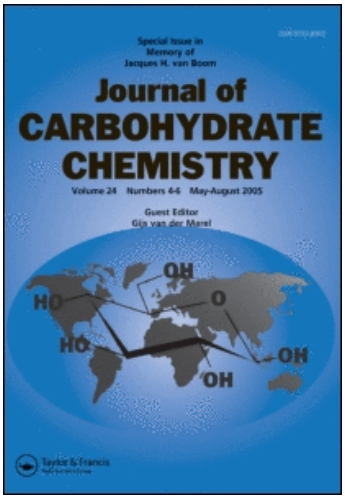

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Structural Elucidation by Fast Atom Bombardment Mass Spectrometry of Multisulfated Oligosaccharides Isolated from Human Respiratory Mucous Glycoproteins

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STRUCTURAL ELUCIDATION BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF MULTISULFATED OLIGOSACCHARIDES ISOLATED FROM HUMAN RESPIRATORY MUCOUS GLYCOPROTEINS

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ABSTRACT

Underivatized mono- and multisulfated oligosaccharides obtained by the alkaline-borohydride treatment of human respiratory mucous glycoproteins were analyzed by positive ion fast atom bombardment mass spectrometry (FAB MS). Employing three unique and structurally homologous groups, the FAB mass spectra of a mono- and a disulfated tri- and tetrasaccharide and a mono-, di- and trisulfated branched hexasaccharide were compared. Each produced mass spectra displaying molecular weight-related and structurally significant fragment ions including fragments differing in mass by multiples of 102 amu reflecting the loss of one or more sulfate esters. From these data, combined with monosaccharide composition, the carbohydrate sequence and number and location of sulfate esters can readily be determined. These findings, with other chemical and enzymatic analyses, make FAB MS a valuable tool applicable to the unambiguous structural elucidation of underivatized reduced, linear and branched, mucous glycoprotein oligosaccharides that vary in degree of sulfation.

INTRODUCTION

Increased carbohydrate sulfation on high molecular weight tracheobronchial mucous glycoproteins in response to chronic lung disease, such as in chronic bronchitis, in chronic lung infection and irritation, and in cystic fibrosis (CF),[1-8] suggests that oxyanionicity may play important roles related to defense, gelation, hydration, mobility, and charge density. Current investigations in our laboratories pertain to defining the structures of oligosaccharides possessing varying degrees of sulfation isolated from these tracheobronchial mucous glycoproteins of patients with CF.[9-12] This structural information is important if we are to understand the physiochemical, biochemical and physiological roles that the sulfate ester may play.

Complete structural characterization of sulfated oligosaccharides from respiratory mucous glycoproteins is an extremely difficult endeavor. This is due to the availability of very low quantities of purified sulfated oligosaccharide of a single structure, and to the lability of the sulfate esters when subjected to unmodified routine carbohydrate sequencing methods. Low yields of purified sample reflect the limited amount of crude sample available and also the considerable oligosaccharide heterogeneity with regards to carbohydrate composition, size, the number of branches it may possess, whether or not other anionic sugars may be present, *i.e.*, sialic acid, and, lastly, the number of sulfate esters present[9-12] attest to this difficulty.

For these reasons, methods of analyses are sought that are capable of utilizing low sample quantities while providing meaningful structural information that will add to, and also confirm, other chemical and enzymatic results. Since the application of readily available fast atom bombardment mass spectrometry (FAB MS) to the structural analysis of sulfated glycosaminoglycans,[13-16] methylated oligosaccharides,[17,18] and methylated glycolipids[19] has proven to be a useful technique in providing valuable information pertaining to primary carbohydrate sequences and sites of sulfation, we now apply positive ion FAB MS to comparatively analyze the underivatized mono- and novel disulfated forms of both a trisaccharide and a tetrasaccharide and also the mono-, and unique di- and trisulfated forms of a branched hexasaccharide which were previously isolated from CF tracheobronchial

mucous glycoproteins and structurally characterized via methylation analysis and sequential exoglycosidase degradation.[10-12] The results of these ongoing studies indicate that positive ion FAB MS will be an effective tool for the structural characterization of underivatized linear and branched mono-, and especially, multisulfated oligosaccharides isolated from glycoproteins via β-elimination by alkaline-borohydride cleavage and reduction.

