Carbohydrate Research, 79 (1980) 103–113

COMPARISON OF ALKALI-LABILE OLIGOSACCHARIDE CHAINS OF M AND N BLOOD-GROUP GLYCOPEPTIDES FROM HUMAN ERYTHROCYTE MEMBRANE

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(Received January 22nd, 1979; accepted for publication, April 1st, 1979)

ABSTRACT

Alkali–borohydride degradation of M or N blood-group active, tryptic glycopeptides and glycoproteins was performed under conditions giving the reduced oligosaccharides in a yield significantly improved over that reported earlier. Degradation of desialosylated glycoproteins yielded β-D-Gal*p*-(1→3)-D-GalNAcol, D-GalNAcol, and Galol in a ratio of ~30:1:1. GalNAc was shown to be α-D-linked to the polypeptide chain. Degradation of the glycopeptides gave the tetrasaccharide, NeuAc-(2→3)-β-D-Gal*p*-(1→3)-[NeuAc-(2→6)]-D-GalNAcol, and two trisaccharides, NeuAc-(2→3)-β-D-Gal*p*-(1→3)-D-GalNAcol and β-D-Gal*p*-(1→3)-[NeuAc-(2→6)]-D-GalNAcol, in a molar ratio of ~8:3:1. These oligosaccharides were accompanied by minor amounts of unidentified compounds showing identical electrophoretic mobility when derived from M and N glycopeptides. During isolation of the reduced oligosaccharides, the release of sialic acid did not exceed 5.5%, indicating that only a part of the trisaccharide portion might have arisen as a result of desialosylation of the tetrasaccharide. No differences between the degradation products derived from M and N glycoproteins were found, and the presence of significant amounts of larger, alkali-labile oligosaccharides was not observed.

INTRODUCTION

The alkali-labile oligosaccharides of M and N blood-group glycopeptides from human erythrocyte membranes were characterized by Thomas and Winzler[1], who found the major product of alkali-borohydride degradation to be the tetrasaccharide NeuAc-(2→3)-β-D-Gal*p*-(1→3)-[NeuAc-(2→6)]-D-GalNAcol, and considered the other identified compounds to be degradation products of this tetrasaccharide. An

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alternative structure for the tetrasaccharide with a (2→2 or 4)-linkage between the sialic acid and D-galactose residues was also proposed[2]. Despite the observation that the alkali-labile oligosaccharides are involved in M and N blood-group activity[3-6], no difference between oligosaccharides derived from these two antigens was found[1-3]. This agrees with the observations[6-14], that the genetically determined differences between M and N glycoproteins are located in the polypeptide chains. Springer *et al.*[15-19] suggested, however, that M and N glycoproteins, apart from common oligosaccharides, contain blood-group specific, alkali-labile oligosaccharides having 1 and 2 residues of sialic acid in the N and M antigens, respectively, and that these M- or N-specific oligosaccharides are more complex than the tetrasaccharide described previously; however, neither isolation nor structural studies on these compounds were reported. According to Springer and Yang[18], the complex oligosaccharides were not detected by Thomas and Winzler[1] because they were eluted from Sephadex columns prior to the alkali-labile tetrasaccharide, and together with the alkali-stable portion of the degraded glycopeptides. Indeed, the yield of reduced oligosaccharides in the procedure of Thomas and Winzler[1] was only 25–30% of the total carbohydrate content of the glycopeptides.

Therefore, we decided to characterize the oligosaccharides released from M and N antigens by the alkali–borohydride degradation of Iyer and Carlson[20]. This procedure, in contrast to the methods employed previously[1-3], results in an extensive cleavage of the serine (or threonine)–2-acetamido-2-deoxy-D-galactose linkage with minimal degradation of the released oligosaccharides[21]. Since we were primarily interested in the characterization of the oligosaccharides involved in blood-group specificity, we used for most experiments the purified amino-terminal, tryptic glycopeptides, which carry the M and N blood-group determinants and most of the oligosaccharide chains[9-14,22,23]. Some studies were also performed with whole glycoproteins.

EXPERIMENTAL

Colorimetric determinations. — Neutral sugars were determined by the phenol–sulfuric acid[24] or orcinol–sulfuric acid[25] method. Sialic acid, total or free, was determined by the periodate–resorcinol[26] or thiobarbituric acid[27] method, respectively; and 2-acetamido-2-deoxygalactose by the procedure of Ludowieg and Benmaman[3,28].

