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Structural polymorphism within the amino-terminal region of MM, NN, and MN glycoproteins (glycophorins) of the human erythrocyte membrane

(MN antigens/CNBr peptides)

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ABSTRACT MM, NN, and MN glycoproteins of human erythrocytes from single donors were cleaved by cyanogen bromide into three fragments—A, B, and C—which, upon gel electrophoresis, appeared to be common to the three antigens. Phenol/aqueous urea partitioning and gel filtration were used to separate the peptides quantitatively. Peptide C lacked carbohydrate and homoserine and represented the carboxyl-terminal portion of the glycoproteins. Peptides A and B contained one homoserine each and accounted for all the carbohydrate of the glycoproteins. The peptide portion of glycopeptide A from MM, NN, or MN antigens consisted of eight amino acid residues, of which six were homologous and two varied according to blood type. The variants were serine and glycine in glycopeptide A(MM), leucine and glutamic acid in A(NN), and half-residues of serine, glycine, leucine, and glutamic acid in A(MN). Serine was the amino-terminal residue in A(MM), leucine in A(NN), and one half residue of serine and leucine in A(MN). Each glycopeptide carried two tetrasaccharides (2 NANA, 1 Gal, 1 GalNAc) and one trisaccharide (NANA, Gal, GalNAc) linked *O*-glycosidically to one serine and two threonines as determined by β-elimination and sulfite addition. The carbohydrate units were attached to serine and threonine located in the invariant region, because the amino-terminal serine residue could be oxidized by periodate. The M-N antigens are believed to be products of allelic genes which are expressed exclusively in homozygotes and equimolarly in heterozygotes.

A linear sequence of the polypeptide portion of the major sialoglycoprotein from pooled human erythrocytes has been reported by Tomita and Marchesi (1) with apparent substitutions occurring at positions 1 and 5 of the sequence. We have been studying the covalent structure of similar glycoproteins isolated from individual donors in order to document anticipated structural polymorphism because these proteins carry distinct blood group specificities and are in fact the M-N antigens of erythrocytes. It has been shown that the carbohydrate portion of MM, NN, and MN specific glycoproteins appears to be similar in content, distribution, and indeed linear structure (2–5) and could not alone explain differences in blood group specificities. Two recent reports (6, 7) have presented evidence, based on amino-terminal determinations and compositional analyses of proteolytic fragments, that suggested that type-specific amino acid substitutions may occur at positions 1 and 5 of the amino-terminal region of MM and NN glycoproteins and that such substitutions may in part account for the observed specificities.

Because the M-N antigens contain two methionine residues per polypeptide, it has been possible to examine in some detail the structure of CNBr cleavage products of each protein. In this communication we report a simple procedure for the isolation of the three CNBr peptides in nearly quantitative yields and confirm unequivocally that the amino-terminal region contains amino acid substitutions that are characteristic of each blood group. The data indicate that the MM and NN glycoproteins are products of allelic genes and that the heterozygous form (MN) occurs as an equimolar expression of the two alleles.

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MATERIALS AND METHODS

Preparation of Erythrocyte Membranes. Erythrocytes separated from freshly drawn typed human blood were lysed in hypotonic buffer containing 0.02% NaN_3, 1 mM phenylmethylsulfonyl fluoride, and 2 mM *N*-ethylmaleimide, and the cell ghosts were collected as described by Dodge *et al.* (8).

Preparation of M-N Glycoproteins. Erythrocyte membranes were solubilized in aqueous pyridine and the MM, NN, and MN antigens were isolated and purified essentially as described (9) except for the following modifications. The glycoproteins (~50 mg) were extracted with 10 ml of chloroform/methanol, 2:1 (vol/vol), dissolved in 1 ml of water, and redelipidated according to Folch *et al.* (10). Lipid-free glycoproteins (upper phase) were further purified on a Bio-Gel P-100 column (2.5 × 80 cm) in 0.1 M pyridine acetate, pH 5.0. Fractions were monitored for the presence of sialic acid (NANA) after hydrolysis of appropriate aliquots in 0.05 M H_2SO_4 (1 hr, 80°) (11). One NANA-containing peak[*] eluted in the void volume of the column and amounted to a yield of approximately 30 mg of glycoprotein per unit of whole blood. The purified glycoproteins were tested for hemagglutination inhibition activity against specific antisera.