RESULTS AND DISCUSSION

The positive FAB MS of the mono- and disulfated trisaccharides (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol and (4-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol, abbreviated SVII and SSVII, respectively, which uniquely possess the same trisaccharide structure but differ in their degree of sulfation, are presented in Figure 1. Both mass spectra are dominated by intense structurally significant fragment and molecular ions. Though both SVII and SSVII were analyzed as their sodium salts, it was anticipated that the sulfate esters could potentially exist in either protic or sodium forms and thus produce molecular weight related ions that present themselves as multiplets separated by 22 daltons; as was observed for the FAB MS of chondroitin sulfate oligosaccharides.[13] The relatively large intensities of the molecular weight related ions $(M + Na)^+$ for SVII and SSVII of *m/z* 713 and 815, and the absence of *m/z* 691 in the mass spectra of SVII and the relatively low abundance of the molecular weight related ion at *m/z* 793 for SSVII, indicate that the complete sodium cationic form predominates over all other countercationic possibilities (Table 1). For these reasons, and ease of discussion, the structures presented in Figure 1 and latter Figures are drawn with sodium as the counterion for each sulfate ester. Analogous to glycosaminoglycans,[13,14] though more prominent and intense for sulfated oligosaccharides, a striking and useful feature observable when comparing the FAB mass spectra of the monosulfated SVII and the disulfated SSVII, Figures 1a and 1b, respectively, is the direct correlation of the number of

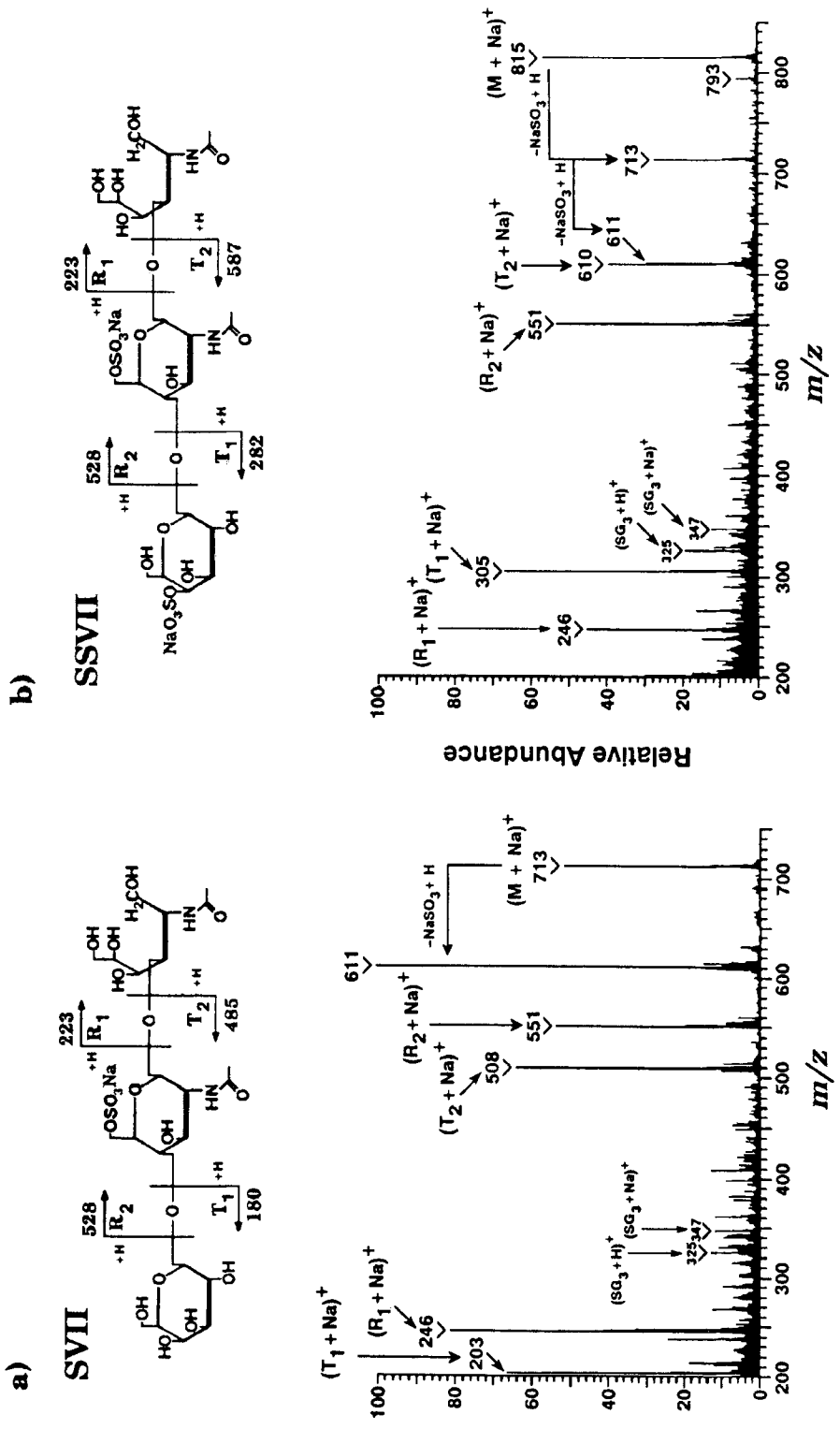

Figure 1. Positive ion FAB mass spectra of a) SVII and b) SSVII in thioglycerol (SG).