Gas–liquid chromatography. — Galactose and 2-acetamido-2-deoxygalactitol were determined as alditol acetates[29], after hydrolysis with 2M hydrochloric acid for 2 h at 100°, in the presence of inositol as an internal standard on a column (3 m) packed with ECNSS-M on Gas-Chrom Q at a temperature programmed from 150 to 220° (1°/min). Otherwise, monosaccharides were determined[30] as per-*O*-trimethylsilyl derivatives on OV-17 and SE-30 columns, at a temperature of 125° for 10 min, and then at a gradient from 125 to 230° (2°/min). Reduced disaccharides were determined[31], after trimethylsilylation, on OV-17 and SE-30 columns at 250°. The disaccharide 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose from

brain gangliosides was reduced and served as standard. The g.l.c.–m.s. of reduced disaccharides was performed on a 5992 A Hewlett–Packard apparatus equipped with an OV-17 column.

High-voltage electrophoresis and paper chromatography. — Electrophoresis was performed on Whatman No. 3MM paper at 70 V/cm for 30 min, in 5:2:43 (v/v) pyridine–acetic acid–water at pH 5.3. The following solvent systems were used for descending paper chromatography: (*A*) 6:4:3 1-butanol–pyridine–water and (*B*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water, all v/v. Whatman papers No. 3MM and No. 1 were used for preparative and analytical purposes, respectively. For the separation of monosaccharides and oligosaccharides, chromatograms were irrigated for 40 h and 3–4 days, respectively. Carbohydrates were visualized with the periodate–benzidine reagent[32]. Hexosamines and their derivatives were also detected with ninhydrin.

Preparation of glycoproteins and glycopeptides. — M, N, or MN glycoproteins were obtained from the respective erythrocytes of blood group O by phenol–water extraction of membranes[33,34]. M- or N-active tryptic glycopeptides were isolated from trypsin-digested glycoproteins as described previously[9].

Isolation of reduced oligosaccharides. — A 0.4% solution of M or N glycopeptide in 50mM NaOH–M $NaBH_4$ was kept for 18 h at 50°. The excess of borohydride was decomposed with acetic acid. The solution was concentrated *in vacuo* and desalted on a Sephadex G-10 column eluted with 50mM pyridine acetate buffer, pH 5.3. The carbohydrate-containing fractions were pooled and fractionated on a Sephadex G-50 (fine) column eluted with the same buffer. Fractions containing reduced oligosaccharides (the second carbohydrate peak) were pooled and concentrated *in vacuo*. Oligosaccharides were subsequently fractionated by preparative, high-voltage electrophoresis, which was repeated until a homogeneous material was obtained. Oligosaccharides that were not homogeneous by paper chromatography were also fractionated by preparative paper chromatography.

Desialosylation. — Sialic acid was split off from glycoproteins[35] and oligosaccharides by hydrolysis with 50 or 25mM H_2SO_4 for 1 h at 80°. Solutions of oligosaccharides were neutralized with $BaCO_3$, centrifuged, and the supernatant was evaporated *in vacuo*.

Periodate oxidation. — Glycoproteins, *N*-succinylated in 6M guanidine hydrochloride, were oxidized with periodate for 18 h and degraded under conditions previously described[35]. Oligosaccharides (0.1%) were oxidized with 0.3M $NaIO_4$ for 18 h at 4° in darkness, and then treated with $NaBH_4$ (4 mg/mL) for 1 h at 20°. The excess of borohydride was removed with acetic acid. The samples, after evaporation *in vacuo*, were fractionated by high-voltage electrophoresis.

Alkali–borohydride degradation of glycoproteins. — Glycoproteins were degraded under the conditions of Iyer and Carlson[20], including desalting the samples by treatment with Dowex 50 (H^+), followed by coevaporation of boric acid with methanol.

