The structure of five sulfated oligosaccharide units of highly anionic tracheobronchial mucous glycoproteins, isolated from a cystic fibrosis patient's sputum, was established. Reduced oligosaccharides (84%) were released under alkaline borohydride conditions, and the acidic oligosaccharides (63%) were isolated by Dowex 1-X2 chromatography. Following the removal of acidic oligosaccharides possessing N-acetylneuraminic acid and L-fucose by lectin affinity chromatography, a heterogeneous mixture of sulfated oligosaccharides was obtained. From this fraction, five short chain monosulfated oligosaccharides (S-I to S-V) were purified by sequential separation by SynChroprep AX300 anion exchange high pressure liquid chromatography, gel filtration on Bio-Gel P-2, and high voltage paper electrophoresis. Based on the results of carbohydrate composition, sequential exoglycosidase degradation, permethylation analysis, lectin affinity chromatography, and periodate oxidation, the following structures (where GalNAcol is N-acetylgalactosaminitol) were proposed for these oligosaccharides.

S-I \[\text{SO}_3(6)\text{Gal} \beta(1\rightarrow3)\text{GalNAcol}\]
S-II \[\text{SO}_3(6)\text{Gal} \beta(1\rightarrow3)\text{GlcNAc} \beta(1\rightarrow3)\text{GalNAcol}\]
S-III \[\text{SO}_3(6)\text{Gal} \beta(1\rightarrow4)\text{GlcNAc} \beta(1\rightarrow3)\text{GalNAcol}\]
S-IV \[\text{SO}_3(6)\text{Gal} \beta(1\rightarrow4)\text{GlcNAc} \beta(1\rightarrow3)\text{Gal} \beta(1\rightarrow3)\text{GalNAcol}\]
S-V \[\text{SO}_3(6)\text{Gal} \beta(1\rightarrow4)\text{GlcNAc} \beta(1\rightarrow3)\text{Gal} \beta(1\rightarrow4)\text{GlcNAc} \beta(1\rightarrow3)\text{GalNAcol}\]

Tracheobronchial mucous glycoproteins are a class of large molecular weight components that are the primary macromolecular constituents of a mucous viscoelastic gel among whose functions may include protection of the pulmonary airways from inhaled particulate matter, irritants, microorganisms, and rapid drying by evaporation. In the absence of pulmonary disease, tracheobronchial mucous glycoproteins are secreted in low quantities and have been shown to possess both an anionic nature and side-chain oligosaccharides of small length (1). Notably, the acidic characteristic of these tracheobronchial mucous glycoproteins is primarily due to the presence of N-acetylgalactosaminic acid and sulfate esters.

A major feature observed in many chronic obstructive pulmonary diseases is the hypersecretion of tracheobronchial mucous glycoproteins (2). Several reports indicate that in certain pathological states, such as chronic bronchitis and cystic fibrosis, these hypersecreted tracheobronchial mucous glycoproteins reflect an increased anionic characteristic due to their increased content of N-acetylgalactosaminic acid and sulfate esters (3). These same investigations have suggested that the content of sulfate esters was greatest in the larger oligosaccharides isolated from these glycoproteins and that the N-acetylgalactosaminic acid content was greatest in the smaller oligosaccharides (3, 4). Although structures of several oligosaccharides containing N-acetylgalactosaminic acid isolated from these glycoproteins have been reported (5), there have been no similar reports on the primary structures of oligosaccharides containing sulfate esters.

Since normal human tracheobronchial secretions are attainable only in small quantities and possess relatively low percentages of sulfate esters (6), we chose to investigate the sulfated oligosaccharides isolated from tracheobronchial mucous glycoproteins found in the sputum of patients suffering from cystic fibrosis. From these studies we hope to accrue structural data that will provide better understanding of the roles of the sulfate ester and sulfated oligosaccharides in health and disease. In the present investigation, we report on the separation and complete structural determination of five sulfated oligosaccharides consisting of a disaccharide, two trisaccharides, a tetrasaccharide, and a pentasaccharide, purified from the tracheobronchial mucous glycoproteins isolated from the sputum of a patient suffering from cystic fibrosis. Though the general acceptance, based upon compositional analyses, is that the sulfate ester is predominant on long-chain oligosaccharides the current study indicates that they can be present on oligosaccharide structures as short as a disaccharide. Sputum from a single patient was employed in this investigation rather than a pool from several individuals in an effort to decrease the introduction of any heterogeneity due to individual variation.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