TABLE 1. Countercation Composition of Molecular Weight-Related Ions from SVII and SSVII

Countercation composition	*m/z* [a]
Oligosaccharide - SVII	
$(2Na)^{2+}$	713
$(H^{1+},Na^{1+})^{2+}$	691
Oligosaccharide - SSVII	
$(3Na)^{3+}$	815
$(H^{1+},2Na^{2+})^{3+}$	793
$(2H^{2+},Na^{1+})^{3+}$	771

a. Double underlines indicate ions observed in the respective mass spectra.

sulfate esters on the molecule with the number (n) of 102 atomic mass units (amu) losses representing the elimination of sodium sulfite with hydrogen replacement (*i.e.*, $[(M + Na)\ (-NaSO_3\ + H)_n]^+$. For SVII, in Figure 1a, a very intense single loss of 102 amu is observed (*i.e.*, *m/z* 713/611) whereas for SSVII, in Figure 1b, two prominent losses of 102 amu each are clearly noted (*i.e.*, *m/z* 815/713, 713/611) indicating mono- and disulfated structures, respectively.

The intense fragment ions resulting from the simple cleavage on either side of the glycosidic oxygen with hydrogen attachment provide the sequences of SVII and SSVII. Fragment ions are identified as being either T_n to signify nonreducing end fragment ions or as R_n to indicate reducing end fragment ions.[13] In contrast to glycosaminoglycans,[13-16] oligosaccharides arising from mucous glycoproteins may be linear or branched. For smaller oligosaccharides with dp < 8 an important indicator that the oligosaccharide is not branched is the presence of the fragment ion at *m/z* 246 $(R_1 + Na)^+$ (Figures 1a and 1b) indicating that the terminal reducing sugar 2-acetamido-2-deoxy-D-galactitol does not possess more than one glycosidic linkage or substitution. Key fragment ions at *m/z* 203 in Figure 1a and *m/z* 305

in Figure 1b both represent $(T_1 + Na)^+$ nonreducing terminal fragment ions with the latter fragment ion possessing a sulfate ester.

Similar to the trisaccharides SVII and SSVII, above, the novel linear tetrasaccharides (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (SXIV) and (6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (SSXIV) are also unique structural homologs that are mono- and disulfated, respectively. The FAB mass spectra of SXIV and SSXIV, presented in Figure 2a and 2b, are also dominated by the fully natriated molecular weight related ions $(M + Na)^+$ at *m/z* 875 and 977, respectively, and demonstrate significant sequence-related fragment ions that represent all T_n and R_n glycosidic cleavage possibilities for these structures. Only SSXIV demonstrated a molecular weight related ion corresponding to the replacement of a sodium cation with a proton at *m/z* 955 (Table 2). The number of sulfate esters present are readily indicated by the number of sequential 102 amu losses observed from the initial $(M + Na)^+$ fragment ion. For the monosulfated SXIV a singular 102 amu loss is seen, *i.e.*, *m/z* 875/773, whereas the disulfated SSXIV distinctly displayed two 102 amu losses at *m/z* 977/875 and 875/773. A single glycosidic linkage to 2-acetamido-2-deoxy-D-galactitol occupying the reducing end terminus for both SXIV and SSXIV is suggested by the presence of *m/z* 246 $(R_1 + Na)^+$. The nonreducing terminal end fragment ion (*i.e.*, $(T_1 + Na)^+$) for SXIV at *m/z* 203 and for SSXIV at *m/z* 305 shows the latter residue to be sulfated.

It should be noted, although no specific fragment ion loss from *m/z* 305 fragment ion for either SSXIV or SSVII, in Figures 2b and 1b, respectively, was identified that could help identify the position of the sulfate ester as being 6-*O*-sulfate or 4-*O*-sulfate, that the 4-*O*-sulfate bearing fragment ion for SSVII possessed a much higher relative abundance to all other fragment ions within its own FAB mass spectrum. This is in sharp contrast to the relatively low abundance of the 6-*O*-sulfate bearing *m/z* 305 $(T_1 + Na)^+$ fragment ion compared to other fragment ions within the mass spectrum for SSXIV in Figure 2b.