Treatment with N-*acetylhexosaminidases.* — Desialosylated or desialosylated–

Smith-degraded glycoproteins were incubated with *β-N*-acetyl-D-hexosaminidase or *α-N*-acetyl-D-galactosaminidase of *Charonia lampas* (Seikagaku Fine Biochemicals, Tokyo) (0.01 enzyme unit per 1 mg of glycoprotein) in 0.2M acetate buffer (pH 4.0) for 48 h at 37° under toluene. The samples were desalted with Dowex 1 X-4 (HCO_3^-) and 50W X-8 (H^+), and the released monosaccharides were quantitatively determined by gas–liquid chromatography. Enzyme alone and boiled enzyme with glycoprotein served as controls.

Inhibition of hemagglutination. — The assay systems as well as the animal and plant agglutinins used have been described previously[35].

RESULTS

Products of alkali–borohydride degradation of desialosylated glycoproteins. — It was shown by gas–liquid chromatography that during alkaline degradation of desialosylated glycoproteins 83% (±15%, n = 5) of the 2-acetamido-2-deoxygalactose were converted into 2-acetamido-2-deoxygalactitol residues. The main product released from disialosylated M, N, and MN glycoproteins, which accounted for ~89% of the 2-acetamido-2-deoxygalactose content present in the undegraded substances, was identified as 2-acetamido-2-deoxy-3-*O*-*β*-D-galactopyranosyl-D-galactitol as follows: gas–liquid chromatography–mass spectrometry yielded a fragmentation pattern, reportedly[36] characteristic for hexosyl-(1→3)-2-acetamido-2-deoxyhexitol, and the *β*-D configuration of the D-galactosyl group was deduced by comparison with the reduced standard disaccharide from brain gangliosides, as *α*-D-Gal*p*-(1→3)-D-GalNAcol shows a different retention time on the columns used[36]. The samples of degraded asialoglycoproteins, after treatment with Dowex 1 (X-4, HCO_3^-), were examined by paper chromatography. The reduced disaccharide showed an R_{Lac} 1.6 (solvent *A*) and no compounds with slower mobility were observed.

Small amounts of 2-acetamido-2-deoxygalactitol (3.5% ±0.5% relative to the reduced disaccharide, n = 5) and galactitol (3.2% ±0.6%, n = 3) were detectable by gas–liquid chromatography in the products of alkali degradation of desialosylated glycoproteins, indicating a negligible degradation by the alkaline reagent of the liberated oligosaccharides. A faint spot corresponding to 2-acetamido-2-deoxygalactitol was also observed on paper chromatograms developed in solvents *A* and *B*. 2-Acetamido-2-deoxygalactitol could not be detected by gas–liquid chromatography among the products of the alkali degradation of native glycoproteins. This indicates that the glycoproteins contain a small proportion (less than one per molecule) of shorter oligosaccharides, presumably NeuAc-(2→6)-D-GalNAc*p*.

Treatment of glycoproteins with N-*acetylhexosaminidases.* — To confirm the presence of 2-acetamido-2-deoxy-D-galactose unsubstituted with galactose residues, and to establish the anomeric configuration of the 2-acetamido-2-deoxy-D-galactopyranosyl groups, desialosylated or desialosylated–Smith degraded glycoproteins were treated with *α*- and *β-N*-acetyl-D-hexosaminidases. No 2-acetamido-2-deoxy-D-hexose was released by the latter enzyme. The *α-N*-acetyl-D-galactosaminidase re-

TABLE I

EFFECTS OF VARIOUS TREATMENTS OF DESIALOSYLATED GLYCOPROTEINS ON THEIR ACTIVITY TOWARD 2-ACETAMIDO-2-DEOXYHEXOSE-SPECIFIC AGGLUTININS[a]

Treatment of desialosylated glycoproteins	*Agglutinin[b] from*			
	Helix pomatia[c] (*α or β*)	Soja hispida (*α or β*)	Cepaea hortensis (*α*)	Dolichos biflorus (*α*)
None	0.08	>10	>10	>10
α-*N*-Acetylgalactosaminidase	5	>10	>10	>10
Smith degradation	0.02	0.01	0.01	0.08
Smith degradation and *α*-*N*-acetylgalactosaminidase	1.3	0.7	0.7	2.5
$NaIO_4$ oxidation–$NaBH_4$ reduction	5.0			
NaOH–$NaBH_4$ degradation	>10			

