Analysis of the N-linked oligosaccharides of human C1s using electrospray ionisation mass spectrometry

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Abstract Information on the structures of the oligosaccharides linked to Asn residues 159 and 391 of the human complement protease C1s was obtained using mass spectrometric and monosaccharide analyses. Asn[159] is linked to a complex-type biantennary, bisialylated oligosaccharide NeuAc2 Gal2 GlcNAc4 Man3 (molecular mass = 2206 ± 1). Asn[391] is occupied by either a biantennary, bisialylated oligosaccharide, or a triantennary, trisialylated species NeuAc3 Gal3 GlcNAc5 Man3 (molecular mass = 2861 ± 1), or a fucosylated triantennary, trisialylated species NeuAc3 Gal3 GlcNAc5 Man3 Fuc1 (molecular mass = 3007 ± 1), in relative proportions of approximately 1:1:1. The carbohydrate heterogeneity at Asn[391] gives rise to three major types of C1s molecules of molecular masses 79,318 ± 8 (A), 79,971 ± 8 (B), and 80,131 ± 8 (C), with an average mass of 79,807 ± 8. A minor modification, yielding an extra mass of 132 ± 2, is also detected within positions 1–153.

Key words: Complement; C1; N-Linked oligosaccharide; Electrospray ionisation mass spectrometry

1. Introduction

The first component of human complement, C1, is a complex enzyme comprising two serine proteases, C1r and C1s, that are sequentially activated upon binding of C1 to various activators (for reviews, see [1–3]). Proenzyme C1s, a single-chain protein, is converted through cleavage of a single Arg–Ile bond into C1s, the active protease responsible for the enzymic activity of C1, which contains two disulfide-linked chains A and B. The C-terminal B chain is a trypsin-like serine protease domain, whereas the N-terminal A chain is a mosaic-like polypeptide composed of five structural modules, including two pairs of internal repeats (I/III and IV/V) and one epidermal growth factor-like motif II [4,5]. The latter contains, at position 134, an Asn residue that undergoes partial (approx. 50%) β-hydroxylation [6]. The native C1s molecule can be split into two regions, the α region (comprising motifs I, II, and part of motif III) and the γ-B region (comprising motifs IV, V, and the catalytic B chain) [6,7]. The amino acid sequence of human C1s has been determined [4,5], indicating that the protein (673 amino acid residues) contains two N-glycosylation consensus sequences (-Asn159-Cys-Ser- and -Asn391-Gly-Ser-). Further studies [8] have shown that both positions are occupied by an oligosaccharide chain, but that only the carbohydrate linked to Asn[159] is susceptible to cleavage by PNGase F. Since early carbohydrate analyses performed on intact C1s [9], no precise information on the nature of the two N-linked oligosaccharides has been obtained.

Electrospray ionisation MS has proved to be a valuable technique for precise mass determination of biopolymers [10,11]. The present work is based on the combined use of this technique and of monosaccharide analyses performed on C1s or various of its fragments and peptides containing one of the two oligosaccharides. Our data provide precise information on the composition of the oligosaccharides linked to Asn[159] and Asn[391], indicating both to be of the complex glycotype, with the latter showing three major glycoforms. They also provide indirect evidence for a minor modification occurring within the N-terminal region of C1s.

2. Materials and methods

2.1. Materials

Human plasmin was obtained from Kabi Vitrum, Stockholm, Sweden. Trypsin (treated with 1-chloro-4-phenyl-3-(L-tosylamido)-butan-2-one) was from Sigma. PNGase F was purified from cultures of *Flavobacterium meningosepticum* according to the method of Tarentino et al. [12], modified as described in [8]. DFP was obtained from Sigma. ConA-Sepharose was purchased from Pharmacia, Uppsala, Sweden. α-Methyl-D-mannopyranoside was from Janssen Chimica (Noisy le Grand, France).

2.2. Proteins, fragments, and peptides

C1s was isolated from pooled human plasma as described previously [13], and its concentration was estimated from absorbance at 280 nm by using E(1%,1 cm) = 14.5 [7] and an M_r value of 79,800, derived from the present study. Recombinant human C1s was obtained from a baculovirus/insect cells expression system, and activated by incubation with human C1r as described previously [14]. The γ-B fragment from both human plasma C1s and recombinant C1s was obtained by limited proteolysis with plasmin, and the reaction was terminated in each case by blocking the active site serine residues of both γ-B and plasmin with DFP, as described in [6]. Both γ-B fragments were purified as described in [7]. The C1sα fragment was obtained by limited proteolysis with trypsin, and purified as described in [7]. Deglycosylation of C1sα with PNGase F was carried out as described previously [7]. Peptide C1sA 154–195 was obtained from CNBr cleavage of fragment C1s α2 [6].