Tracheobronchial glycoproteins are readily purified from contaminating proteins, cell debris, and DNA typically found in purulent sputum obtained from patients suffering from cystic fibrosis following thiol reduction, treatment with deox-
yribonuclease, and Bio-Gel A-5m gel chromatography (20). The highly anionic glycoproteins can then be separated by anion exchange chromatography (20). Alkaline borohydride treatment of these acidic tracheobronchial mucous glycoproteins has been shown to produce a heterogenous population of β-eliminated and reduced oligosaccharides (4, 39). In the present study, separation of monosulfated oligosaccharides from this mixture was accomplished by anion exchange chromatography (Fig. 3), by lectin affinity chromatography (Fig. 4) to remove oligosaccharides possessing either N-acetyllneuraminic acid or L-fucose residues, and by high pressure liquid chromatography (Fig. 5). Further separation by Bio-Gel P-2 (Fig. 6) chromatography produced four well-defined low molecular weight oligosaccharide fractions with elution patterns corresponding to standard disaccharides, trisaccharides, tetrasaccharides, and pentasaccharides. Though oligosaccharides of higher degree of polymerization were present, no distinct separation could be achieved on this column. By subsequent high voltage paper electrophoresis (Fig. 7) of these four fractions, five sulfated oligosaccharides of high purity were attained. Determination of the primary carbohydrate structure of each of the five sulfated oligosaccharides was performed by analyzing them and their respective desulfated analogs by classical carbohydrate chemistry employing total carbohydrate analysis, specific glycosidase degradation, methylation analysis, and periodate oxidation and analysis. Determination that the sulfate ester on each oligosaccharide resided on the C6 primary alcohol of the nonreducing terminus galactose residue was based upon several analytical results. These included methylation and periodate oxidation analysis of the intact sulfated and desulfated oligosaccharides, periodate oxidation analysis of the sulfated galactose residue that was liberated from each oligosaccharide by β-galactosidase, the generation of 3,6-anhydrogalactose following alkaline hydrolysis, and, last, the time required to acid hydrolyze the sulfate ester.

To our knowledge, this is the first report presenting the complete structural analysis of sulfated oligosaccharides isolated from human tracheobronchial mucous glycoproteins. It should be noted, though, that the neutral carbohydrate cores of each sulfated oligosaccharide presented have been reported. The neutral disaccharide Galβ(1→4)GalNAcβ(2→6)GalNAc, which is the neutral carbohydrate core of the sulfated oligosaccharide S-I, has been isolated from sputum from a patient suffering from chronic bronchitis (40) and also from a patient suffering from cystic fibrosis (41). It also has been isolated from hog submaxillary gland mucin glycoproteins (42) and from human salivary mucin oligosaccharides from normal patients and patients with cystic fibrosis (43). The triasaccharides Galβ(1→3)GlcNAcβ(1→3)GalNAc and Galβ(1→4)GlcNAcβ(1→3)GalNAc, which form the neutral carbohydrate cores of S-II and S-III, respectively, have both been identified from bronchial glycoproteins from a patient suffering with cystic fibrosis (41). S-III has also been isolated from human colonic mucin (44, 45) and from bronchial glycoproteins from a patient with chronic bronchitis (40). The tetrasaccharide Galβ(1→4)GlcNAcβ(1→3)Galβ(1→3)GlcNAc, the neutral carbohydrate core of S-IV, has been identified from sputum glycoproteins from a patient with cystic fibrosis (41). Last, the pentasaccharide Galβ(1→4)GlcNAcβ(1→3)Galβ(1→4)GlcNAcβ(1→3)GalNAc, which forms the neutral core of the sulfated oligosaccharide S-V has been reported from human colonic mucins (44, 45).