[a]The activity is given as the minimal inhibitory concentration in mg/mL. [b]Specificity denotes the anomeric configuration of the 2-acetamido-2-deoxy-D-galactopyranosyl groups required for the reaction with the agglutinin; it is indicated between parentheses. [c]Similar results were obtained with agglutinins from *Helix lucorum* and *Helix aspersa*. These three agglutinins are not inhibited by sialoglycoproteins[35].

leased 3.6% (±0.6%, n = 2) of the total 2-acetamido-2-deoxy-D-galactopyranosyl groups from desialosylated glycoproteins and 68% (±7.1%, n = 2) from desialosylated–Smith-degraded compounds. The effect of *α*-*N*-acetyl-D-galactosaminidase treatment on the inhibitory activity of glycoproteins toward 2-acetamido-2-deoxy-D-hexose-specific heterophile agglutinins is shown in Table I. The results suggest that desialosylated glycoproteins inhibit *Helix pomatia* agglutinin owing to the content of terminal 2-acetamido-2-deoxy-*α*-D-galactopyranosyl groups. The release of only two thirds of the 2-acetamido-2-deoxy-D-galactopyranosyl groups from the desialosylated–Smith-degraded glycoprotein correlates with the incomplete loss of its activity toward 2-acetamido-2-deoxy-*α*- and -*α*,*β*-D-galactose-specific agglutinins. This suggests that the residual 2-acetamido-2-deoxy-D-galactopyranosyl groups left on the enzyme-treated glycoprotein also have the *α*-D configuration.

Release of sialic acid during isolation of alkali-labile sialyloligosaccharides. — During the course of alkali–borohydride and subsequent Dowex 50 (H^+) treatment of sialo-glycoproteins or -peptides, appreciable amounts (5–30%) of sialic acid were released. Alkali–borohydride degradation of sialoglycoproteins resulted in the formation of 9% (±3%, n = 5) of free *β*-D-Gal*p*-(1→3)-D-GalNAcol, in comparison with an equivalent amount of asialoglycoprotein. This reduced disaccharide was predominantly an artefact generated by desialosylation, as similar proportions (6% ±3%, n = 4) of the compound were detectable after alkali–borohydride treatment of periodate oxidized–borohydride reduced sialoglycoproteins, where the terminal galactopyranosyl groups would be degraded. These data show the importance of avoiding Dowex 50 (H^+) in the isolation of alkali-labile sialyloligosaccharides.