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Abbreviations: The nomenclature of complement components is that recommended by the World Health Organization; activated components are indicated by an overhead bar, e.g. C1s. ConA, concanavalin A; DFP, di-isopropyl phosphorofluoridate; MS, mass spectrometry; PNGase F, peptide *N*-glycosidase F.

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Peptide C1sA 379–422 was obtained from CNBr cleavage of fragment γ-B and purified as described in [15]. CNBr cleavage was carried out with a 30/1 (w/w) CNBr/protein ratio for 24 h at 4°C, either in 70% formic acid or in 70% trifluoroacetic acid.

2.3. Affinity chromatography on ConA-Sepharose

Fragment $C\bar{1}s$ γ-B (0.5–1.5 mg), in 50 mM triethanolamine-hydrochloride/145 mM NaCl, pH 7.4, was applied to a ConA-Sepharose column (1 × 5 cm) equilibrated with the same buffer. The bound protein fraction was eluted by a 5% (w/v) solution of α-methyl-D-mannopyranoside. Fractions were monitored by absorbance at 280 nm.

2.4. Monosaccharide analysis

Samples were dialyzed exhaustively against 0.5% (v/v) acetic acid and dried down under vacuum. After methanolysis [16] and trimethylsilylation, duplicate analyses of the methylglycosides trimethylsilyl derivatives were performed by gas–liquid chromatography on a CP Sil 5 CB capillary column (0.25 mm × 25 m) [17].

2.5. N-Terminal sequence analysis

N-Terminal sequence analysis of fragment C1sα was performed using an Applied Biosystems Model 470 A gas-phase protein sequencer, as described previously [7].

2.6. Electrospray ionisation MS

Mass spectra were obtained on an API III triple-quadrupole mass spectrometer (PE/Sciex; Thornhill, Ont., Canada) equipped with a nebulizer-assisted electrospray source (ionspray). A 5 kV voltage was applied to the electrospray needle. The mass spectrometer was scanned from m/z 1000 to 2000, with steps of 1 m/z unit for proteins, and from m/z 700 to 1800, with steps of 0.5 m/z unit for peptides. The dwell time was 2 ms and the resolution was one mass unit. For each mass spectrum, 5–10 scans were accumulated in the multiple channel acquisition mode. Each molecular species produced a series of multiply charged protonated ions. The reconstructed molecular mass profiles were obtained using a deconvolution algorithm (PE/Sciex). Horse heart myoglobin (16951.5 Da) and insulin (5733.6 Da) were used for calibration. Molecular masses are given as isotope-average values. For flow injection analyses, samples (80–200 pmol) were dissolved in methanol/water/acetic acid (25:74:1). The liquid chromatography-MS system comprised a syringe pump Phoenix 20 CU (Carlo Erba Fison Instruments) fitted with a homemade capillary column (Nucleosil C8, 0.25 × 150 mm fused silica). The flow rate was 5 μl/min, with a linear gradient of 5–90% acetonitrile in 0.1% trifluoroacetic acid in 60 min. Proteins and fragments, dissolved in 6 M urea or triethanolamine-hydrochloride buffers were infused into the column prior to gradient elution, and the solvent front containing buffers and salts was bypassed before the column effluent was directed to the mass spectrometer.

3. Results

3.1. MS analysis of intact $C\bar{1}s$

The mass spectrum of intact $C\bar{1}s$ (Fig. 1) showed a coherent series of multiply protonated ions extending from 45 to 60 H^+. Using this series of ions, a complex molecular mass pattern was derived, showing three major peaks A, B, and C of molecular masses 79,318 ± 8, 79,971 ± 8, and 80,131 ± 8, and two minor peaks a and c of masses 79,475 ± 8 and 80,272 ± 8, respectively. Given that about half of the $C\bar{1}s$ molecules undergo hydroxylation at Asn^{134} [6], the molecular mass of the polypeptide moiety of $C\bar{1}s$, calculated from the amino acid sequence, was 74,888. From the values determined in Fig. 1, the deduced mass of the carbohydrate moiety of $C\bar{1}s$ ranged from 4430 ± 8 (species A) to 5243 ± 8 (species C). Given the presence of two N-linked carbohydrates in $C\bar{1}s$, this suggested that species A corresponded to molecules with a complex-type biantennary,