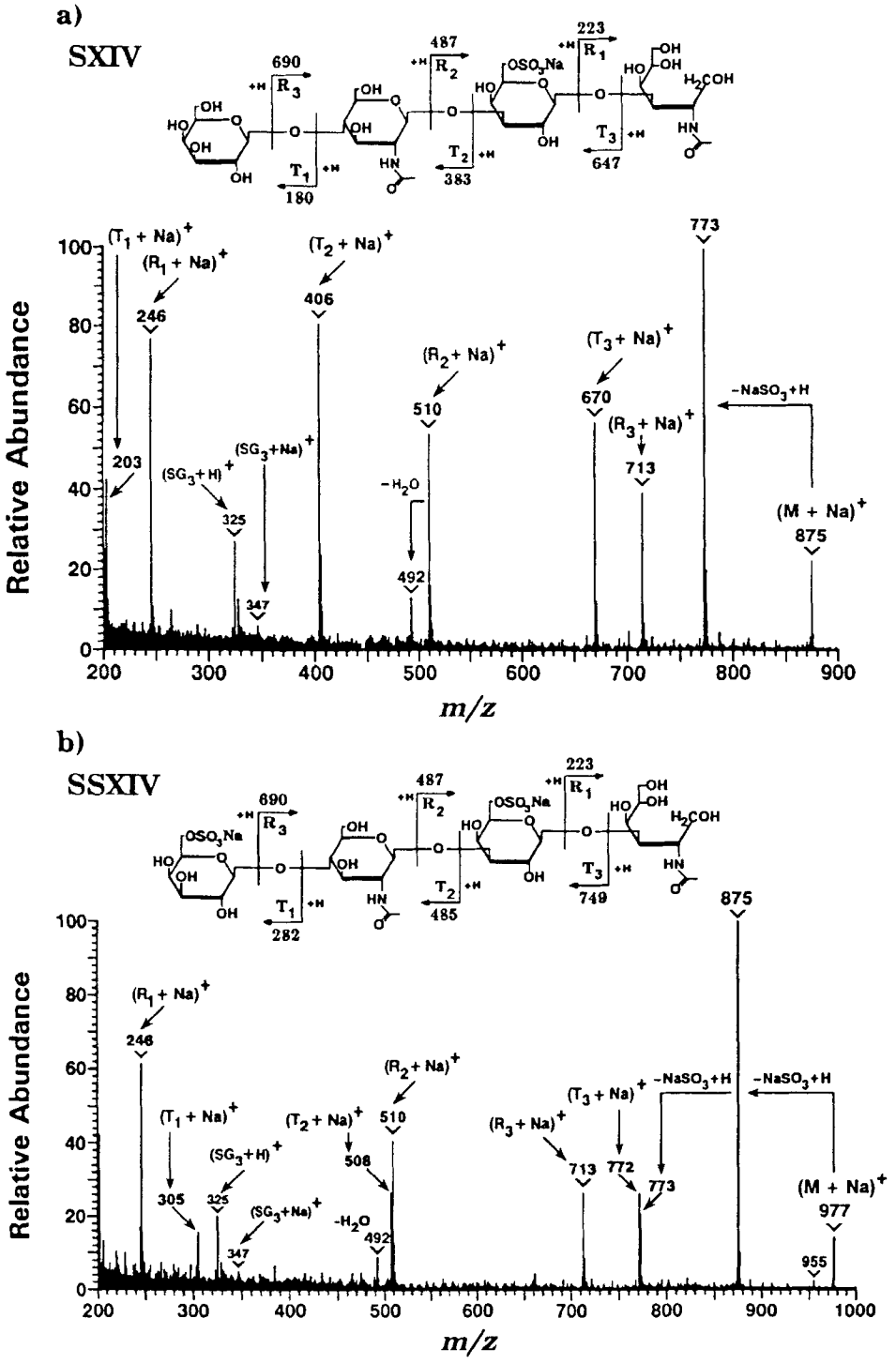

Figure 2. Positive ion FAB mass spectra of a) the monosulfated tetrasaccharide SXIV and of the disulfated tetrasaccharide SSXIV.

TABLE 2. Countercation Composition of Molecular Weight-Related Ions from SXIV and SSXIV

Countercation composition	*m/z* [a]
Oligosaccharide - SXIV	
$(2Na)^{2+}$	<u>875</u>
$(H^{1+}, Na^{1+})^{2+}$	853
Oligosaccharide - SSXIV	
$(3Na)^{3+}$	<u>977</u>
$(H^{1+}, 2Na^{2+})^{3+}$	<u>955</u>
$(2H^{2+}, Na^{1+})^{3+}$	933

a. Double underlines indicate ions observed in the respective mass spectra.