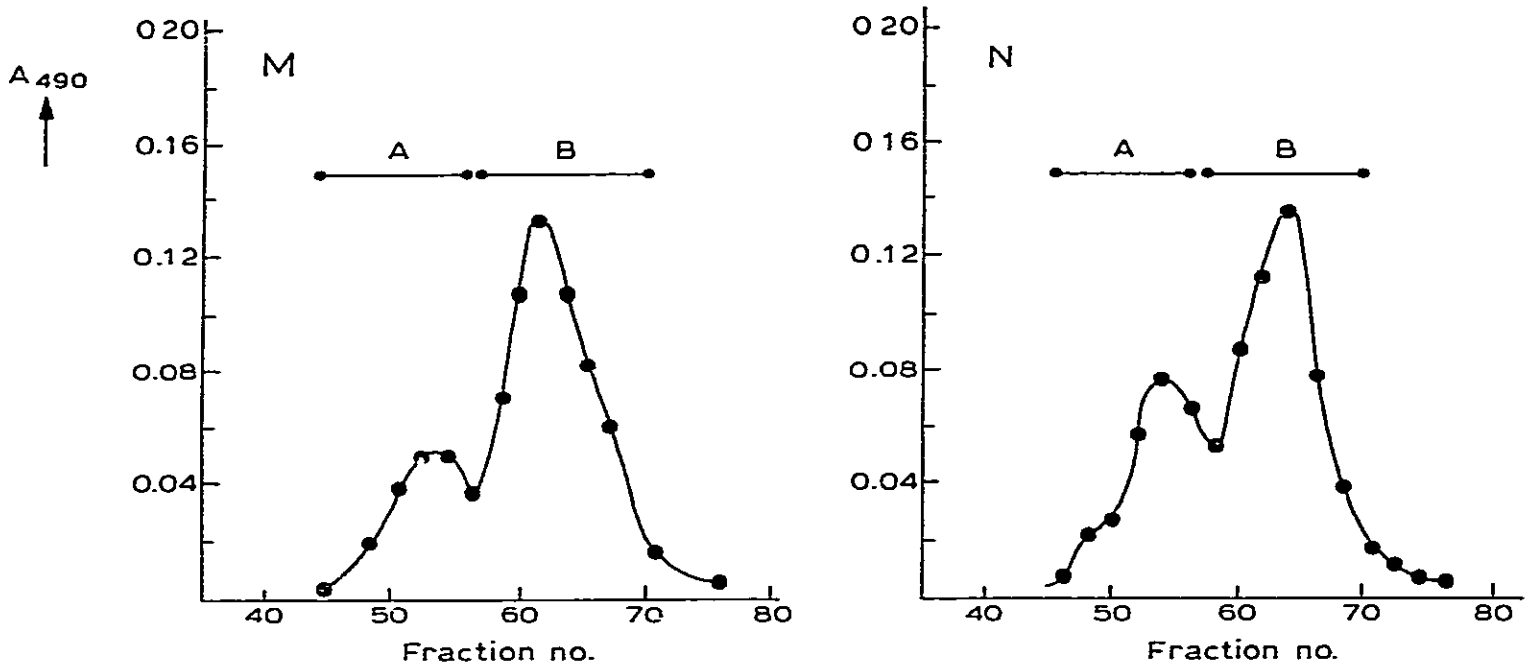

Fig. 1. Fractionation of the products of alkali degradation of M and N tryptic glycopeptides (20 mg) on Sephadex G-50 (fine) column (2 × 45 cm). Fractions of 1.8 ml were collected and analyzed for neutral sugars (50-μl aliquots) by the phenol–sulfuric acid method.