In a report on the primary-structure determination of 14 neutral oligosaccharides isolated from human bronchial glycoproteins utilizing 500-MHz 1H NMR spectroscopy, it was suggested that bronchial glycoprotein oligosaccharides could be grouped into two classes based upon the type of core they possessed (41). These two cores were, specifically, galactose or N-acetylglucosamine linked β(1→3) to N-acetylgalactosaminol. Both of these cores were found to be present in this study. S-I and S-IV were found to have Galβ(1→3)GalNAc at their original reducing termini, whereas S-II, S-III, and S-V were found to possess GlcNAcβ(1→3)GalNAc. It should also be noted that with regard to the four common types of O-glycosidic glycoprotein linkages found in nature, as described and categorized by Schachter and Williams (46), S-I and S-IV possess type 1 cores, whereas S-II, S-III, and S-IV are type 3 core structures.

Last, it has been generally accepted within recent years that N-acetyllneuraminic acid is the primary anionic moiety on short chain oligosaccharides and that sulfate is predominant on longer chains. This view has been based primarily on compositional analysis of column chromatographic fractions possessing oligosaccharides of different molecular weight ranges (3, 4). The present study clearly shows that, though present in small quantities in the present study, oligosaccharides as small as a disaccharide can possess a sulfate ester.

The overall goal of our research is to elucidate the structures of these sulfated oligosaccharides that occur on tracheobronchial mucous glycoproteins in man and to determine what changes occur from the normal to the disease state. This information is fundamental to our understanding of the role(s) that mucinous glycoproteins play in the defense mechanisms that are triggered by chronic lung disease.

REFERENCES

1. Lafitte, J. J., Lamblin, G., Lhermitte, M., Humbert, P., Degand, P., and Rousell, P. (1977) Carbohydr. Res. 56, 383-389
2. Lopata, M., Barton, A. D., and Lourenco, R. V. (1974) Am. Rev. Respir. Dis. 110, 709-739
3. Roberta, G. P. (1974) Eur. J. Biochem. 50, 266-269
4. Rousseau, P., Lamblin, G., Degand, P., Walker-Nasir. E., and Jehlou, R. W. (1975) J. Biol. Chem. 250, 2114-2122
5. Lamblin, G., Boersma, A., Klein, A., Rousseau, P., van Halibeek, H., and Vliegenthart, J. F. G. (1984) J. Biol. Chem. 259, 9051-9058
6. Sachdev, G. P., Myers, F. J., Horton, F. O., Fox, O. F., Wen, G., Rogers, R. M., and Carubelli, R. (1980) Biochem. Med. 24, 82-94
7. Neuberger, A. (1941) J. Chem. Soc. 47-61
8. Kuhn, R., Zilliken, G., and Gauhe, A. (1953) Chem. Ber. 86, 466-475
9. Lloyd, A. G. (1962) Biochem. J. 83, 455-460
10. Lewis, B. A., Smith, F., and Stephen, A. M. (1963) Methods Carbohydr. Chem. 2, 172-188
11. Mawhinney, T. P., Feather, M. S., Barbero, G. J., and Martinez, J. R. (1980) Anal. Biochem. 101, 112-117
12. Mawhinney, T. P. (1986) J. Chromatogr. 351, 91-102
13. Antonopoulos, C. A. (1962) Acta Chem. Scand. 16, 1521-1522
14. Mawhinney, T. P. (1983) J. Chromatogr. 257, 37-44
15. Aminoff, D. (1961) Biochem. J. 81, 384-392
16. Mawhinney, T. P., Madison, M. A., Rice, R. H., Feather, M. S., and Barbero, G. J. (1982) Carbohydr. Res. 104, 169-181
17. Park, T. S., and Johnson, M. J. (1949) J. Biol. Chem. 181, 149-154
18. Ghelregabיחה, M., Rusini, S., Monaldi, B., and Lato, M. (1976) J. Chromatogr. 127, 133-132
19. Lloyd, A. G. (1980) Biochem. J. 175, 478-482
20. Do, T. F., Cheng, P. W., Iver, P. N., Carlson, D. M., and Polony, I. (1976) Arch. Biochem. Biophys. 177, 95-104
21. Dache, Z., and Shettles, L. B. (1971) J. Biol. Chem. 242, 579-585
22. Roche, A. C., Schauer, R., and Monsigny, M. (1975) FEBS Lett. 57, 245-249