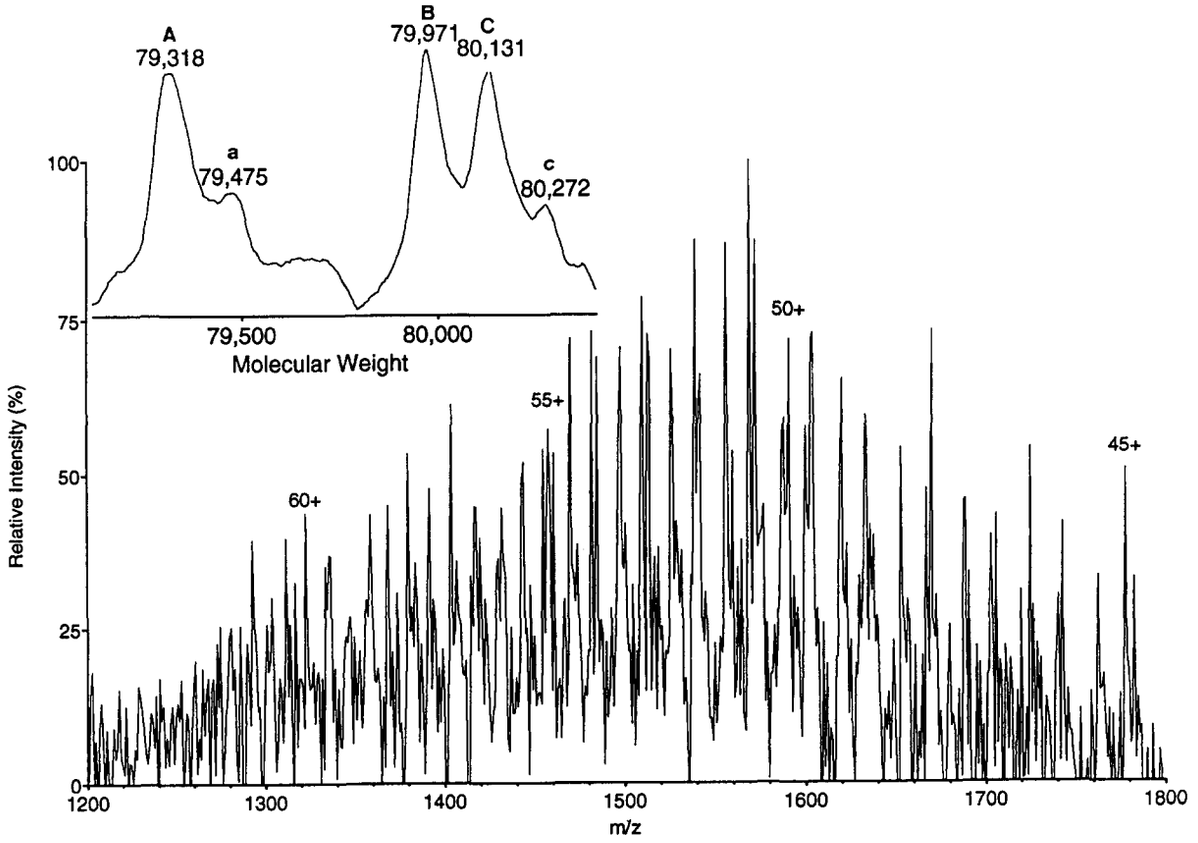

Fig. 1. Electrospray ionisation mass spectrum of intact $C\bar{1}s$. The inset represents the reconstructed molecular mass profile derived from the multiply charged ions series. The spectrum was obtained as described in section 2.

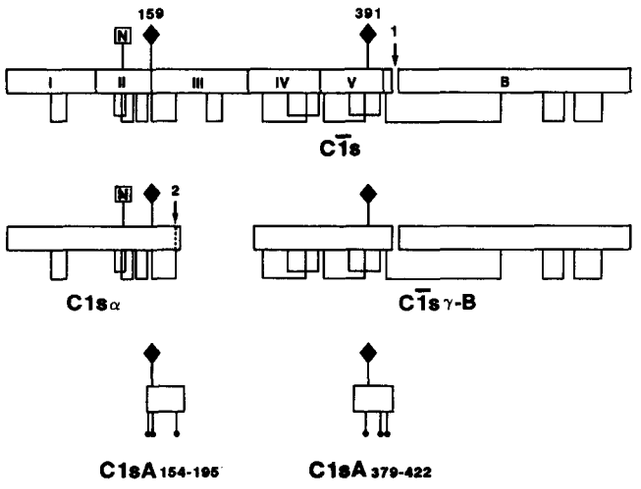

Fig. 2. Schematic representation of the structure of C1s and of the various oligosaccharide-containing fragments and peptides used in this study. I, II, III, IV, and V indicate the modules of the N-terminal A chain, and N the asparagine residue at position 134 that undergoes partial hydroxylation. The disulfide bridges present in C1s and its α and γ-B fragments are represented. Arrow 1 shows the Arg–Ile bond split upon C1s activation, and arrow 2 shows the Arg–Cys bond that is partially cleaved in C1sα. (◆) Asn-linked oligosaccharides. (●) Reduced and alkylated cysteine residues.

bisialylated oligosaccharide (expected mas = 2206) at each site, whereas the occurrence of the higher molecular weight species B and C suggested heterogeneity at one or both site(s). Further studies were performed on fragments and peptides containing one of the two carbohydrate attachment sites. These fragments and peptides are represented on Fig. 2.