A consistent FAB mass spectral feature for the mono- and disulfated tetrasaccharide homologs SXIV and SSXIV (Figures 2a and b) is the loss of water from the reducing end fragment $(R_2 + Na)^+$ as indicated by the presence of m/z 492. The formation of this $[(R_2 + Na) - H_2O]^+$ fragment ion arising from the dehydration of the galactose-6-sulfate residue which possesses a free hydroxyl at C-4 is similar to, but more prominent, than that observed for internal 2-acetamido-2-deoxy-D-glucosamine-6-sulfate residues in glycosaminoglycans[13] which also possess a free hydroxyl at C-4. No such indication of dehydration was noted for both SVII and SSVII which possess internal 2-acetamido-2-deoxy-D-glucosamine-6-sulfate residues that do not have a free C-4 hydroxyl group.

Figures 3a, 3b and Figure 4 present the positive ion FAB mass spectra of the novel mono-, di- and trisulfated branched hexasaccharide homologs (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-2-acetamido-2-deoxy-D-galactitol (SXXI), (6-*O*-

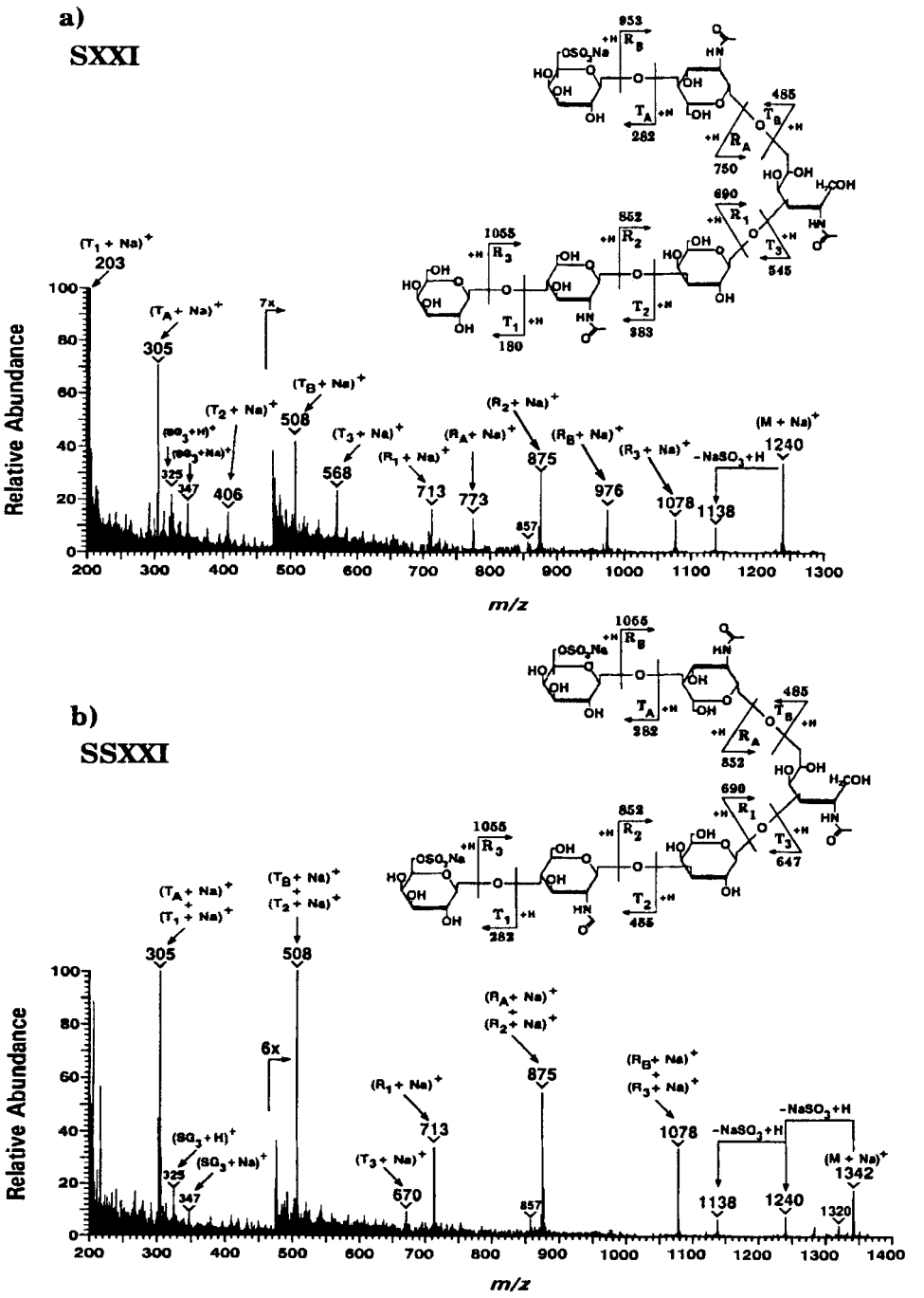

Figure 3. Positive ion FAB spectra of a) the monosulfated hexasaccharide SXXI and of b) the disulfated hexasaccharide SSXXI in thioglycerol (SG).