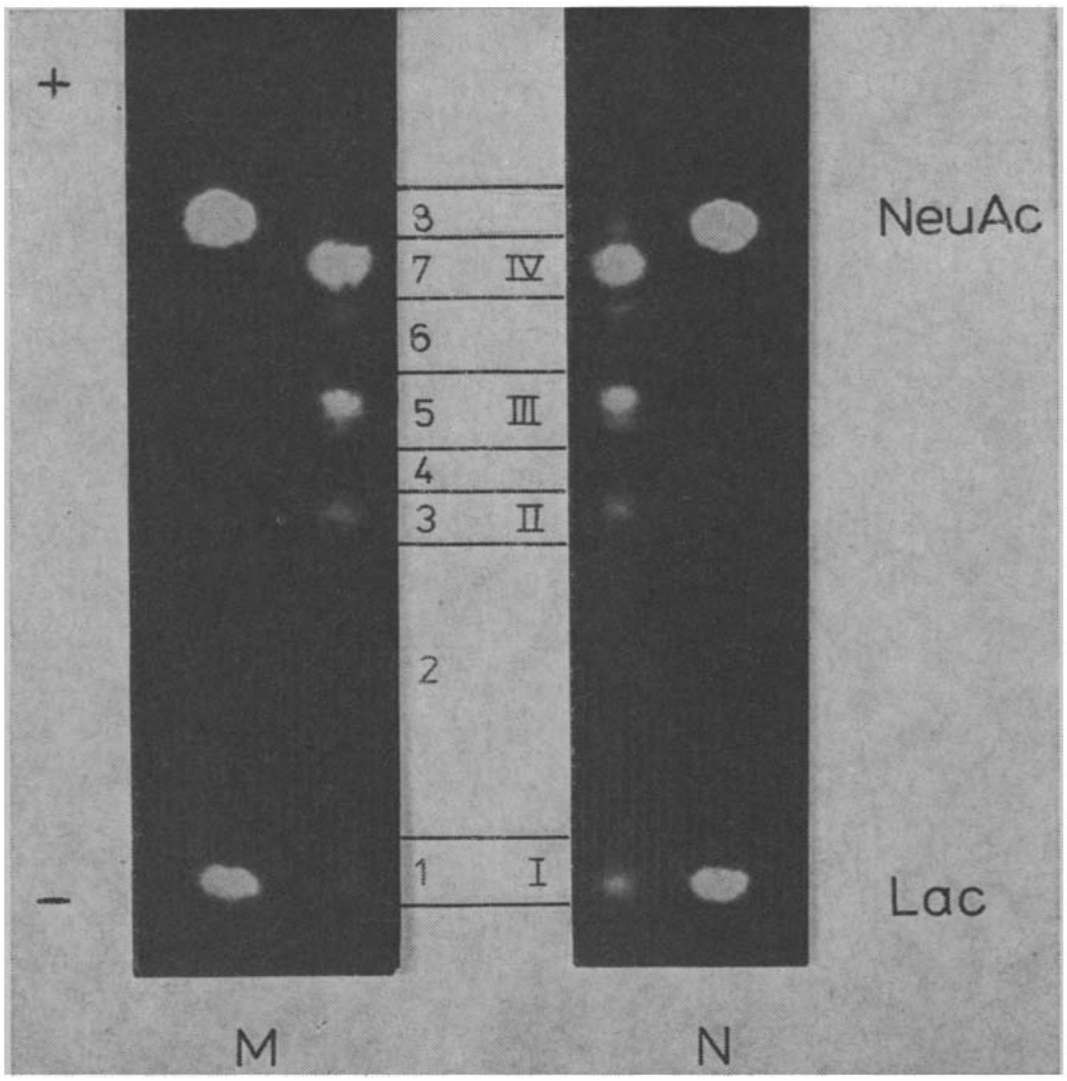

Fig. 2. High-voltage electrophoresis of the oligosaccharide fractions obtained from M and N tryptic glycopeptides. Staining with the periodate–benzidine reagent. Between the electropherograms: the sections of the paper from the preparative electrophoresis that were eluted for the quantitative determination of the electrophoretic fractions (see Table II).

TABLE II

DISTRIBUTION OF GALACTOSE AND *N*-ACETYLNEURAMINIC ACID CONTENT AFTER HIGH-VOLTAGE ELECTROPHORESIS OF REDUCED OLIGOSACCHARIDE FRACTIONS DERIVED FROM M AND N GLYCOPEPTIDES[a]

Strip No.	*Spot No.*	*M*			*N*		
		Gal (μg)	*NeuAc (μg)*	*NeuAc:Gal ratio (mol)*	*Gal (μg)*	*NeuAc (μg)*	*NeuAc:Gal ratio (mol)*
Paper electrophoresis:							
1	I	51	36	0.4	50	43	0.5
2		4	7		20	15	
3	II	56	156	1.6	63	167	1.5
4		0	0		13	28	
5	III	109	171	0.9	130	231	1.0
6		20	68		33	92	
7	IV	207	721	2.0	300	986	1.9
8		4	4		7	51	
Amount applied		520	1431		690	1920	
Total recovered		442	1163		616	1613	
Paper chromatography:							
	IIIa	18	24	0.7	21	28	0.8
	IIIb	52	77	0.9	63	116	1.1

[a]See Fig. 2.

TABLE III

CARBOHYDRATE COMPOSITION OF REDUCED OLIGOSACCHARIDES OBTAINED FROM M AND N GLYCOPEPTIDES

Glycopeptide	*Oligosaccharide*	*NeuAc (%)*	*Gal (%)*	*GalNAcol (%)*	*NeuAc:Gal:GalNAcol ratio (mol)*
M	IIIa	38.7	21.4	24.3	1.0:1:0.8
	IIIb	37.7	19.6	28.2	1.1:1:1.2
	IV	58.6	14.6	18.7	2.3:1:1.1
N	IIIa	38.9	22.7	26.5	1.0:1:0.9
	IIIb	36.2	19.3	23.2	1.1:1:1.1
	IV	59.3	15.9	19.9	2.2:1:1.0

Isolation of alkali-labile oligosaccharides from M and N tryptic glycopeptides. — The degradation of glycopeptides was fairly complete, as no 2-amino-2-deoxygalactose was detectable by colorimetric reaction[28]. The elution profile of the degradation products from a Sephadex G-50 column is shown in Fig. 1. The retarded Fraction B, derived from M or N glycopeptide, contained 63 or 62% of the neutral sugars and

93 or 87% of the total sialic acid, respectively. The content of free sialic acid in the degradation products was 4.3% (±1.2%, n = 5).

Oligosaccharide Fraction B produced several spots on high-voltage electrophoresis (Fig. 2). The eluates from the strips corresponding to the four main spots and to adjacent areas (see Fig. 2) were analyzed for neutral sugars and sialic acid. The results summarized in Table II show that ~¾ of the neutral sugars and sialic acid recovered from the paper were found in Fractions III and IV. The electrophoretic patterns of alkali-degraded M and N glycopeptides were closely similar.