3.2. MS analysis of fragments containing the oligosaccharide linked to Asn^{391}

Fragment C1s γ-B, arising from cleavage of the Lys^{269}–Leu^{270} bond in the A chain [6], contains the oligosaccharide linked to Asn^{391} (Fig. 2). The molecular mass of its polypeptide moiety was calculated to be 44,825, including an extra mass of 164 accounting for the di-isopropyl phosphate group linked to the active site serine (see section 2). The mass spectrum of fragment C1s γ-B (Fig. 3) showed three major peaks of molecular masses 47,037 ± 5, 47,690 ± 5, and 47,834 ± 5, present in equivalent amounts. The corresponding deduced masses of the carbohydrate moieties of C1s γ-B were 2212 ± 5, 2865 ± 5, and 3009 ± 5, suggesting the occurrence of a complex-type biantennary, bisialylated oligosaccharide (mass = 2206), a triantennary, trisialylated oligosaccharide (mass = 2863), and a fucosylated triantennary, trisialylated oligosaccharide (mass = 3009).

In order to discriminate biantennary and triantennary oligosaccharide species, fragment C1s γ-B was loaded on a ConA-Sepharose column. The major part of the protein (γ-BI) was not retained on the column, whereas the rest of the material (γ-BII) was bound, and then subsequently eluted by α-methyl-D-mannopyranoside. The mass spectrum of γ-BI showed two major peaks with molecular masses of 47,687 ± 5 and 47,838 ± 5, corresponding to species B and C of Fig. 3, whereas γ-BII yielded a single major peak with a mass of 47,037 ± 5, corresponding to species A of Fig. 3. These results were therefore consistent with the occurrence at position 391 of three types of complex oligosaccharides, namely a biantennary, bisialylated

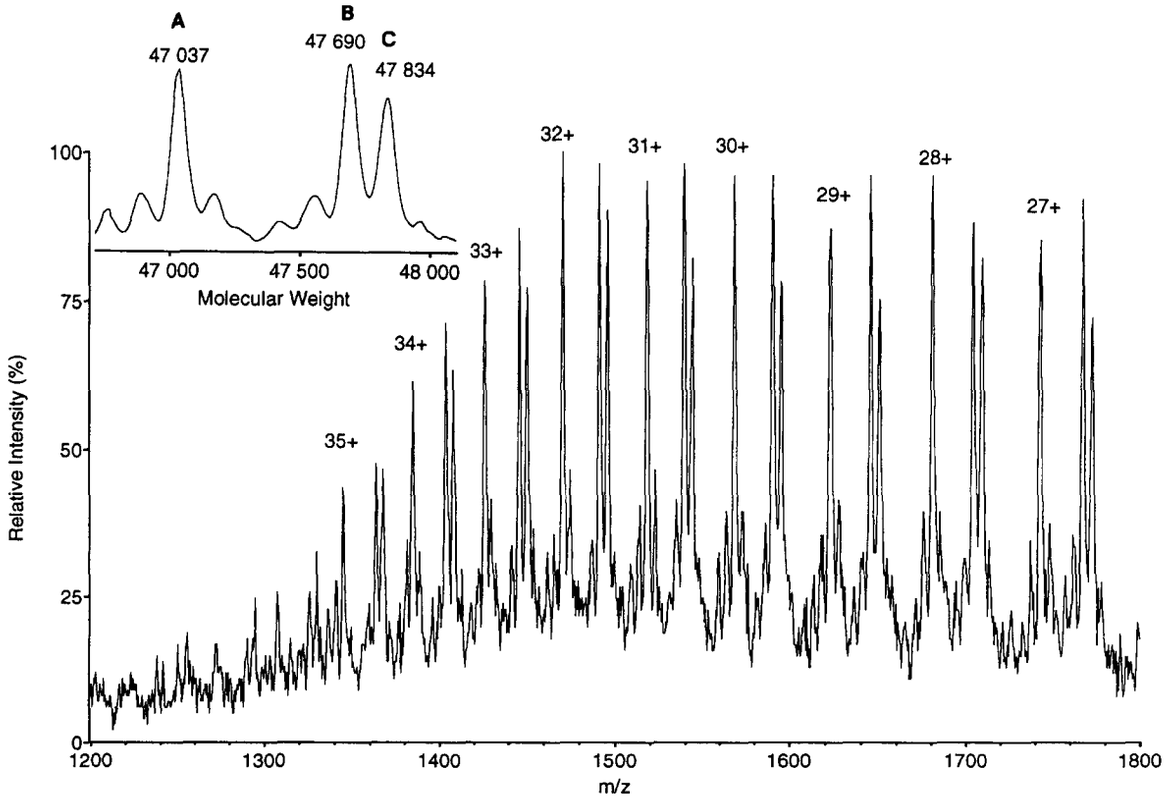

Fig. 3. Electrospray ionisation mass spectrum of fragment C1s γ-B.

species (about 1/3), and a triantennary, trisialylated species, either containing or lacking one fucose residue (about 1/3 each). This conclusion was further supported by monosaccharide analyses (Table 1), indicating ratios of galactose and *N*-acetylglucosamine relative to mannose in γ-BI and γ-BII consistent with the presence of triantennary and biantennary species, respectively, and the presence of fucose in γ-BI.