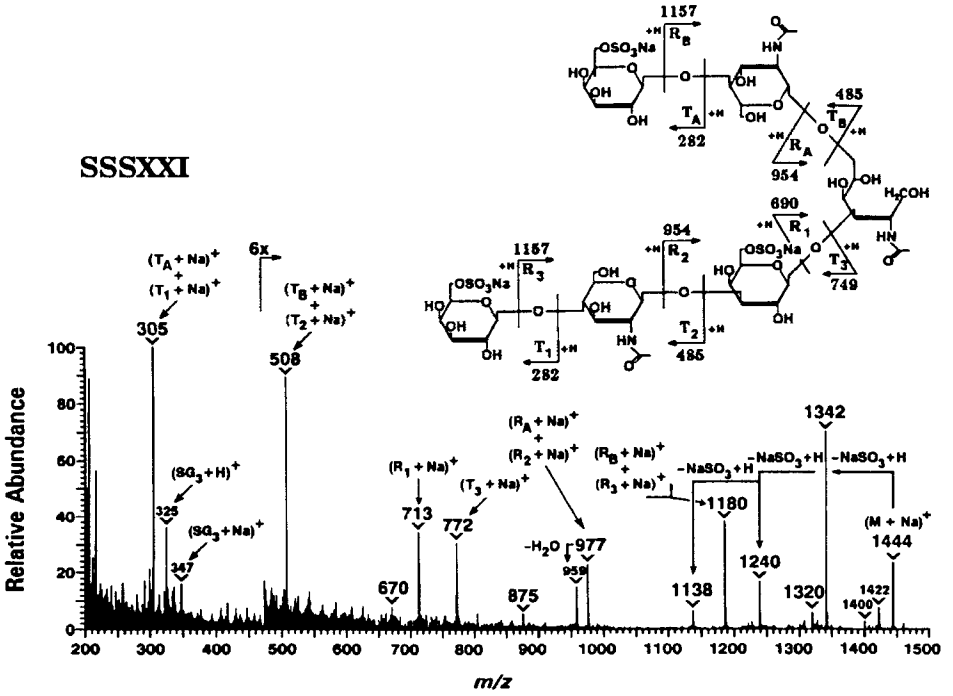

Figure 4. Positive ion FAB spectra of the trisulfated hexasaccharide SSSXXI in thioglycerol (SG).

sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-2-acetamido-2-deoxy-D-galactitol (SSXXI), and (6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-2-acetamido-2-deoxy-D-galactitol (SSSXXI). Because of the existence of two glycosidic linkages to 2-acetamido-2-deoxy-D-galactitol, a sequential alphabetical letter (*l*) subscript (*i.e.*, T_l and R_l) was employed to help facilitate the identification of the fragment ions related to the 1→6 branch. The positive FAB MS of SXXI, SSXXI and SSSXXI, in Figures 3a, 3b and 4, respectively, display prominent fully natriated molecular weight related ions $(M + Na)^+$ at *m/z* 1240, 1342 and 1444, respectively, and

clearly demonstrate significant sequence-related fragment ions that represent all T_n, T_l, R_n and R_l glycosidic cleavage possibilities for these structures. The distinctive absence of *m/z* 246 supports the presence of more than one glycosidic linkage to the terminal reducing-end sugar 2-acetamido-2-deoxy-D-galactitol. The number of sequential losses of 102 amu from the respective molecular ion $(M + Na)^+$ for SXXI, SSXXI and SSSXXI at *m/z* 1240/1138, *m/z* 1342/1240, 1240/1138; and *m/z* 1444/1342, 1342/1240, 1240/1138, respectively, is readily distinquishable and agrees with the indicated degrees of sulfation. Notably, the FAB mass spectrum of the unique trisulfated SSSXXI oligosaccharide (Figure 4) also displayed a readily discernable molecular weight related fragment ion cluster (*i.e.*, at *m/z* 1444, 1422, 1400) reflecting variations in their countercation composition separated by 22 amu. SSXXI also presents a similar cluster at relatively weak abundance (*i.e.*, at *m/z* 1342,1320) and no related cluster being observed for SXXI. The countercation compositions of these molecular weight-related ions for SXXI, SSXXI and SSSXXI are presented in Table 3. The disulfated fragment ion *m/z* 1342 resulting from the loss of sodium sulfite with hydrogen replacement from the molecular ion *m/z* 1444 of SSSXXI (Figure 4) also reveals a -22 amu fragment ion at *m/z* 1320 that is attributable to countercation variation. Additionally, like the mono- and disulfated tetrasaccharides SXIV and SSXIV, the trisulfated hexasaccharide SSSXXI demonstrates the loss of a water molecule with the fragment ion $[(R_2 + Na) - H_2O]^+$ arising from the dehydration of the galactose-6-sulfate residue.