CNBr Cleavage of the M-N Glycoproteins. Glycoproteins obtained from individual donors were treated with 30% CNBr in 75% formic acid for 35 min at 25° under nitrogen, after which the digests were lyophilized to remove all reagents. These conditions were found optimal in that they resulted in quantitative conversion of methionine to homoserine and released less than 5% of NANA residues from the glycoproteins.

Isolation of CNBr Peptides. CNBr-cleaved products from about 30 mg of glycoprotein were dissolved in 7 ml of 6 M urea and treated with an equal volume of anhydrous phenol at 67° for 10 min. After cooling, 0.2 vol of water was added to effect partitioning of phases. The phases were separated by centrifugation at 600 × *g*. The lower phase was washed twice with the upper phase of mixture of equal volumes of phenol and 6 M urea. The aqueous phase contained the hydrophilic peptides (glycopeptides A and B) and the phenol layer contained the

Abbreviations: NANA, sialic acid; GalNAc, *N*-acetylgalactosamine.

[*] Glycoprotein preparations isolated from membranes that were processed in the absence of proteolytic inhibitors were found to contain, in addition to the major glycoprotein, a second lower molecular weight component which was retarded on Bio-Gel P-100 and contained 5–19% of the protein-bound NANA placed on the column. Those preparations were considered to have been modified by limited proteolysis and will not be described further.

carbohydrate-free hydrophobic peptide (peptide C). Details of purification of peptides B and C will be described elsewhere. Glycopeptides A and B were fractionated on a column of Bio-Gel P-6 (1.4 × 84 cm) with 0.1 M pyridine acetate (pH 5.0) as elutant.

Introduction of ^3H into NANA Residues of CNBr Glycopeptides.† Prior to fractionation of CNBr digests with phenol/urea, a portion (5%) equivalent to about 1–2 mg of glycoprotein was removed and treated sequentially with $NaIO_4$ and NaB^3H_4 as described (12, 13). Periodate was used in amounts equivalent to the total NANA content of the CNBr fragments. NaB^3H_4 (200 μCi/μmol) was added in 25-fold molar excess in relation to periodate and was followed, after 5 min, by a further addition of a similar amount of unlabeled borohydride. The reduction was terminated with excess acetone, and the mixture was neutralized with acetic acid and then combined with the unlabeled digest. This procedure labeled the glycoprotein fragments to the extent of 25–30 × 10^6 dpm/mg and provided a sensitive monitor for subsequent fractionation of the CNBr glycopeptides. Radioactivity was measured by a liquid scintillation spectrometer (Packard Instruments) with a solution containing 0.4% Omnifluor (New England Nuclear) and 2% Bio-Solv (Beckman) in toluene as scintillant.

Hemagglutination and Hemagglutination Inhibition Activity. Cell typing was performed by using antisera obtained from Ortho Pharmaceutical Co. (Raritan, NJ). Hemagglutination inhibition activity of the glycoprotein and the CNBr fragments was determined by their ability to inhibit agglutination of typed cells by corresponding antisera (14).

Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out on 10% and 15% slab gels in 0.1% sodium dodecyl sulfate by the procedure of Maizel (15). Prior to electrophoresis, samples were incubated in running buffer containing 1% sodium dodecyl sulfate for 1 hr at 37°. Gels were stained with Coomassie or Schiff stains (16). Radioactive sample gels were sliced into 1-mm sections and solubilized in 30% H_2O_2 (17), and the radioactivity was measured by liquid scintillation.

Amino Acid Analysis. Glycopeptides were hydrolyzed in peroxide-free constant-boiling HCl under nitrogen for 24 hr at 107° and then analyzed on a JLC-6AH amino acid analyzer (JEOLCO) adapted to a Durrum DC-6A column (0.9 × 30 cm) utilizing Pico buffer system II and a program in which buffer A was adjusted to pH 2.82 and run through the analyzer for 140 min. This schedule resolved homoserine completely from glutamic acid. To quantitate homoserine the hydrolysates were evaporated to dryness *in vacuo*, treated with 0.1 M NaOH for 5 min at 100°, neutralized, and placed on the analyzer. The alkaline treatment was necessary to hydrolyze homoserine lactone but it also resulted in complete destruction of hexosamines. Such treatment was therefore utilized in situations in which selective destruction of the amino sugars was desired. Cysteic acid or norleucine was used as an internal standard.

