Purification and Structures of Oligosaccharide Chains in Swine Trachea and Cowper's Gland Mucin Glycoproteins*

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Mucin glycoproteins were purified from extracts of swine trachea mucosa and Cowper's gland. The gelatinous extracts were solubilized by reduction and carboxymethylation and then purified by chromatography on Sepharose CL-6B and DEAE-Sepharose. The structure of some of the carbohydrate units in these glycoproteins were determined and compared.

Alkaline borohydride treatment indicated that more than 86% of the carbohydrate chains in these glycoproteins were linked to serine or threonine residues in the polypeptide chain through O-glycosidic bonds with N-acetylgalactosamine. Reduced oligosaccharides released by treatment with alkaline borohydride were isolated by gel filtration on Bio-Gel P-6 and chromatography on DEAE-cellulose and paper. The structures of the oligosaccharides were established by methylation analysis, gas chromatography, and sequential hydrolysis with specific exoglycosidases. The major oligosaccharides in Cowper's gland mucin glycoproteins were sialylated short chains: NeuAca2,6GalNAcol and NeuAca2,3Galβ1,3(NeuAca2,6)GalNAcol.

In marked contrast, branched chains containing a Galβ1,3(GlcNAcβ1,6)GalNAcol core unit were the major components of trachea mucin glycoprotein. Ten of these chains had the following structures:

1. Fuca1,2Galβ1,3GalNAcol
2. GlcNAcβ1,6
   \GalNAcol
   Galβ1,3
3. GalcNAcβ1,6
   \GalNAcol
4. Galβ1,4GlcNAcβ1,6
   \GalNAcol
   Galβ1,3
5. Fuca1,2Galβ1,3GalNAcol
6. Fuca1,2Galβ1,4GlcNAcβ1,6
   \GalNAcol
   GlcNAcβ1,3Galβ1,3
7. NeuAca2,3Galβ1,3GalNAcol
8. NeuAca2,6
   \GalNAcol
   Galβ1,3
9. GlcNAcβ1,6
   \GalNAcol
   NeuAca2,3Galβ1,3
10. Galβ1,4GlcNAcβ1,6
   \GalNAcol
   NeuAca2,3Galβ1,3

The mammalian respiratory tract is protected by mucus secretions which are synthesized in the surface epithelium...
mucin glycoprotein which is present in the gel phase of this secretion (4). This acidic mucin glycoprotein is the principal constituent of the seminal gel and is primarily responsible for the rheological properties of boar semen.

The glycoproteins from trachea mucosa and Cowper’s gland are representative of two distinct types of mucin glycoproteins in mammalian tissues. The oligosaccharide chains in trachea mucin glycoprotein are clustered at one end of the polypeptide chain (5), whereas in Cowper’s gland mucin glycoprotein the oligosaccharide chains are distributed all along the polypeptide chain (4). Furthermore, the oligosaccharide chains in trachea mucin glycoprotein are much larger and less sialylated than those in Cowper’s gland mucin glycoproteins, and they have blood group A* and H* activity (6, 7).

This communication reports the purification, chemical composition, and immunological properties of these mucin glycoproteins. Also, we describe and compare the structures of some of the O-glycosidically linked oligosaccharide chains isolated from these purified mucin glycoproteins.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

The mucin glycoproteins in trachea epithelium and Cowper’s gland are present in insoluble gel secretions. The solubilization of these gels required reduction of sulfydryl groups and carboxymethylation, as well as disruption of hydrophobic interactions with guanidine hydrochloride. The reduced mucin glycoproteins were separated from lower molecular-weight serum proteins by gel filtration in the presence of 2 M guanidine hydrochloride. The major mucin glycoproteins purified from trachea epithelium and Cowper’s gland are eluted near the void volume during gel filtration on Sepharose CL-4B columns and show very high buoyant densities during ultracentrifugation in CsCl gradients. The molecular weight of these glycoproteins was estimated to be in the range of 6 × 10^6.

The purity of the mucin glycoproteins was examined by gel electrophoresis. These high-molecular-weight glycoproteins barely entered 5% polyacrylamide gel after electrophoresis in the presence of 1% sodium dodecyl sulfate. However, none of the lower-molecular-weight proteins present in the initial gels were detected by polyacrylamide gel electrophoresis or analytical sucrose density centrifugation. Electrophoresis in 5% polyacrylamide gel after electrophoresis in 1% agarose gels in the presence of dodecyl sulfate yielded single polydisperse bands which stained weakly for protein and intensely for carbohydrate. Purified trachea mucin glycoprotein gave a single precipitin line on immunodiffusion with homologous antibody, whereas Cowper’s gland mucin glycoprotein gave two precipitin lines. The bands may represent two forms of the same mucin glycoprotein. A number of studies have shown the presence of two immunologically distinct components in highly purified preparations of mucin glycoproteins (38–41). The trachea epithelium and Cowper’s gland mucin glycoproteins from swine were immunologically distinct and no cross-reaction between these glycoproteins and their respective antibodies were observed.