Further analyses were performed on peptide C1sA 379–422 (Fig. 2), obtained from CNBr cleavage of fragment γ-B after reduction and alkylation with 4-vinyl pyridine. The calculated mass of its polypeptide moiety is 5041.7. The mass spectrum of this peptide revealed several peaks, including species of molecular masses 7247 ± 1, 7902 ± 1, and 8048 ± 1. The deduced masses for the oligosaccharide moieties of these species are 2206 ± 1, 2861 ± 1, and 3007 ± 1, corresponding respectively to the biantennary, triantennary, and fucosylated triantennary species already identified (calculated masses = 2206, 2863, and 3009, respectively). A species of mass 7612 most likely arose from the loss, during CNBr cleavage, of one *N*-acetylneuraminic acid unit from species 7902 (expected difference = 291, observed = 290). Another species of mass 7572 could not be identified.

Other analyses were performed on fragment γ and the B chain, both reduced and alkylated with iodoacetamide. Fragment γ yielded three peaks corresponding to the major oligosaccharides described above. In contrast, the B chain yielded a single peak of molecular mass 28,112 ± 3, consistent with the mass deduced from the amino acid sequence (28,100.9, i.e. 27,936 plus 164 accounting for the di-isopropyl phosphate group linked to the active-site serine), providing evidence that the B chain undergoes no post-translational modification.

3.3. MS analysis of the γ-B fragment obtained from recombinant $C\bar{1}s$

Analysis by MS of the γ-B fragment obtained from recombinant $C\bar{1}s$ expressed in the baculovirus/insect cells system showed a single major peak with a mass of 45,879 ± 5, corresponding to a deduced mass for the carbohydrate moiety of 1054 ± 5, consistent with a short oligosaccharide structure comprising 2 *N*-acetylglucosamine and 4 mannose residues (calculated mass = 1055).

Table 1
Analysis of the monosaccharides (residues/mol) of the C1s fragments α and γ-B

Fragment	Fucose	Galactose	Mannose	*N*-Acetylglucosamine	*N*-acetyl neuraminic acid
γ-BI	0.3 ± 0.1	2.8 ± 0.1	3.0	4.6 ± 0.1	1.2 ± 0.2
γ-BII	N.D.	2.0 ± 0.1	3.0	3.6 ± 0.1	1.1 ± 0.1
α	N.D.	2.0 ± 0.1	3.0	3.5 ± 0.3	1.4 ± 0.1

Analyses were performed in duplicate on 80–130 μg of each sample, as described in section 2. Values are calculated on the basis of 3.0 mannose residues/mol, and given as mean values ± S.D. The low relative contents in *N*-acetylneuraminic acid (expected values for γ-BI, γ-BII, and α = 3.0, 2.0, and 2.0, respectively) are most likely due to partial loss during dialysis and/or drying of the samples, which were performed under acidic conditions (see section 2) known to favour removal of sialic acids. The expected relative fucose content of γ-BI is 0.5. N.D., not detected.

3.4. MS analysis of fragments containing the oligosaccharide linked to Asn^{159}

Fragment α of C1s extends from positions 1–192 of the A chain (Fig. 2). In addition to the oligosaccharide linked to Asn^{159}, approximately half of the C1sα molecules were expected to contain β-hydroxyasparagine at position 134 [6]. Also, it is known from previous studies [7] that partial cleavage of the Arg^{186}–Cys^{187} bond occurs during limited proteolysis of $C\bar{1}s$ with trypsin. N-Terminal sequence analysis of the C1sα fragment used in this study indicated that approx. 56% of the molecules were cleaved at this position.