Consistent for all sulfated oligosaccharides in this positive FAB MS study are unambiguous mass spectra dominated by intense, fully natriated, molecular ions, the prominent losses of 102 amu reflecting the elimination of sodium sulfite with hydrogen replacement, and the presentation of sequence-related fragment ions. With the exception of monosulfated oligosaccharides SVII, SXIV and SXXI, all mutisulfated oligosaccharides clearly displayed molecular weight-related ions reflecting countercation dispersions. The ready determination of the number of sulfate esters present on an oligosaccharide by the number of $[(M + Na) \ (-NaSO_3 \ + H)_n]^+$ fragment ions is very convenient in that it can potentially spare the required sample amount now required for sulfate quantification via other colorimetric[20] and chromatographic[21] procedures standardly employed in our structural studies.

TABLE 3. Countercation Composition of Molecular Weight-Related Ions from SXXI, SSXXI and SSSXXI

Countercation composition	*m/z* [a]
Oligossaccharide - SXXI	
$(2Na)^{2+}$	1240
$(H^{1+}, Na^{1+})^{2+}$	1218
Oligosaccharide - SSXXI	
$(3Na)^{3+}$	1342
$(H^{1+}, 2Na^{2+})^{3+}$	1320
$(2H^{2+}, Na^{1+})^{3+}$	1298
Oligosaccharide - SSXXI	
$(4Na)^{4+}$	1444
$(H^{1+}, 3Na^{3+})^{4+}$	1422
$(2H^{2+}, 2Na^{2+})^{4+}$	1400
$(3H^{3+}, Na^{1+})^{4+}$	1378

a. Double underlines indicate ions observed in the respective mass spectra.

Furthermore, positive ion FAB mass spectrometry is proven informative for branched as well as straight chain multisulfated oligosaccharides, emphasizing its analytical versatility. These results clearly demonstrate, for the first time, the utility of readily available positive FAB MS for the structural analysis of underivatized multisulfated oligosaccharides isolated from tracheobronchial mucous glycoproteins via alkaline-borohydride reduction.

Lastly, though less available to the standard research laboratory, it should be noted that other MS techniques employing relatively mild ionization precesses, such

as thermospray (TS) and electrospray ionization (ES) mass spectrometry, may possess significant potential in the characterization of mucous glycoprotein oligosaccharides. The versatility of ES and TS demonstrated with peptides,[22,23] glycopeptides[24] and in initial studies of glycosaminoglycans[25,26] and N-linked oligosaccharides[27] is encouraging of these ionization techniques as a future tool in O-linked oligosaccharide analysis. Ongoing ES MS investigations indicate that, while precise molecular weights are readily attained, more recent modifications such as ES MS/MS[26,27] may better serve to provide structural information.

EXPERIMENTAL

The mono- and disulfated trisaccharides (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (**SVII**) and (4-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-6-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (**SSVII**), the mono- and disulfated tetrasaccharides (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (**SXIV**) and (6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-2-acetamido-2-deoxy-D-galactitol (**SSXIV**) and the mono-, di- and trisulfated hexasaccharides (β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-2-acetamido-2-deoxy-D-galactitol (**SXXI**), (6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]- *O*-2-acetamido-2-deoxy-D-galactitol (**SSXXI**), and (6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(6-*O*-sulfo-β-D-galactopyranosyl)-(1→3)-*O*-[(6-*O*-sulfo-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-2-acetamido-2-deoxy-D-galactitol (**SSSXXI**) were isolated from tracheobronchial mucous glycoproteins by β-elimination via alkaline borohydride

reduction, purified by gel filtration, ion-exchange chromatography and characterized by complete methylation analysis and sequential enzymatic degradation, as previously described.[12]

Samples (10-20 μg) dissolved in approximately 1-2 μL of glass-distilled nanopure grade water (Barnstead, Dubuque IA 52001) were placed onto a stainless-steel probe tip coated with 1-thioglycerol (3-mercapto-1,2-propanediol). Fast atom bombardment mass spectra of all oligosaccharides in the positive ion mode were obtained employing a VG ZAB-HF mass spectrometer equipped with a standard VG FAB ion source and a saddle field gun (Ion-Tech Model B11N) with a xenon atom beam at 8 keV and 1 mA.