The abbreviation used is: GalNAcβ, N-acetylgalactosaminol.
Sulfated Oligosaccharides of Human Tracheobronchial Mucins

23. Baenziger, J. U., and Natowicz, M. (1981) Anal. Biochem. 112, 357–361.
24. Lombart, C. G., and Winder, R. J. (1974) Eur. J. Biochem. 49, 77–86.
25. Gordon, H. T., Thornburg, W., and Werum, L. N. (1956) Anal. Chem. 28, 849–855.
26. Danielsen, I. (1965) Methods Enzymol. 1, 407–409.
27. Guthrie, R. D. (1962) Methods Enzymol. 1, 435–441.
28. Kennedy, J. F. (1972) Methods Enzymol. 1, 93–100.
29. Spec, J. C. (1962) Methods Enzymol. 1, 441–445.
30. Slomiany, A., and Slomiany, B. L. (1978) J. Biol. Chem. 253, 7301–7306.
31. Percival, E., and Wold, J. K. (1983) J. Chem. Soc. 5459–5468.
32. Baenziger, J. U., and Natowicz, M. (1981) Anal. Biochem. 112, 357–361.
33. Lombard, C. G., and Winder, R. J. (1974) Eur. J. Biochem. 49, 77–86.
34. Warner, T. G., and O’Brien, J. S. (1982) J. Biol. Chem. 257, 224–232.
35. Green, E. D., van Halbeck, H., Boime, I., and Baenziger, J. U. (1985) J. Biol. Chem. 260, 15625–15630.
36. Percival, E., and Wold, J. K. (1983) J. Chem. Soc. 5459–5468.

Supplements to Structure Determination of Sulfated Glycoconjugates

Thomas P. Murray, Edward Adams, and James M. Murray

EXPERIMENTAL PROCEDURES

Materials...
Sulfated Oligosaccharides of Human Tracheobronchial Mucins

been reported that when carbohydrates possessing a CA epitope occur and an unpracticed CA-CH reaction is adapted to basic conditions the 3,4-linked deoxy sugars isomerized (38,39). hence, a-sialylated A-3-linked deoxy sugars was employed in flow injection analysis and then hydrolyzed for 3,8-anhydro-glucitol and carbohydrates analysis (11,12).

Lectin affinity of oligosaccharides: Sulfated and desulfated oligosaccharides and residual oligosaccharides were investigated for binding to lectins. A-3-linked deoxy sugars was employed in flow injection analysis and then hydrolyzed for 3,8-anhydro-glucitol and carbohydrates analysis (11,12).

Lectins used were in the presence of 0.1 M sodium acetate buffer, pH 4.0. The amounts of desulfated oligosaccharides were measured by the decrease in absorbance at 576 nm for the unconjugated lectins.

For each reduced oligosaccharide structure with a CA epitope as CA-CH reaction was performed in a reducing solution containing 0.1 M sodium acetate buffer, pH 4.0. The amounts of desulfated oligosaccharides were measured by the decrease in absorbance at 576 nm for the unconjugated lectins.

When the lectin affinity of oligosaccharides was studied by using the following lectins: (a) SNA-CH, (b) SNA-CH, (c) SNA-CH, (d) SNA-CH, (e) SNA-CH, (f) SNA-CH, and (g) SNA-CH. The lectin affinity was studied for each reduced oligosaccharide structure with CA-CH reaction was performed in a reducing solution containing 0.1 M sodium acetate buffer, pH 4.0. The amounts of desulfated oligosaccharides were measured by the decrease in absorbance at 576 nm for the unconjugated lectins.