Carbohydrate Analysis. NANA was released by mild acid hydrolysis and quantitated by the procedure of Warren (11) as described by Spiro (18). Neutral sugars were hydrolyzed in 1 M HCl for 4 hr at 100°, and the hydrolysates were passed through Dowex 50-X4 (H^+)/Dowex 1-X8 (formate). The neutral sugar fraction was quantitated by gas/liquid chromatography as the alditol acetates (19) in a Perkin–Elmer model 910 system equipped with an M-2 integrator and columns of 3% SP-2340 on 100/120 Supelcoport (Supelco Inc., Bellefonte, PA). 2-Deoxyglucose was used as internal standard. Amino sugars were released by hydrolysis of glycopeptides in 4 M HCl for 4 hr at 100° and analyzed directly on the amino acid analyzer or by gas/liquid chromatography of the alditol acetate derivatives on OV 225 columns. In preparation for this analysis, the amino sugars were *N*-acetylated (20), desalted on columns of Dowex 50-X4 (H^+)/Dowex 1-X8 (formate), reduced, and peracetylated. Inositol served as internal standard.

Amino Terminus Analyses. Dinitrophenylation of glycopeptides was carried out according to the procedure of Sanger (21). Glycopeptides were dissolved in 0.4 M $NaHCO_3$ and allowed to react with an equal volume of 5% 2,4-fluorodinitrobenzene in ethanol for 4 hr at room temperature. Dinitrophenylated products were hydrolyzed in constant-boiling HCl for 20 hr and analyzed on the amino acid analyzer. Amino-terminal residues were quantitated by a subtractive procedure. To identify the dinitrophenyl amino acids, hydrolysates were extracted with ether and the extracts were subjected to ascending chromatography on cellulose thin-layer plates (Eastman) with 1.5 M sodium phosphate buffer (pH 6.0) as the irrigating solvent (22).

Periodate Oxidation. Oxidation of glycopeptides was carried out with 0.07 M sodium metaperiodate in 0.035 M acetate buffer (pH 4.5) for 6 hr at 0°–4°. The reaction was stopped by addition of ethylene glycol, and iodate ions were precipitated by addition of barium methoxide. Excess barium was precipitated as the carbonate with CO_2. Solutions were centrifuged after each precipitation, dried, hydrolyzed, and analyzed for amino acid and hexosamine contents.

Alkaline Sulfite Treatment. Glycopeptides were treated with 0.1 M NaOH containing 0.5 M Na_2SO_3 (23) at 37° for 24, 48, 72, and 96 hr, after which they were neutralized, dried, and hydrolyzed in constant-boiling HCl. The hydrolysates were examined on the amino acid analyzer for decreases in serine, threonine, and galactosamine and concomitant appearance of cysteic acid, α-amino-β-sulfonylbutyric acid, and sulfonylhexosamines. Because cysteic acid was not resolved from sulfonylbutyric acid and both eluted in the void volume of the Durrum column, these amino acids were quantitated by using the color factor of cysteic acid. Sulfonylgalactosamine would be expected to derive from *N*-acetylgalactosamine (GalNAc) residues that were linked to serine or threonine and also carried good leaving groups at their 3-hydroxyl functions (24). Sulfonylgalactosamine was retained on the resin and eluted 4 min after cysteic acid. Sulfonylgalactosamine was kindly provided by R. G. Spiro, Harvard Medical School, and was used as standard.

RESULTS

Our procedure for isolating sialoglycoproteins from human erythrocytes obtained from single donors has been modified to include the use of proteolytic inhibitors during the preparation of erythrocyte ghosts, a delipidation sequence, and gel column chromatography of the delipidated product. Reflecting donor type, the glycoproteins possessed MM, NN, or MN specificities but no detectable AB activities as determined by hemagglutination inhibition assays and showed similar gross carbohydrate and amino acid compositions. Consistent with a molecular weight of about 30,000 (25, 26), each protein contained two residues of methionine per polypeptide.

