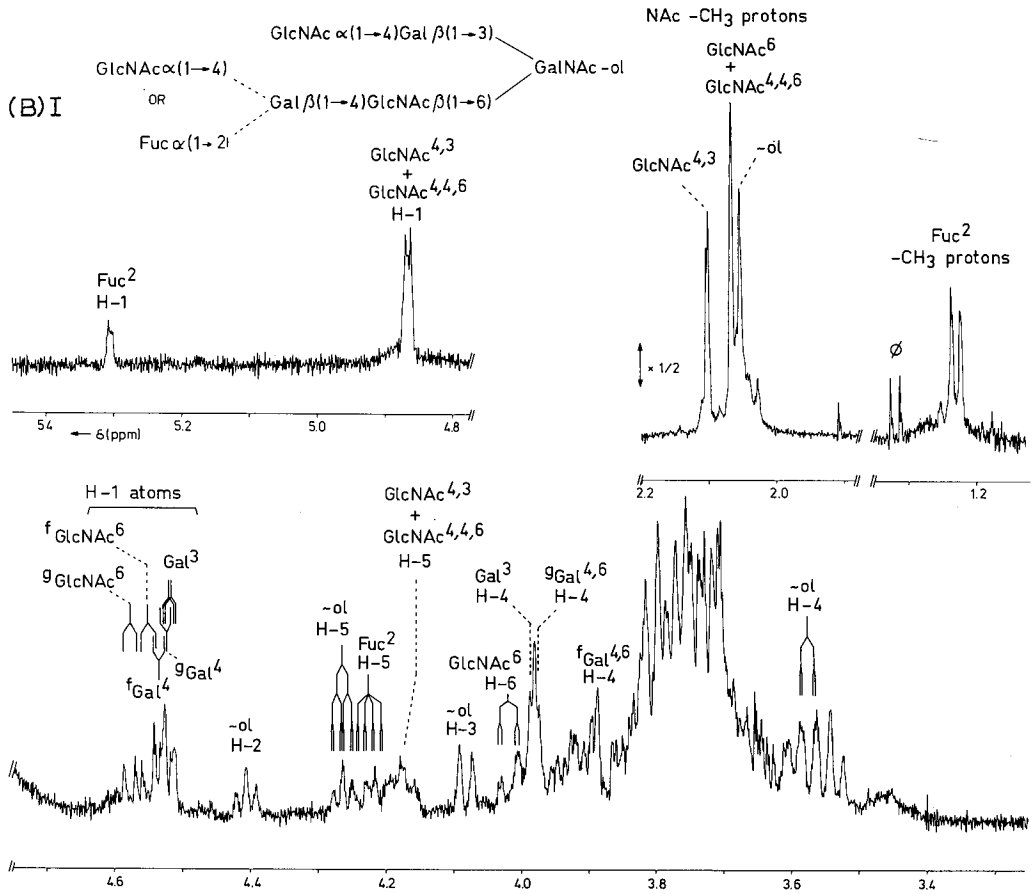
Residue	Reporter group	Chemical shift in							
									
		A [8]	*B* [16,20]	Fraction *III*	*C* [6,8]	*D* [8,28]	Fraction *I*	*E* [16]	*F* [16]
GalNAc-ol	H-2	4.395	4.404	4.405	4.399	4.394	4.406	4.405	4.386
	H-3	4.061	4.081	4.081	4.091	4.060	4.082	4.083	3.991
	H-4	3.468	3.541	3.572	3.522	3.465	3.574	3.574	3.509
	H-5	4.281	4.193	4.261	4.163	4.282	4.264	4.264	4.142
	H-6	3.931	3.76	3.90–3.95	3.68	3.931	3.90–4.00	3.952	3.8
	NAc	2.066	2.058	2.054	2.046	2.067	2.055	2.054	2.045
Gal[3]	H-1	4.468	4.525	4.518	4.584	4.465	4.520	4.521	4.468
	H-4	3.901	3.971	3.976	3.926	3.900	3.985	3.980	4.104
GlcNAc[6]	H-1	4.538	—	4.546	—	4.560	4.577 [g] 4.548 [f]	4.579	4.584
	NAc	2.066	—	2.067	—	2.064	2.067	2.067	2.055
Gal[4]	H-1	—	—	—	—	4.470	4.535 [f] 4.526 [g]	4.526	4.550 4.532
	H-4	—	—	—	—	3.925	3.980 [f] 3.892 [g]	3.987	3.890
GlcNAc[4]	H-1	—	4.869	4.868	—	—	4.867	4.870 4.873	—
	H-5	—	4.183	4.174	—	—	4.176	4.182 4.167	—
	NAc	—	2.089	2.103	—	—	2.102 2.067 [g]	2.104 2.069	— —
Fuc[2]	H-1	—	—	—	5.256	—	5.305 [f]	—	5.313 5.310
	H-5	—	—	—	4.279	—	4.221 [f]	—	4.221
	CH_3	—	—	—	1.243	—	1.230 [f]	—	1.230