As shown in Fig. 4A, the mass spectrum of C1sα showed two groups of peaks, centred on mass values of 24,047 + 3 and 24,181 ± 3. The major group contained three observable peaks with mass values of 24,031 ± 3, 24,047 ± 3, and 24,063 ± 3. In view of the heterogeneities expected at the polypeptide level, it was hypothesized that the species of molecular mass 24,031 corresponded to C1sα molecules lacking both hydroxylation at Asn^{134} and internal cleavage (calculated mass of the polypeptide moiety = 21,824), the species of mass 24,063 corresponded to molecules bearing both modifications (calculated mass = 21,858), and the intermediary species of mass 24,063 represented a mixture of molecules exhibiting either hydroxylation or cleavage only (calculated masses 21,840 and 21,842, respectively). The masses of the carbohydrate moiety deduced for each species are 2207 ± 3, 2205 ± 3, 2207 ± 3, and 2205 ± 3, respectively, consistent with the presence on each of the four species of a complex-type, biantennary, bisialylated oligosaccharide (expected mass = 2206). This hypothesis is in agreement with monosaccharide analyses performed on fragment C1sα, which yielded results comparable with those obtained for fragment C1s γ-BII (Table 1). Although the minor group of peaks observable in Fig. 4A was poorly resolved, the mass of its central component was estimated at 24,181 ± 3, i.e. a difference of 134 ± 3 compared with the intermediate species of the major group (molecular mass = 24,047 ± 3). Considering that both groups of peaks have the same overall shape, the most likely hypothesis is therefore that this double pattern arises from another, minor modification occurring in C1sα.

In agreement with previous data [7,8], treatment of C1sα with PNGase F removed the N-linked oligosaccharide, decreasing the mass of the major peaks observed in Fig. 4A by values ranging from 2204 to 2206 (Fig. 4B). The molecular mass of the lightest species, supposed to contain molecules with no hydroxylation and no internal cleavage, was estimated at 21,825 ± 2, in perfect agreement with the predicted value, considering that, during cleavage of the oligosaccharide by PNGase F, the Asn residue is converted to Asp [8]. The fact that the mass of the central component of the minor group also decreased by a value of 2205 ± 2 (Fig. 4A,B) clearly indicated that the putative minor modification was independent of the N-linked oligosaccharide. Again, after treatment with PNGase F, the difference of mass between this minor species and the intermediate species of the major group was 131 ± 2 (Fig. 4B), in good agreement with the value determined from Fig. 4A.

Further analyses were performed on peptide C1sA 154–195, a reduced and S-carboxymethylated CNBr-cleavage peptide (Fig. 2). The mass spectrum of this peptide showed a major peak with a mass of 7086 ± 1, which, by comparison with the mass calculated for the peptide moiety (4880.4), yields a deduced mass for the oligosaccharide linked to Asn^{159} of 2206 ± 1,