Protein Cleavage with CNBr and Isolation of Fragments. CNBr digestion of each glycoprotein followed by radiolabeling of the digests yielded two major peptides upon slab gel electrophoresis (Fig. 1). The smaller peptide (peptide C) migrated slightly slower than standard cytochrome *c* and stained strongly with Coomassie brilliant blue but not at all with periodic acid-Schiff stain. The larger peptide (glycopeptide B) on the

† Labeling of the glycoprotein in this manner prior to CNBr cleavage is not advisable because it was found to oxidize methionine to a small but significant extent and decreased the yield of homoserine.

other hand stained well with periodic acid-Schiff but only faintly with Coomassie and migrated to an intermediate position between ovalbumin and cytochrome *c* standards. A third expected major peptide (glycopeptide A) did not stain with Coomassie or periodic acid-Schiff but was found to migrate with the tracking dye by slicing the gel and monitoring for radioactivity (see below and Fig. 3). Two apparently minor components that migrated to the vicinity of the original glycoprotein and slightly ahead of ovalbumin are thought to be aggregates of glycopeptide B. Efforts to separate the CNBr fragments by conventional column chromatography were largely unsuccessful because of their tendency to self- and cross-aggregate. However, partitioning of the fragments in phenol/aqueous urea resulted in the complete separation of glycopeptides A and B into the aqueous phase with peptide C remaining exclusively in the phenol layer (Fig. 1, lane 5). Chromatography of the aqueous phase on Bio-Gel P-6 resolved glycopeptides A and B (Fig. 2*A*). The peaks were collected as shown and glycopeptide A was further rechromatographed on Bio-Gel P-6 (Fig. 2*B*). Examination of glycopeptides A and B by slab gel electrophoresis (Fig. 3) showed that glycopeptide A migrated as a single species slightly slower than the marker dye as detected by measurement of radioactivity in gel slices. Glycopeptide B consisted of a major radioactive band and two minor ones as noted earlier. The CNBr fragments did not inhibit anti-M or anti-N antisera as determined by hemagglutination inhibition assays.

Compositional Analysis of Glycopeptide A. The amino acid and carbohydrate compositions of glycopeptide A from MM, NN, and MN glycoproteins are shown in Table 1. Glycopeptide A contained one homoserine residue‡ which derived from one of two methionine residues in the protein and represented nearly 70% recovery of this fragment. Each peptide contained a total of eight amino acid residues, of which six were common to the three types and two varied. The variants were leucine, serine, glycine, and glutamic acid. Thus, in addition to the six common residues, MM peptide contained serine and glycine, NN peptide contained leucine and glutamic acid, and MN contained one half residues of all four.

The glycopeptides contained about three residues each of galactose and GalNAc and nearly five residues of *N*-acetylneuraminic acid.

Amino Terminus Analyses. Dinitrophenylation of the glycopeptides resulted in conversion of 0.78 residue of serine in MM peptide, 0.75 residue of leucine in NN, and 0.55 residue of serine plus 0.41 residue of leucine in the MN glycopeptide to the dinitrophenyl derivatives as quantitated by subtractive amino acid analyses (Table 2) and visualized by thin-layer chromatography (not shown). The data demonstrated a leucine/serine substitution at the amino-terminal position and suggested a glutamic acid/glycine switch at another locus on the peptide. Furthermore, glycopeptide A(MN) apparently consisted of an equimolar mixture of MM and NN peptides. Treatment of the MM and NN glycopeptides with periodate resulted in the loss of one serine residue in the MM peptide but none from the NN peptide, indicating that the amino-terminal serine residue in the former did not carry a carbohydrate substituent on its β-hydroxyl function.