[f] For the branch that is terminated by fucose in $\alpha(1 \rightarrow 2)$-linkage.
[g] For the branch that is terminated by GlcNAc in $\alpha(1 \rightarrow 4)$-linkage.

acid from gastric and duodenal-gland mucous glycoproteins is in contrast to some other reports [27].

The overall 500-MHz ^{1}H-NMR spectra of the three total oligosaccharide fractions obtained from pig gastric, duodenal-gland and small-intestinal mucous glycoproteins, are depicted in Fig. 1. Those for the corresponding rat fractions showed essentially the same patterns. In general, the spectra of the stomach fractions look much more complicated than those of the other two tissues, pointing to the occurrence of larger and/or more-branched structures in gastric mucous glycoproteins. This is in agreement with the relatively low molar proportion of GalNAc-ol in the total oligosaccharide fraction of the mucous glycoproteins from stomach as compared to that of the other


(A)III
GlcNAc α(1→4)Gal β(1→3)
GalNAc-ol
GlcNAc β(1→6)
GlcNAc6 H-1
Gal3 H-1
GlcNAc4 H-1
~ol H-2
~ol H-5
GlcNAc4 H-5
~ol H-3
Gal3 H-4
~ol H-4
GlcNAc6 H-5
GlcNAc6 H-4
NAc -CH3 protons
GlcNAc6
GlcNAc4
~ol
× 1/4
δ(ppm)


(B)I
GlcNAc α(1→4)Gal β(1→3)
GlcNAcα(1→4)
OR
Gal β(1→4)GlcNAc β(1→6)
GalNAc-ol
Fuc α(1→2)
GlcNAc4,3 + GlcNAc4,4,6 H-1
Fuc2 H-1
NAc -CH3 protons
GlcNAc6 + GlcNAc4,4,6
GlcNAc4,3
~ol
Fuc2 -CH3 protons
× 1/2
H-1 atoms
fGlcNAc6
gGlcNAc6
Gal3
gGal4
fGal4
~ol H-2
~ol H-5
Fuc2 H-5
GlcNAc4,3 + GlcNAc4,4,6 H-5
~ol H-3
Gal3 H-4
gGal4,6 H-4
GlcNAc6 H-6
fGal4,6 H-4
~ol H-4
δ(ppm)

tissues (see Table I). Furthermore, the spectra of the total oligosaccharides from corresponding tissues are more complex for pig than for rat.

The overall spectra also permit one to deduce some more detailed structural information. They clearly show GalNAc-ol to be present as the terminal monosaccharide unit in all constituents of the mixtures. This alditol is characterized by the H-2 signal at $\delta \simeq 4.39$ ppm, the H-5 signal at $\delta \simeq 4.28$ ppm and the H-3 signal at $\delta \simeq 4.06$ ppm [8,16,20]. With regard to the terminal sugars at the other side of the chains, the signals at $\delta \simeq 5.3$ ppm (H-1), $\delta \simeq 4.25$ ppm (H-5) and $\delta \simeq 1.24$ ppm (CH_3) point to the occurrence of fucose in $\alpha(1 \rightarrow 2)$-linkage to galactose in several components of the mixtures [8,16]. The oligosaccharides from small-intestinal glycoproteins contain NeuAc in $\alpha(2 \rightarrow 3)$-linkage to galactose (δH-3ax = 1.798 ppm; δH-3eq = 2.762 ppm; see Fig. 1C) [20,21]. However, the most striking outcome of the ^{1}H-NMR spectroscopic analyses of the total oligosaccharide fractions is the presence of an anomeric doublet at $\delta = 4.867$ ppm ($J_{1,2} = 3.8$ Hz) in the spectra of duodenal-gland oligosaccharides (see Fig. 1B), whereas this signal does not occur in the spectra for the other tissues (see Fig. 1A and C). This indicates the presence of terminal, non-reducing GlcNAc in $\alpha(1 \rightarrow 4)$-linkage to a galactose residue in the duodenal-gland fraction (for details, see Ref. 16). It can be concluded that $\alpha(1 \rightarrow 4)$-linked GlcNAc has a restricted distribution; it can be conceived of as a typical constituent of duodenal-gland mucous glycoproteins.