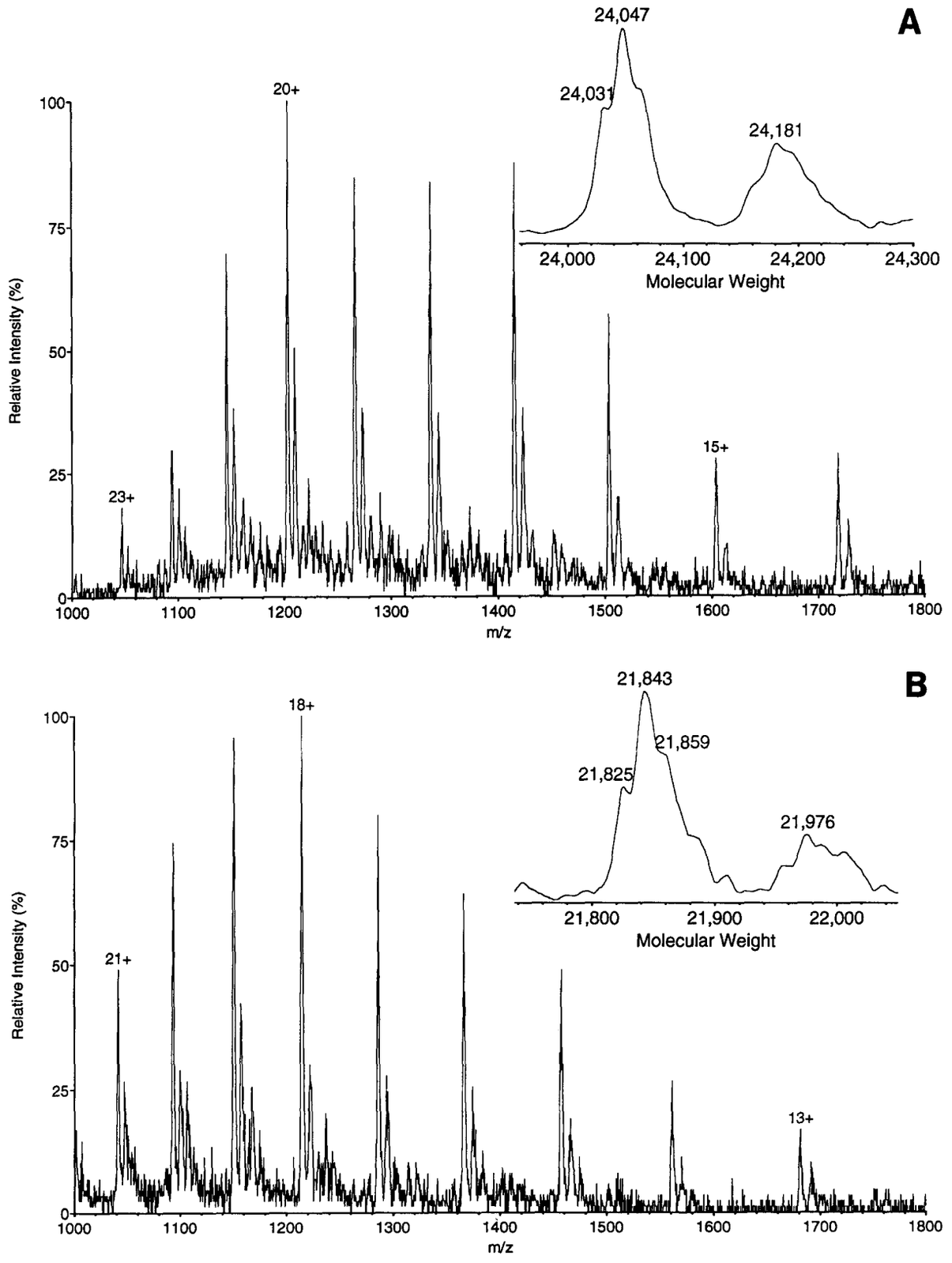

Fig. 4. Electrospray ionisation mass spectra of fragment C1sα. (A) Native fragment C1sα. (B) Fragment C1sα treated with PNGase F.

providing further support to the hypothesis of a biantennary, bisialylated oligosaccharide. Two minor peaks of masses 7102 (+16) and 7118 (+32) were also observed, probably arising from oxidation of amino acid(s) during CNBr cleavage. No evidence for any other modification of the polypeptide backbone was obtained, indicating that the putative minor modification

occurring in the α region must take place within the N-terminal part of this region, more precisely between positions 1 and 153.

4. Discussion

The analyses performed in this study are consistent with the conclusion that: (i) Asn^{159} is linked to a complex-type biantennary, bisialylated oligosaccharide (NeuAc2 Gal2 GlcNAc4 Man3); (ii) Asn^{391} is occupied by either a biantennary, bisialylated species, or triantennary, trisialylated species (NeuAc3 Gal3 GlcNAc5 Man3) containing or lacking a fucose residue. The three species attached to Asn^{391} are present in roughly equimolar amounts. Considering that $C\bar{1}s$ used in this study was purified from human plasma pooled from several donors, it cannot be concluded whether this heterogeneity occurs in each individual or reflects variations from one individual to the other.

This pattern of the N-linked oligosaccharides of $C\bar{1}s$ allows a detailed interpretation of the major peaks observed by MS analysis of intact $C\bar{1}s$ (Fig. 1). Thus, it appears clearly that: (i) species A (molecular mass = 79,318 ± 8) contains a biantennary, bisialylated oligosaccharide at both positions 159 and 391 (theoretical mass = 79,300); (ii) species B (molecular mass 79,971 ± 8) contains a biantennary oligosaccharide at position 159, and a non-fucosylated triantennary oligosaccharide at position 391 (theoretical mass = 79,957); (iii) species C (molecular mass = 80,131 ± 8) contains a biantennary oligosaccharide at position 159, and a fucosylated triantennary species at position 391 (theoretical mass = 80,103). If one only takes into account these species A, B, and C, which represent approx. 75–80% of the $C\bar{1}s$ population, the experimental average mass of $C\bar{1}s$ is 79,807 ± 8 (calculated value = 79,787). On the same basis, the carbohydrate content of $C\bar{1}s$ ranges from 5.56% (species A) to 6.34% (species C), with an average value of 6.13%.

The MS analyses performed on fragment C1sα, together with those performed on peptide C1sA 154–195, provide indirect evidence for the occurrence of a minor post-translational modification taking place within positions 1–153 of $C\bar{1}s$, therefore independent of the oligosaccharide linked to Asn^{159}. The data from Fig. 4A and B, showing minor peaks differing from the major species by an extra mass of 134 ± 2 and 131 ± 2, respectively, appear consistent with the presence, on about 25% of the $C\bar{1}s$ molecules, of a pentose residue (molecular mass = 132) attached to the polypeptide backbone. Considering that $C\bar{1}s$ contains in its N-terminal region (positions 115–160) an epidermal growth factor-like domain, it should be mentioned that several cases of O-glycosylation have been reported on serine residues located in such domains, e.g. in coagulation Factor IX [18–20]. To our knowledge, however, no case of a single O-linked pentose residue has ever been described. The hypothesis that the extra mass arises from heterogeneity at the sequence level appears unlikely, considering that no such heterogeneity has ever been detected by sequence analyses performed at the cDNA [4,5] or protein [21] levels. Further analyses will be required to identify and locate this minor modification. Obviously, the minor peaks a and c seen in Fig. 1 can be interpreted as derivatives of the major species A and C, respectively, containing, in addition to the N-linked oligosaccharides, the extra modification. It can also be postulated that a third minor peak b (expected molecular mass = 80,103), derived from species B, occurs at the level of the major species C.