Carbohydrate–Protein Linkage. Reaction of the glycopeptides with alkaline sulfite effected a quantitative β-elimination of the carbohydrate from the peptide and the formation of sulfite addition products from the amino acids and hexos-

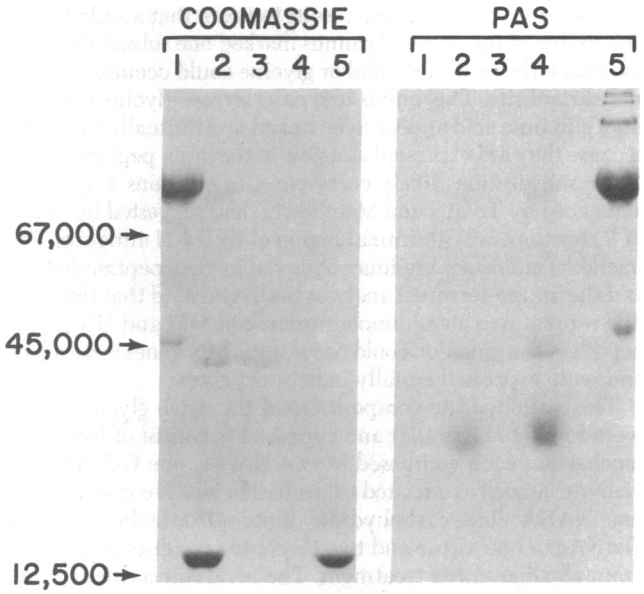

FIG. 1. Sodium dodecyl sulfate/acrylamide gel electrophoresis of MN glycoprotein and its CNBr products on 10% slab gels. (*Left*) Stained with Coomassie brilliant blue. Lanes: 1, MN glycoprotein; 2, unfractionated CNBr products; 3, CNBr glycopeptide B from phenol/urea aqueous phase after Bio-Gel P-6; 4, glycopeptide A after P-6; 5, phenol/urea lower phase after dialysis (peptide C). (*Right*) Stained with periodic acid-Schiff. Lanes: 1, peptide C; 2, glycopeptide B; 3, glycopeptide A; 4, unfractionated CNBr products; and 5, MN glycoprotein. Molecular weight markers: albumin, 67,000; ovalbumin, 45,000; and cytochrome *c*, 12,500.

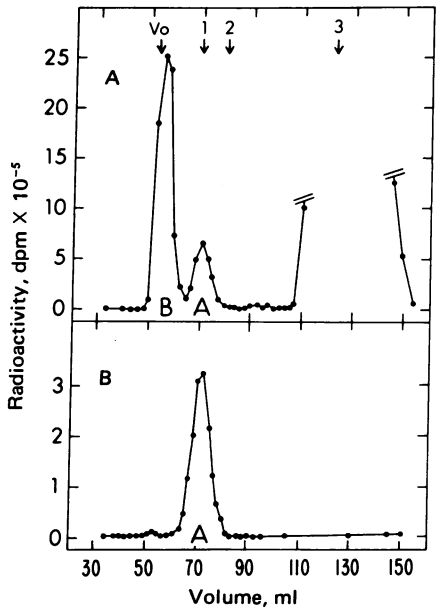

FIG. 2. Gel filtration of a mixture of ^3H-labeled and unlabeled glycopeptides obtained after phenol/urea extraction of CNBr-treated MN glycoprotein. (*A*) Aqueous layer was placed on a Bio-Gel P-6 column (1.4 × 84 cm) and eluted with 0.1 M pyridine acetate (pH 5.0). Peak A, glycopeptide A; peak B, glycopeptide B; peak at 110 ml, reagents from IO_4^-/B^3H_4 treatment. Arrows indicate position of elution of standard glycopeptides: 1, thyroglobulin unit B (M_r 3250); 2, unit A (M_r 2100); 3, Man. (*B*) Rechromatography of glycopeptide A using conditions as in *A*.

‡ Glycopeptide B also contained a homoserine residue which together with that found in A accounted for nearly quantitative recovery of the two methionines present in the unmodified glycoproteins. This fragment was obtained in about 80% yield and was found to aggregate into several polymeric species even in the presence of sodium dodecyl sulfate as shown in Fig. 1. Glycopeptides A and B contained all the carbohydrate of the intact glycoproteins. Peptide C was obtained in about 80% yields; it contained neither homoserine nor carbohydrate and was considered to represent the carboxyl-terminal region of the protein (1).