The major fractions (III and IV) were purified by two-fold electrophoresis. The purified Fraction IV was homogeneous in electrophoresis and paper chromatography (R_{Lac} 0.08 in solvent *A*). Fraction III showed a trailing spot in electrophoresis, and two components in paper chromatography with R_{Lac} 0.19 (IIIa) and 0.43 (IIIb). Oligosaccharides IIIa and IIIb were isolated by preparative paper chromatography. The amount of oligosaccharide IIIb was about three times that of IIIa (Table II).

Attempts to purify Fractions I and II by re-electrophoresis produced several spots (including compounds III and IV) and the electrophoresis had to be repeated 2–3 times. The purified Fractions I and II did not move in paper chromatography, and owing to low yield they were not further characterized.

Characterization of oligosaccharides. — The chemical composition of the oligosaccharides (Table III) indicates that compounds IIIa and IIIb are trisaccharides, and compound IV is a tetrasaccharide. All these oligosaccharides yielded the disaccharide 2-acetamido-2-deoxy-3-*O*-*β*-D-galactopyranosyl-D-galactitol after desialosylation. Periodate oxidation did not destroy the D-galactose residue in oligosaccharides IV and IIIb, and completely destroyed it in the oligosaccharide IIIa. The derivative of 2-acetamido-2-deoxygalactitol (a major periodate–benzidine- and ninhydrine-positive compound), found in the hydrolyzed products of the periodate oxidation of oligosaccharides IIIa, IIIb, and IV, had identical chromatographic (R_{GalN} 1.6 in solvent *A*) and electrophoretic (R_{GalN} 1.35) mobilities. Thomas and Winzler[1] established unequivocally that periodate oxidation–reduction of the tetrasaccharide converted the 2-acetamido-2-deoxygalactitol into a 2-acetamido-2-deoxythreitol residue. Therefore, the identical chromatographic and electrophoretic properties of the 2-acetamido-2-deoxygalactitol derivatives described here allow to conclude that in all of the oligosaccharides studied 2-acetamido-2-deoxygalactitol is oxidized to 2-acetamido-2-deoxythreitol.

The periodate-oxidized and reduced oligosaccharides were fractionated by electrophoresis prior to hydrolysis, and eluates were obtained from two areas of the paper: (a) from the strip corresponding to the lactose standard (the neutral fraction), and (b) from the wide strip covering the area from the position of Fraction III to that of the sialic acid standard (sections 5–8 in Fig. 2, the acidic fraction). The eluates were hydrolyzed and analyzed by chromatography and electrophoresis. In the case of oligosaccharides IIIb and IV, 2-amino-2-deoxythreitol was found only in the acidic fraction together with galactose. For oligosaccharide IIIa, galactose was not detected,

and 2-amino-2-deoxythreitol was found only in the neutral fraction, showing that it was separated from sialic acid during oxidation.

The results obtained indicate that oligosaccharides IV and IIIb are the tetrasaccharide NeuAc-(2→3)-β-D-Gal*p*-(1→3)-[NeuAc-(2→6)]-D-GalNAcol and the linear trisaccharide NeuAc-(2→3)-β-D-Gal*p*-(1→3)-D-GalNAcol, respectively, previously identified by Thomas and Winzler[1]. Oligosaccharide IIIa is also a trisaccharide composed of N-acetylneuraminic acid and a β-D-Gal*p*-(1→3)-D-GalNAcol residue. The finding of 2-amino-2-deoxythreitol in the neutral fraction of its product of periodate oxidation may be explained only by the linkage of the *N*-acetylneuraminosyl group to O-6 of the 2-acetamido-2-deoxygalactitol residue. Therefore, it is suggested that Compound IIIa is the branched trisaccharide β-D-Gal*p*-(1→3)-[NeuAc-(2→6)]-D-GalNAcol.