Fractionation and characterization of rat duodenal-gland oligosaccharides

The relatively simple 500-MHz ^{1}H-NMR spectrum of the total oligosaccharide fraction from rat duodenal-gland glycoproteins suggests that this mixture consists of a limited number of compounds, the structures of which are not too complex. This might allow, after fractionation of the oligosaccharides, a rapid, complete structural characterization of these components, including the localization of the $\alpha(1 \rightarrow 4)$-linked GlcNAc residues.

Bio-Gel P2 filtration of total carbohydrate obtained from rat duodenal-gland mucous glycoproteins afforded three main oligosaccharide fractions, denoted *I*, *II* and *III* (see Fig. 2). Their molar carbohydrate composition is included in Table I.

500-MHz ^{1}H-NMR spectra were recorded of the three oligosaccharide fractions; those of fractions *I* and *III* are depicted in Fig. 3. The ^{1}H-NMR parameters of the pertinent structural-reporter groups of the compounds present, together with those of some reference oligosaccharides, are compiled in Table II. The chemical shift value for H-2 of GalNAc-ol ($\delta \simeq 4.40$ ppm), being the same in all three spectra, reflects the common presence of the Gal$\beta(1 \rightarrow 3)$GalNAc-ol core unit in oligosaccharide fractions *I*, *II* and *III* (see also Fig. 1) [8,16]. Moreover, GalNAc-ol bears a GlcNAc residue in $\beta(1 \rightarrow 6)$-linkage in all compounds. The latter can be deduced from the chemical shifts of GalNAc-ol H-5 ($\delta \simeq 4.26$ ppm) and H-6 ($\delta \simeq 3.92$ ppm) [8,16].

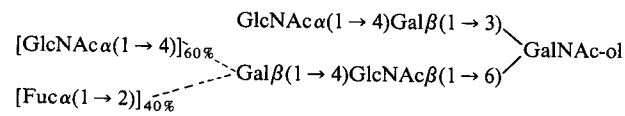
Fraction *III* consists of an almost pure tetrasaccharide which was identified as the aforementioned core trisaccharide extended with GlcNAc in $\alpha(1 \rightarrow 4)$-linkage to galactose. The terminal α-linked GlcNAc residue is characterized by its H-1 ($\delta = 4.868$ ppm), H-5 ($\delta = 4.174$ ppm), H-4 ($\delta = 3.476$ ppm) and *N*-acetyl ($\delta = 2.103$ ppm) signals. The chemical shift of H-1, in conjunction with its coupling constant ($J_{1,2} = 3.8$ Hz), is indicative of the α-configuration of the glycosidic linkage between GlcNAc and galactose (compare compound *B*, Table II) [16,20]. Owing to the presence of this GlcNAc, H-1 of galactose has undergone a considerable downfield shift towards $\delta = 4.518$ ppm; also

Fig. 3. (opposite page) Resolution-enhanced, 500-MHz ^{1}H-NMR spectra (^2H_2O, 27°C, p^{2}H $\simeq$ 7) of (A) oligosaccharide fraction *III* and (B) oligosaccharide fraction *I*, obtained from rat duodenal-gland mucous glycoproteins by alkaline borohydride treatment and Bio-Gel P2 fractionation. The (first) superscript behind the name of a sugar residue indicates the position of the adjacent monosaccharide to which it is glycosidically linked (subsequent superscripts indicating the type of the subsequent linkage in the sugar sequence are used only if necessary for discrimination) [16]. The pre-superscripts f and g refer to residues in a branch that is terminated by fucose or α-GlcNAc, respectively [16]. The relative-intensity scales of the *N*-acetyl methyl-proton regions deviate from those of the other parts of the spectra, as indicated. The doublet at $\delta \simeq 1.32$ ppm, marked by Φ, stems from a frequently occurring non-carbohydrate contaminant.