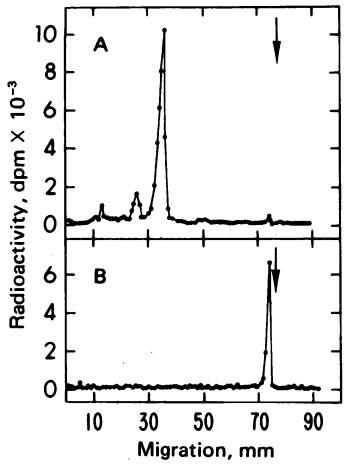

FIG. 3. Sodium dodecyl sulfate/acrylamide gel electrophoresis of CNBr glycopeptides from MN glycoprotein. Labeled glycopeptides A and B obtained after Bio-Gel P-6 were electrophoresed on 15% slab gels, sliced, and assayed for radioactivity. (*A*) Glycopeptide B; (*B*) glycopeptide A. Arrows indicate position of marker dye.

amine involved in the glycopeptide bond. It was observed (Table 3) that two threonines and one serine were lost from each glycopeptide and were converted nearly quantitatively (77%) to α-amino-β-sulfonylbutyric acid and to cysteic acid. All three GalN residues were converted in 90–96% yields to the sulfonyl derivative, thus indicating that the amino sugars were linked glycosidically to serine and threonine and that they were also substituted at the 3-*O* positions, presumably by Gal residues (3, 4). The carbohydrate composition and the β-elimination data suggested the presence on each peptide of two tetrasaccharides, each containing two NANA, one GalNAc, and one Gal, as well as a trisaccharide unit composed of one NANA, one Gal, and one GalNAc.

DISCUSSION

Digestion of the M-N sialoglycoproteins from single donors with CNBr resulted in quantitative scission of the peptide portion at the two methionine residues and permitted the formation of three distinct peptide fragments, A, B, and C, which could be resolved by acrylamide gel electrophoresis and appeared to be common to the MM, NN, and MN antigens. The conditions of digestion permitted quantitative conversion of methionine to homoserine yet liberated fewer than 5% of NANA residues from the glycoproteins. Quantitative separation of the fragments was achieved by using a sequence of phenol/aqueous urea partitioning and gel column chromatography. The phenol/urea treatment was necessary to remove the strongly hydrophobic peptide C and prevent its interaction with glycopeptide B. This simple purification scheme permitted comparative studies on analogous fragments of glycoproteins obtained from single donors. This report is focused on the chemistry of the smallest of the three fragments, CNBr fragment glycopeptide A.

Table 1. Composition of glycopeptide A obtained from CNBr-treated MM, NN, and MN glycoproteins*

Component	Glycopeptide μmol/μmol MM	NN	MN
Hse†	1.00	1.00	1.00
Thr	1.88	1.98	1.84
Ser	1.71	0.95	1.46
Glu	0.08	1.20	0.55
Gly	1.03	0.21	0.61
Ala	1.03	1.22	1.12
Val	0.96	1.02	1.02
Leu	0.02	0.97	0.55
NANA	4.61	5.14	4.84
GalNAc	2.98	3.28	3.13
Gal	2.86	3.41	3.09

* Although three preparations of each type were assayed in triplicate, data are presented for preparations from single donors.
† Homoserine was quantitated after treatment with base.

Table 2. Effect of dinitrophenylation on amino acid composition of glycopeptide A obtained from CNBr-treated MM, NN, and MN glycoproteins

Amino acid	Glycopeptide μmol/μmol MM Control	MM Dnp	NN Control	NN Dnp	MN Control	MN Dnp
Val	1.00	1.00	1.00	1.00	1.00	1.00
Hse	1.05	0.99	0.98	0.98	0.94	0.94
Ser	1.73	0.95	0.93	1.06	1.38	0.83
Leu	0.06	0.12	0.95	0.20	0.52	0.11

Dnp, dinitrophenylated amino acid.

Glycopeptide A from MM, NN, or MN glycoprotein was an octapeptide that contained six homologous amino acid residues (one serine, two threonines, one alanine, one valine, and one homoserine) but varied in the remaining two. Thus, glycopeptide A(MM) contained serine and glycine in addition to the six common residues, and glycopeptide A(NN) contained leucine and glutamic acid. Interestingly, glycopeptide A(MN) was found to contain four fractional yet equimolar residues of each of the two variant pairs in addition to the six homologous amino acids.