INDIVIDUAL SULFATED OligosACCHARIDE RESULTS

Sulfated Oligosaccharides

Carbohydrate and sulfate analysis demonstrated that sulfated oligosaccharide S1 contained galactose, fucose, and sulfate. The sulfate and galactose contents were determined as S1 is a carbohydrate isolated from a sialic acid enriched fraction. The carbohydrate composition of the sulfated oligosaccharide S1 was determined as S1 is a carbohydrate isolated from a sialic acid enriched fraction. The carbohydrate composition of the sulfated oligosaccharide S1 was determined as S1 is a carbohydrate isolated from a sialic acid enriched fraction. The carbohydrate composition of the sulfated oligosaccharide S1 was determined as S1 is a carbohydrate isolated from a sialic acid enriched fraction. The carbohydrate composition of the sulfated oligosaccharide S1 was determined as S1 is a carbohydrate isolated from a sialic acid enriched fraction.
Sulfated Oligosaccharides of Human Tracheobronchial Mucins

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Sulfated Oligosaccharides of Human Tracheobronchial Mucins

As shown in Table II, treatment of S-Y with strong acid caused a 79% loss of the oligosaccharide's sulfate. Additionally, a 41% decrease of the oligosaccharide's galactose was seen which was mainly present on the N-acetylglucosamine residues. For this reason, the mixture was treated with 1 M NaOH to de-sulfate the mixture and isolate only the intact disaccharide. The isolated disaccharide was then treated with 1 M NaOH to de-sulfate the mixture and isolate only the intact disaccharide.

### Table I

| Sulfated Oligosaccharide Composition | Disaccharide Content (mol%) | Monosaccharide Content (mol%) |
|-------------------------------------|----------------------------|-------------------------------|
| S-I                                 | 9.01, 9.25                  | 7.28, 7.75                     |
| S-II                                | 9.45, 9.67                  | 7.28, 7.75                     |
| S-III                               | 9.77, 10.00                 | 7.28, 7.75                     |
| S-IV                                | 9.77, 10.00                 | 7.28, 7.75                     |
| S-V                                 | 9.77, 10.00                 | 7.28, 7.75                     |

**C**

| Sulfated Oligosaccharide Composition | Disaccharide Content (mol%) | Monosaccharide Content (mol%) |
|-------------------------------------|----------------------------|-------------------------------|
| S-I                                 | 9.01, 9.25                  | 7.28, 7.75                     |
| S-II                                | 9.45, 9.67                  | 7.28, 7.75                     |
| S-III                               | 9.77, 10.00                 | 7.28, 7.75                     |
| S-IV                                | 9.77, 10.00                 | 7.28, 7.75                     |
| S-V                                 | 9.77, 10.00                 | 7.28, 7.75                     |

**D**

| Sulfated Oligosaccharide Composition | Disaccharide Content (mol%) | Monosaccharide Content (mol%) |
|-------------------------------------|----------------------------|-------------------------------|
| S-I                                 | 9.01, 9.25                  | 7.28, 7.75                     |
| S-II                                | 9.45, 9.67                  | 7.28, 7.75                     |
| S-III                               | 9.77, 10.00                 | 7.28, 7.75                     |
| S-IV                                | 9.77, 10.00                 | 7.28, 7.75                     |
| S-V                                 | 9.77, 10.00                 | 7.28, 7.75                     |

### Table II

| Monosaccharide Analysis of Sulfated Oligosaccharides from Human Bronchial Mucins |
|--------------------------------------|----------------------------------|
| Sulfated Oligosaccharide             | GalNAc-Gal-GalNAc-Sulfate         |
| S-I (9.81, 9.71)                     | GalNAc-Gal-GalNAc-Sulfate         |
| S-II (9.67, 9.71)                    | GalNAc-Gal-GalNAc-Sulfate         |
| S-III (9.77, 10.00)                  | GalNAc-Gal-GalNAc-Sulfate         |
| S-IV (9.77, 10.00)                   | GalNAc-Gal-GalNAc-Sulfate         |
| S-V (9.77, 10.00)                    | GalNAc-Gal-GalNAc-Sulfate         |

### Table III

| Monosaccharide Analysis of Sulfated Oligosaccharides from Human Bronchial Mucins |
|--------------------------------------|----------------------------------|
| Sulfated Oligosaccharide             | GalNAc-Gal-GalNAc-Sulfate         |
| S-I (9.81, 9.71)                     | GalNAc-Gal-GalNAc-Sulfate         |
| S-II (9.67, 9.71)                    | GalNAc-Gal-GalNAc-Sulfate         |
| S-III (9.77, 10.00)                  | GalNAc-Gal-GalNAc-Sulfate         |
| S-IV (9.77, 10.00)                   | GalNAc-Gal-GalNAc-Sulfate         |
| S-V (9.77, 10.00)                    | GalNAc-Gal-GalNAc-Sulfate         |