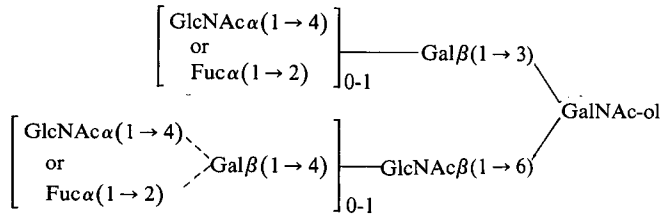
its H-4 has shifted downfield to $\delta = 3.976$ ppm (compare with compound *A*, Table II). These shift effects are identical to those observed previously [16] for extensions of oligosaccharides by GlcNAc in $\alpha(1 \rightarrow 4)$-linkage to a galactose residue. The $\beta(1 \rightarrow 6)$-linked core GlcNAc residue is present in terminal position (δH-1 = 4.546 ppm, δH-4 = 3.422 ppm and δH-5 $\simeq$ 3.46 ppm), which can be deduced from comparison with reference compound *A* (see Table II and Ref. 8). Therefore, the structure of the main component of fraction *III* has been established to be:

GlcNAc$\alpha(1 \rightarrow 4)$Gal$\beta(1 \rightarrow 3)$[GlcNAc$\beta(1 \rightarrow 6)$]GalNAc-ol.

The structures of the constituents of fraction *I* could be identified to be extensions of that of fraction *III*, first of all with galactose in $\beta(1 \rightarrow 4)$-linkage to the β-linked GlcNAc (denoted Gal^4 and GlcNAc^6, respectively; see also footnote to Table II). The upper branch Gal^3 residue, like in fraction *III*, is terminated by α-linked GlcNAc in all components in the mixture (compare chemical shifts of upper branch with data for Gal^3 and GlcNAc^4 for compounds *B* and *E* and for fraction *III*; see Table II). In the α-anomeric region of the spectrum ($\delta > 4.8$ ppm), besides the GlcNAc^4 H-1 signal at $\delta = 4.867$ ppm, another H-1 signal is observed at $\delta = 5.305$ ppm; this is attributable to fucose in $\alpha(1 \rightarrow 2)$-linkage to Gal^4 [8,16,21]. From the intensity ratio of the fucose and α-GlcNAc H-1 signals being 1:4 (see Fig. 3B), it can be inferred that this Gal^4 residue is substituted either by terminal fucose in $\alpha(1 \rightarrow 2)$-linkage, or by terminal GlcNAc in $\alpha(1 \rightarrow 4)$-linkage, in the ratio of 2:3. Therefore, this fraction exhibits an interesting type of microheterogeneity. While the upper branch is homogeneous in all components of the mixtures, the lower branch consists of Fuc$\alpha(1 \rightarrow 2)$Gal$\beta(1 \rightarrow 4)$GlcNAc$\beta(1 \rightarrow 6)$ only in 40% of the structures; this structural element is characterized by the chemical shifts of H-1, H-5 and CH_3 of fucose, together with that of H-1 of the accompanying Gal^4 (see also compound *F*, Table II). In the remaining 60% of the structures, this Gal^4 is terminated by $\alpha(1 \rightarrow 4)$-linked GlcNAc. The chemical shifts of the structural-reporter groups of this branch are identical to those described for reference compound *E* (see Table II) [16]. It should be noted that the trend in the chemical shifts for Gal^4 and GlcNAc^6 reporter groups, going from the main to the minor component of *I*, is in accord with that observed for the step from *16.2(a)* to *16.2(b)*, when comparing the corresponding shifts for Gal^{4,6} and GlcNAc^{6,3} reporter groups [16]. In summary, the following set of structures is proposed for fraction *I*:



It is remarkable that fraction *II* (spectrum not shown) shows a high degree of heterogeneity. First of all, in the upper branch, Gal^3 may be present as terminal residue (δH-1 = 4.468 ppm), or it may be substituted either by GlcNAc in $\alpha(1 \rightarrow 4)$-linkage (for Gal^3, δH-1 = 4.521 ppm) or by fucose in $\alpha(1 \rightarrow 2)$-linkage (for Gal^3, δH-1 = 4.582 ppm). The reporter groups of α-linked GlcNAc and fucose residues themselves are found at essentially the same positions as for compounds *B* and *C*, respectively (see Table II). The lower branch of fraction *II* structures may consist of GlcNAc^6 in terminal position (like in fraction *III*), or of an *N*-acetyllactosamine unit terminated either by fucose in $\alpha(1 \rightarrow 2)$-linkage or by GlcNAc in $\alpha(1 \rightarrow 4)$-linkage to galactose (compare with the minor and the major product of fraction *I*, respectively). The complexity of the mixture does not permit one to give the ratios of the various components of mixture *II*. However, the following comprehensive structure may be proposed for the various compounds of rat duodenal-gland oligosaccharide fraction *II*:



Discussion

A comparative ^{1}H-NMR study of the oligosaccharide chains of the mucous glycoproteins from

rat and pig stomach, duodenal gland and small intestine revealed the following.

1. For both species, the carbohydrate moieties of the mucous glycoproteins from the three histologically well-characterized tissues differ in typical terminating residues. Duodenal-gland oligosaccharides contain $\alpha(1 \rightarrow 4)$-linked GlcNAc, whereas small-intestinal mucus contains $\alpha(2 \rightarrow 3)$-linked NeuAc. Both of these terminating residues are absent from gastric mucin. One might wonder whether sialic acid-containing glycans were removed in our CsCl-purification step of the glycoproteins [11,15], or whether sialic acid previously reported to occur also in gastric mucins [27] might be derived from contaminating tissues and/or glycoproteins. The oligosaccharide chains from the mucous glycoproteins of the stomach are more complex than those of the other two investigated parts of the digestive tract. This may be a general phenomenon for higher mammals.

2. On comparison of rat and pig oligosaccharides for corresponding tissues, it appears that the carbohydrate chains of pig mucous glycoproteins are in general more complex than those of rat origin. This is in accordance with the complexity of the structures found by Derevitskaya and coworkers [3,16] for pig oligosaccharides containing $\alpha(1 \rightarrow 4)$-linked GlcNAc. In view of our results, it could be that the latter type of oligosaccharides originate from pig duodenal gland, present as contaminating material of pig gastric mucous glycoprotein preparations.

3. The rat duodenal-gland oligosaccharides exhibit an interesting kind of microheterogeneity, occurring in the upper as well as in the lower branch. All chains share the trisaccharide core of Gal$\beta(1 \rightarrow 3)$[GlcNAc$\beta(1 \rightarrow 6)$]GalNAc-ol (type-*iv* core [29]). The lower branch is mostly extended with galactose in $\beta(1 \rightarrow 4)$-linkage to GlcNAc. Both galactose residues may bear either a fucose in $\alpha(1 \rightarrow 2)$-linkage, or a GlcNAc in $\alpha(1 \rightarrow 4)$-linkage.

4. High-resolution ^{1}H-NMR spectroscopy is a suitable, non-destructive method for such comparative studies as mentioned above. First, already in an early stage, that is, on crude β-elimination products, information can be gained on typical constituents present. Then, with guidance from the ^{1}H-NMR spectra, a strategy for separation of the different components can be set up. In addition, the spectrum of the starting mixture provides us with the possibility of checking that all signals in the spectrum of the unfractionated material can be adequately explained on the basis of the sum of the spectra of the purified components. This shows whether or not a compound has become lost in the purification procedure. Valuable additional information during all steps stems from quantitative sugar analysis.

5. It has been suggested that the extraordinarily large resistance of the initial portion of the duodenum to acid-peptic digestion is due to the mucus secretions of duodenal (Brunner's) gland [30]. This could be associated with the typical structural features of the carbohydrate moieties of the mucin glycoproteins of duodenal glands established in our study. However, the physiological meaning of the observed tissue- and species-specificity of mucous glycoprotein oligosaccharide structures requires further investigation.

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