Dinitrophenylation studies identified serine as the amino terminus in glycopeptide A(MM), leucine in glycopeptide A(NN), and only one half residue each of leucine and serine in glycopeptide A(MN). These results indicate that a serine/leucine switch at the amino terminus marked one substitution site and that either glutamic acid or glycine could occupy the second variant site. The amino acid pairs serine/glycine and leucine/glutamic acid appear to be linked and mutually exclusive because they are expressed as a pair in the same peptide chain. These substitutions likely correspond to positions 1 and 5 as sequenced by Tomita and Marchesi (1) and suggested by others (6, 7) for the amino-terminal region of the M-N antigens. The fractional amino acid residues observed in glycopeptide A(MN) and the amino-terminal analyses both indicated that this peptide represented an equimolar mixture of MM and NN glycopeptides. This situation could occur if the MN genes were allelic and were expressed equally in heterozygotes.

The carbohydrate compositions of the three glycopeptides were found to be similar and appeared to consist of two tetrasaccharides each composed of two NANA, one Gal, and one GalNAc, as well as a related trisaccharide which contained only one NANA. The carbohydrate units are attached through GalNAc to one serine and two threonine residues as deduced from alkaline sulfite treatment. The invariant serine is glycosylated in the three peptides whereas the amino-terminal serines of MM and MN glycopeptides are unsubstituted because they were resistant to β-elimination and were oxidized with periodate.

It has been shown previously (3, 4, 27) that the M-N antigens contain tetrasaccharide units and a lesser number of trisaccharide chains linked to serine and threonine. The carbohydrate composition and the alkaline sulfite results indicated that at least one threonine in each glycopeptide carries a tetrasaccharide

Table 3. Effect of alkaline sulfite (AS) treatment* on glycopeptide A obtained from CNBr-treated MM, NN, and MN glycoproteins

	Glycopeptide μmol/μmol					
	MM		NN		MN	
Component	Control	AS	Control	AS	Control	AS
Val	1.00	1.00	1.00	1.00	1.00	1.00
Thr	1.96	0.16	1.99	0.21	1.97	NC†
Ser	1.78	0.81	1.16	0.14	1.48	0.49
GalNAc	3.11	NC†	3.22	NC†	3.07	NC†
Cysteic + α-NH_2-β-SO_3-butyric acid	—	2.10	—	1.94	—	2.00
SO_3-GalNAc	—	3.00	—	2.89	—	2.69

* Duration of alkaline sulfite (AS) treatment was 72 hr.
† NC = not calculable; peak size was below limits of quantitation.

unit; but the position of linkage of the trisaccharide is not known. Interestingly, Spiro and Bhoyroo (23) have described similar tri- and tetrasaccharide units in fetuin and found the larger unit attached only to serine but the trisaccharide type attached to both.

Unequivocal differences in the peptide portion of the M and N antigens render unlikely a product/precursor relationship (28) between the two, because such a situation could occur only as a post-translational event. Clearly, the antigens are expressions of an allelomorphic gene, two forms of which are transcribed equally in the heterozygous (MN) form. A recent report (29) also described heterogeneity at positions 1 and 5 of the M-N glycoproteins and suggested that, in addition to M antigen, *M* gene produced some N antigen whereas *N* gene produced only N glycoprotein. We have observed significant fractional residues only in MN peptides and propose therefore that expression of the homozygous genes is faithful.

After this manuscript was submitted, a review by Furthmayer (30) appeared which presented the sequence of 31 amino acid residues from the amino-terminal region of glycophorin A from individual donors, and type-specific serine/leucine and glutamic/glycine substitutions at positions 1 and 5 were observed. The report also presented the amino-terminal sequence of the minor glycoprotein, glycophorin B, which showed a NN-like sequence for at least the first 10 residues and suggested that this protein was present on all M-N erythrocytes. Glycophorin B would be expected to give a CNBr fragement identical to glycopeptide A(NN) and, if present in our glycoprotein preparations, it should be detected in glycopeptide A(MM), after CNBr digestion, as fractional residues of leucine and glutamic acid. Table 1 shows that glycophorin B could account for no more than 2% of our MM glycoprotein, indicating that our fractionation scheme may select for glycophorin A-like glycoproteins.

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