The MS analysis performed on fragment γ-B obtained from recombinant $C\bar{1}s$ expressed in the baculovirus/insect cells system shows that, in contrast with $C\bar{1}s$ purified from human serum, the oligosaccharide linked to Asn^{391} is homogeneous and has a molecular mass consistent with a short high mannose-type structure containing 4 mannose and 2 *N*-acetylglucosamine residues. This result is in agreement with our previous data [14], and consistent with studies performed on recombinant HIV-1 gp120 derived from the same expression system, showing that all of the N-linked oligosaccharides are of the high mannose glycotype [22]. This confirms the limitations of the baculovirus-based expression system with respect to the synthesis of complex-type oligosaccharides. In the case of C1s, however, it should be mentioned that the substitution of short high-mannose oligosaccharides for complex-type oligosaccharides, or even the lack of glycosylation at Asn^{159}, do not significantly impair the functional properties of the protein [14].

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References

[1] Cooper, N.R. (1985) Adv. Immunol. 37, 151–216.
[2] Schumaker, V.N., Zavodszky, P. and Poon, P.H. (1987) Annu. Rev. Immunol. 5, 21–42.
[3] Arlaud, G.J., Colomb, M.G. and Gagnon, J. (1987) Immunol. Today 8, 106–111.
[4] Mackinnon, C.M., Carter, P.E., Smyth, J., Dunbar, B. and Fothergill, J.E. (1987) Eur. J. Biochem. 169, 547–553.
[5] Tosi, M., Duponchel, C., Meo, T. and Julier, C. (1987) Biochemistry 26, 8516–8524.
[6] Thielens, N.M., van Dorsselaer, A., Gagnon, J. and Arlaud, G.J. (1990) Biochemistry 29, 3570–3578.
[7] Thielens, N.M., Aude, C.A., Lacroix, M.B., Gagnon, J. and Arlaud, G.J. (1990) J. Biol. Chem. 265, 14469–14475.
[8] Aude, C.A., Lacroix, M.B., Arlaud, G.J., Gagnon, J. and Colomb, M.G. (1988) Biochemistry 27, 8641–8648.
[9] Sim, R.B. (1981) Methods Enzymol. 80, 26–42.
[10] Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M. (1989) Science 246, 64–71.
[11] Chait, B.T. and Kent, S.B. (1992) Science 257, 1885–1894.
[12] Tarentino, A.L., Gomez, C.M. and Plummer Jr., T.H. (1985) Biochemistry 24, 4665–4671.
[13] Arlaud, G.J., Sim, R.B., Duplaa, A.-M. and Colomb, M.G. (1979) Mol. Immunol. 16, 445–450.
[14] Luo, C., Thielens, N.M., Gagnon, J., Gal, P., Sarvari, M., Tseng, Y., Tosi, M., Zavodszky, P., Arlaud, G.J. and Schumaker, V.N. (1992) Biochemistry 31, 4254–4262.
[15] Carter, P.E., Dunbar, B. and Fothergill, J.E. (1983) Biochem. J. 215, 565–571.
[16] Zanetta, J.P., Breckenridge, W.C. and Vincendon, G. (1972) J. Chromatogr. 69, 291–304.
[17] Kamerling, J.P., Gerwig, G.T., Vliegenthart, J.F.G. and Clamp, J.R. (1975) Biochem. J. 151, 491–495.
[18] Nishimura, H., Kawabata, S., Kisiel, W., Hase, S., Ikenata, T., Takao, T., Shimonishi, Y. and Iwanaga, S. (1989) J. Biol. Chem. 264, 20320–20325.
[19] Harris, R.J., van Halbeek, H., Glusha, J., Basa, L.J., Ling, V.T., Smith, K.J. and Spellman, M.W. (1993) Biochemistry 32, 6539–6547.
[20] Lis, H. and Sharon, N. (1993) Eur. J. Biochem. 218, 1–27.
[21] Spycher, S.E., Nick, H. and Rickli, E.E. (1986) Eur. J. Biochem. 156, 49–57.
[22] Yeh, J.-C., Seals, J.R., Murphy, C.I., van Halbeek, H. and Cummings, R.D. (1993) Biochemistry 32, 11087–11099.