### Table IV

| Enzyme Removal of Sulfated Oligosaccharide S-I and its de-sulfated product D-S-I |
|-------------------------------|----------------------------------|
| Treatment                      | Carbohydrate composition          |
|--------------------------------|----------------------------------|
| GalNAc-Gal-GalNAc-Sulfate       | GalNAc-Gal-GalNAc-Sulfate         |
| Sulfated oligosaccharide S-I    | 1.00, 1.00                        |
| 2. Galactose                   | 0.96, 0.90                       |
| 3. S-galactosamine             | 1.00, 1.00                       |
| Desulfated oligosaccharide D-S-I| 1.00, 1.00                        |

### Table V

| Enzyme Removal of Sulfated Oligosaccharide S-II and its de-sulfated product D-S-II |
|-------------------------------|----------------------------------|
| Treatment                      | Carbohydrate composition          |
|--------------------------------|----------------------------------|
| GalNAc-Gal-GalNAc-Sulfate       | GalNAc-Gal-GalNAc-Sulfate         |
| Sulfated oligosaccharide S-II   | 1.00, 1.00                        |
| 2. Galactose                   | 0.96, 0.90                       |
| 3. S-galactosamine             | 1.00, 1.00                       |
| Desulfated oligosaccharide D-S-II| 1.00, 1.00                        |

### Table VI

| Enzyme Removal of Sulfated Oligosaccharide S-III and its de-sulfated product D-S-III |
|-------------------------------|----------------------------------|
| Treatment                      | Carbohydrate composition          |
|--------------------------------|----------------------------------|
| GalNAc-Gal-GalNAc-Sulfate       | GalNAc-Gal-GalNAc-Sulfate         |
| Sulfated oligosaccharide S-III  | 1.00, 1.00                        |
| 2. Galactose                   | 0.96, 0.90                       |
| 3. S-galactosamine             | 1.00, 1.00                       |

### Table VII

| Enzyme Removal of Sulfated Oligosaccharide S-IV and its de-sulfated product D-S-IV |
|-------------------------------|----------------------------------|
| Treatment                      | Carbohydrate composition          |
|--------------------------------|----------------------------------|
| GalNAc-Gal-GalNAc-Sulfate       | GalNAc-Gal-GalNAc-Sulfate         |
| Sulfated oligosaccharide S-IV   | 1.00, 1.00                        |
| 2. Galactose                   | 0.96, 0.90                       |
| 3. S-galactosamine             | 1.00, 1.00                       |

### Table VIII

| Enzyme Removal of Sulfated Oligosaccharide S-V and its de-sulfated product D-S-V |
|-------------------------------|----------------------------------|
| Treatment                      | Carbohydrate composition          |
|--------------------------------|----------------------------------|
| GalNAc-Gal-GalNAc-Sulfate       | GalNAc-Gal-GalNAc-Sulfate         |
| Sulfated oligosaccharide S-V    | 1.00, 1.00                        |
| 2. Galactose                   | 0.96, 0.90                       |
| 3. S-galactosamine             | 1.00, 1.00                       |
Table X

| Oligosaccharide | Carbohydrate Methyl Ester | Glycosylated | Carbohydrated | Lactose | Fixed |
|-----------------|---------------------------|--------------|---------------|---------|-------|
| Gal-1 after S-galactosidase | 3.04 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-1 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-2 after S-galactosidase | 3.08 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-2 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-3 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-3 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-4 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-4 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-5 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-5 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4

Table XI

| Lactose Affination of Carbohydrated and Desulfated Oligosaccharides from Human Tracheobronchial Mucins | Reaction Conditions | Reaction Conditions | Reaction Conditions | Reaction Conditions |
|------------------------------------------------|-------------------|-------------------|-------------------|-------------------|
| Gal-1 after S-galactosidase | 3.04 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-1 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-2 after S-galactosidase | 3.08 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-2 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-3 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-3 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-4 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-4 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-5 after S-galactosidase | 3.05 | 0.79 | 0.56 | Gal-C6H13-C6H4
| Gal-5 | 4.48 | 0.79 | 0.56 | Gal-C6H13-C6H4