DISCUSSION

The results of alkaline degradation of desialosylated glycoproteins show that the alkali-labile oligosaccharides of M and N glycoproteins are predominantly sialyl derivatives of the disaccharide residue 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranosyl. Oligosaccharides larger than a disaccharide were not detected in desialosylated glycoproteins, in accordance with our earlier investigations[3], and in contradistinction with the suggestions of Springer *et al.*[15–19]. Recently, Takasaki *et al.*[37] using enzymic and isotopic methods found, in human blood-group O red-cell membrane glycoproteins, alkali-labile oligosaccharides having a 2-*O*-α-L-fucopyranosyl-β-D-galactopyranosyl nonreducing end-group. This finding does not contradict our results, however, since the yields of the fucosyloligosaccharides were low, *i.e.*, one oligosaccharide per 15–45 mol of the glycoprotein[37]. It has not been established whether these oligosaccharides are constituents of the MN glycoprotein or of other minor membrane components.

Our results are basically consistent with those of Thomas and Winzler[1], confirming that the major alkali-labile oligosaccharide is a tetrasaccharide (compound IV) and that the second significant compound is a linear trisaccharide (compound IIIb). Our initial experiments employing a desalting of the alkaline degradation products with Dowex 50 (H^+) showed, in addition to compound IV, substantial amounts of a linear (compound IIIb) and a branched (compound IIIa) trisaccharide, 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactitol, and free sialic acid. Therefore, strongly acidic conditions were avoided during the isolation of oligosaccharides described in this paper. However, the release of sialic acid, albeit low, could not be completely eliminated. Under the conditions used, the disaccharide was not detected in the degradation products, but the tetrasaccharide (compound IV) was still accompanied by two trisaccharides (compounds IIIa and IIIb). Based on the quantitative determination of oligosaccharides after electrophoretic and chromatographic separation, the ratio of IV to IIIb to IIIa was approximately 8:3:1. The yield of compound IIIb was too large to consider it as an artefact. However,

it cannot be ruled out that the branched trisaccharide (IIIa) is a product of the desialosylation of the tetrasaccharide, which is formed during isolation. This finding does not invalidate the main goal of this work, as compound IIIa was obtained in the same yield from both M and N glycopeptides.

Within the limits of experimental error, no difference between the alkali-labile oligosaccharides of M and N substances was found. The tryptic glycopeptides contain 11 or 12 alkali-labile oligosaccharide chains per molecule[23]. The present results show that the methods used can easily detect one oligosaccharide chain per glycopeptide molecule. The reduced oligosaccharide fraction accounted for >70% of the total carbohydrate content of the glycopeptides, but oligosaccharides larger than a tetrasaccharide were not found. The unidentified compounds having a low chromatographic mobility, present in electrophoretic Fractions I and II, may be larger oligosaccharides (or possibly degraded glycopeptide fragments), but their yields after purification were too low to correspond to one oligosaccharide chain per glycopeptide molecule. Moreover, the identical electrophoretic properties and the similar sialic acid-to-galactose ratios of Fractions I and II derived from M and N glycopeptides rule out the possibility that the unidentified compounds are oligosaccharides responsible for the difference between M and N antigens based on a different proportion of sialyl groups, as suggested by Springer *et al.*[15–19]. In addition, the carbohydrate compositions of the M- or N-active cyanogen bromide fragments are compatible with the presence of three alkali-labile tetrasaccharides on the amino-terminal octapeptides[11,12].

The lack of difference between the oligosaccharides from M and N glycopeptides is in agreement with the differences found between their polypeptide chains[9–14]. These alkali-labile oligosaccharides, which are the same in M and N glycoproteins, play an important role in the antigenic properties[3–6,8], but, for the expression of the M and N blood-group determinants, the combination of oligosaccharide(s) with the specific polypeptide fragment is required[11,12].

ACKNOWLEDGMENTS

This work was supported by grant no. 10.5 of the Polish Academy of Sciences, by a U.S. National Institutes of Health PL-480 Research Agreement (no. 05-001-0), and by the Deutsche Forschungsgemeinschaft (SFB 68/D1). Work by one of us (W.D.) was performed at the Department of Immunobiology, Medical University Clinic Cologne. The authors thank Prof. G. Uhlenbruck for his support, Mrs. R. Schmalisch and E. Janssen for their expert technical assistance, Prof. W. Gielen for a gift of β-D-Gal*p*-(1→3)-D-GalNAc, and Prof. W. Donicke for performing the g.l.c.–m.s. analyses.

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