REFERENCES

1. G. P. Roberts, *Eur. J. Biochem.*, **50**, 265 (1974).

2. P. Roussel, G. Lamblin, P. Degand, E. Walker-Nasir and R. W. Jeanloz, *J. Biol. Chem.*, **250**, 2114 (1975).

3. K. V. Chace, B. Desai, V. C. Naziruddin, M. Flux and G. P. Sachdev, *Exp. Lung Res.*, **15**, 721 (1989).

4. T. F. Boat, P. W. Cheng, R. N. Iyer, D. M. Carlson and I. Polony, *Arch. Biochem. Biophys.*, **177**, 95 (1976).

5. K. C. Chace, M. Flux and G. P. Sachdev, *Biochemistry*, **24**, 7334 (1985).

6. T. F. Boat, H. I. Kleinerman, D. M. Carlson, W. H. Maloney and L. W. Matthews, *Am. Respir. Dis.*, **110**, 428 (1974).

7. P. W. Cheng, T. F. Boat, K. Cranfill, J. R. Yankaskas and R. C. Boucher, *J. Clin. Invest.*, **84**, 68 (1989).

8. A. Klein, G. Lamblin, M. Lhermitte, P. Roussel, J. Breg, H. Van Halbeek and J. F. G. Vliegenthart, *Eur. J. Biochem.*, **171**, 631 (1988).

9. T. P. Mawhinney, G. J. Barbero, M. S. Feather and J. R. Martinez, *Cystic Fibrosis Club Abstr.*, **24**, 33 (1983).

10. T. P. Mawhinney, E. Adelstein, D. A. Morris, A. M. Mawhinney and G. J. Barbero, *J. Biol. Chem.*, **262**, 2994 (1987).

11. T. P. Mawhinney, E. Adelstein, D. A. Gayer, D. C. Landrum and G. J. Barbero, *Carbohydr. Res.*, **223**, 187 (1992).

12. T. P. Mawhinney, D. C. Landrum, D. A. Gayer and G. J. Barbero, *Carbohydr. Res.*, **235**, 179 (1992).

13. S. A. Carr and V. N. Reinhold, *J. Carbohydr. Chem.*, **3**, 381 (1984).

14. V. N. Reinhold, S. A. Carr, B. N. Green, M. Petitou, J. Choay and P. Sinay, *Carbohydr. Res.*, **161**, 305 (1987).

15. L. M. Mallis, H. M. Wang, D. Loganathan and R. J. Linhardt, *Anal. Chem.*, **61**, 1453 (1989).

16. R. J. Lindardt, H. M. Wang, D. Loganathan, D. J. Lamb and L. M. Mallis, *Carbohydr. Res.*, **225**, 137 (1992).

17. A. Dell, H. R. Morris, F. Greer, J. M. Redfern, M. E. Rogers, G. Weisshaar, J. Hiyama and A. G. C. Renwick, *Carbohydr. Res.*, **209**, 33 (1991).

18. T. Krusius, V. N. Reinhold, R. K. Margolis and R. U. Margolis, *Biochem. J.*, **245**, 229 (1987).

19. H. Murakami, Z. Lam, B. C. Furie, V. N. Reinhold, T. Asano and B. Furie, *J. Biol. Chem.*, **266**, 15414 (1991).

20. C. A. Antonopoulos, *Acta Chem. Scand.*, **16**, 1521 (1962).

21. T. P. Mawhinney, *J. Chromatogr.*, **257**, 37 (1983).

22. A. Tsarbopoulos, B. N. Pramanik, J. E. Labdon, P. Reichert, G. Gitlin, S. Patel, V. Sardana, T. L. Nagabhushan and P. P. Trotta, *Protein Sci.*, **2**, 1948 (1993).

23. R. P. Evershed, D. H. Robertson, R. J. Beynon and B. N. Green, *Rapid Commun. Mass Spectrom.*, **7**, 882 (1993).

24. A. W. Guzzetta, L. J. Basa, W. S. Hancock, B. A. Keyt and W. F. Bennett, *Anal. Chem.*, **65**, 2953 (1993).

25. T. Keiichi, K. Kaoru, M. Mitsuo, N. Toskhiya, K. Ikunoshi and E. Masahiko, *Glycocongugate J.*, **9**, 174 (1992).

26. R. Da Col, L. Silvestro, A. Naggi, G. Torri, C. Baiocchi, D. Moltrasio, A. Cedro and I. Viano, *J. Chromatogr.*, **647**, 289 (1993).

27. K. L. Duffin, G. W. Lange, J. K. Welply, R. Florman, P. J. O'Brien, A. Dell, A. J. Reason, H. R. Morris and S. J. Fliesler, *Glycobiology*, **3**, 365 (1993).

28. A. Fura, J. A. Leary, *Anal. Chem.*, **65**, 2805 (1993).

29. M. J. Huddleston, M. F. Bean and S. A. Carr, *Anal. Chem.*, **65**, 877 (1993).