Figure 1

Representative Bar-Graph A: The separation of the different carbohydrates from the tracheobronchial mucins by the described method. After the separation, the carbohydrates were stained with toluidine blue and examined with a microscope. The different carbohydrates are indicated by the different colors. The carbohydrates were identified by comparison with authentic standards. The results were expressed as the percentage of each carbohydrate in the total carbohydrate content of the mucins. The carbohydrate content was determined by the phenol-sulfuric acid method. The data are expressed as the mean ± SD of three experiments.

Figure 2

DEAE-cellulose chromatographic fractionation of the pooled mucous glycoproteins. The purified glycoproteins were eluted from the DEAE-cellulose column with a linear gradient of potassium phosphate buffer (pH 7.0) at a flow rate of 1 ml/min. The fractions were collected every 1 ml. The carbohydrate content of each fraction was determined by the phenol-sulfuric acid method.
Sulfated Oligosaccharides of Human Tracheobronchial Mucins

Figure 3. Fractionation of the alkaline/hydrolyzed cleared and reduced oligosaccharides into neutral and acidic fraction by anion exchange chromatography. Partially digested oligosaccharides (0.1 ml) were applied to a Dowex 1 × 2 (80-200 mesh) column, 1.0 × 20 cm, pre-equilibrated with distilled water. The neutral oligosaccharides were eluted using sodium chloride, while the acidic oligosaccharides (fraction numbers 15 to 26) were eluted in a single step with 0.5 N NaOH and were pooled. The pooled acidic oligosaccharides were desalted on a Bio-Gel P-3 (Bio, 3.0 × 40 cm) column.

Figure 4. Lectin affinity chromatographic isolation of human tracheobronchial oligosaccharides free of N-acetylglucosaminic acid and L-fucose and possessing only a single sialic acid residue. Oligosaccharides isolated by anion-exchange chromatography on a Dowex 1 × 2 (80-200 mesh) column (Figure 3) were subjected to sequential chromatographic isolation through lectin-polyethylene lectin on Sepharose 4B (A) and immobilized soybean agglutinin lectin on Sepharose 4B (BD columns.

Figure 5. High voltage paper electrophoresis of the immunoaffinity isolated fraction F1 - F4 from the Bio-Gel P-3 column (Figure 4A). Approximately 4 μg of each fraction (F1 - F4) were individually applied and run on Whatman 3M filter paper in 3 cm strips. Paper was stained using 0.1% (w/v) periodic acid or sodium toluidine blue at pH 4.8 (pH 4.8). A periodate-periodate reagent was used to visualize oligosaccharides and carboxylic groups. Numbers 1 to 9 indicate partially purified sulfated oligosaccharides which were separated for structural analysis.

Figure 6. Representative anion exchange high performance liquid chromatographic separation of oligosaccharides possessing only a single sialic acid residue on a Bio-Gel P-3 column (Figure 4A). Approximately 4 μg of each fraction (F1 - F4) were individually applied and run on Whatman 3M filter paper in 3 cm strips. Paper was stained using 0.1% (w/v) periodic acid or sodium toluidine blue at pH 4.8 (pH 4.8). A periodate-periodate reagent was used to visualize oligosaccharides and carboxylic groups. Numbers 1 to 9 indicate partially purified sulfated oligosaccharides which were separated for structural analysis.

Figure 7. Representative anion exchange high performance liquid chromatographic separation of oligosaccharides possessing only a single sialic acid residue on a Bio-Gel P-3 column (Figure 4A). Approximately 4 μg of each fraction (F1 - F4) were individually applied and run on Whatman 3M filter paper in 3 cm strips. Paper was stained using 0.1% (w/v) periodic acid or sodium toluidine blue at pH 4.8 (pH 4.8). A periodate-periodate reagent was used to visualize oligosaccharides and carboxylic groups. Numbers 1 to 9 indicate partially purified sulfated oligosaccharides which were separated for structural analysis.