The amino acid composition of both mucin glycoproteins showed the well-documented predominance of hydroxyamino acids serine, threonine, and proline. They contained only small amounts of cysteine and methionine. The carbohydrate content and structure of the two glycoproteins were very different. Cowper’s gland mucin glycoprotein contained mainly NeuAc, GalNAc, and galactose. These three components were present in two highly sialylated oligosaccharide chains, NeuAcα2,6GalNAc and NeuAcα2,3Galβ1,3( NeuNAcα2,6)GalNAc. Cowper’s gland mucin glycoprotein did not contain fucose or sulfate. Trachea mucin glycoprotein contained fucose, galactose, GlcNAc, GalNAc, NeuAc, and sulfate. β-Elimination released a very heterogeneous group of reduced oligosaccharides which ranged in size from a disaccharide to species with at least 15 sugar residues. The mucin glycoprotein purified from trachea mucosa and Cowper’s gland contained carbohydrate substituted exclusively at threonyl and serinyl residues in the polypeptide chain. Base-catalyzed β-elimination released more than 85% of the carbohydrate from these glycoproteins.

Some of the oligosaccharides isolated in the present study may represent biosynthetic intermediates in the synthesis of more complex chains present in trachea mucin glycoprotein. Thus, oligosaccharides Fn and Enα which contain terminal galactose and GlcNAc units could act as acceptors for a number of glycosyltransferases and both chains could be extended. The data summarized in Table III show that relatively small amounts of these oligosaccharide chains are present in trachea mucin glycoprotein. Other chains, also present in small amounts, contain only one terminal sialic acid or fucose residue. The presence of these components may stop the further elongation of one chain, while the other may still be extended. The most abundant chains isolated from trachea mucin glycoprotein thus far were oligosaccharides Enα and En (Table III). To our knowledge, the presence of oligosaccharides with these structures in respiratory mucin glycoproteins has not yet been reported. Both of these oligosaccharides contain the complete blood group H determinant, Fucα1,2Galβ1,4GlcNAc. The addition of terminal fucose residues to both chains in oligosaccharide Enα could stop the growth of these chains and result in the accumulation of this oligosaccharide. The addition of β-1,3-linked GlcNAc instead of fucose to the galactose-attached β-1,3 to GalNAc yields an intermediate, oligosaccharide En1, which can still be extended. Thus, the relative activities or accessibility of α-1,2-fucosyltransferase and β-1,3-GlcNAc transferase could determine the amount of oligosaccharide En which is present in trachea mucin glycoprotein, and the amount of oligosaccharide Enα which is

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**Table III**

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Amount of oligosaccharide chains</th>
<th>Relative distance*</th>
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</thead>
<tbody>
<tr>
<td>Galβ1,3GalNAcol Std</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>Galβ1,3[(GlcNAc)β1,6]GalNAcol Std</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Fn1</td>
<td>1.46</td>
<td>1.63</td>
</tr>
<tr>
<td>Fn2</td>
<td>2.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Enα</td>
<td>7.92</td>
<td>0.88</td>
</tr>
<tr>
<td>Enβ</td>
<td>1.11</td>
<td>0.54</td>
</tr>
<tr>
<td>En1</td>
<td>11.91</td>
<td>0.30</td>
</tr>
<tr>
<td>En2</td>
<td>4.34</td>
<td>0.19</td>
</tr>
<tr>
<td>Enαα</td>
<td>0.92</td>
<td>1.93</td>
</tr>
<tr>
<td>Enββ</td>
<td>1.63</td>
<td>1.93</td>
</tr>
<tr>
<td>Enααα</td>
<td>1.30</td>
<td>1.00</td>
</tr>
<tr>
<td>Enβββ</td>
<td>1.73</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* Distance of migration relative to Galβ1,3[(GlcNAc)β1,6]GalNAcol in solvent system n-butyl alcohol/pyridine/water (6:4:3).
* After desialylation in 0.1 N HCl at 90 °C for 1 h.

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1 Portions of this paper (including "Experimental Procedures," "Results," Table I, II, IV, and V, and Figs. 1-10) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3332, cite the authors, and include a check or money order for $14.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
converted to oligosaccharides with much longer chains. Oligosaccharide synthesis may be terminated by the addition of sialic acid or fucose. In these oligosaccharides the terminal galactose is substituted with either sialic acid or with fucose. This observation supports recent data on the biosynthesis of mammalian glycoproteins (42) which indicate that the actions of siaIytransferases and fucosyltransferases are mutually exclusive. Sialic acid in oligosaccharides Ema and Ema is attached to position 6 of GalNAcol or to position 3 of galactose. All of the oligosaccharides characterized thus far contain only 1 eq of sialic acid. The addition of sialic acid to position 3 of galactose or to position 6 of GalNAcol may also serve to terminate further extension of these chains.

The results presented in this report show that most of the carbohydrate chains of trachea mucin glycoprotein contain a Galβ1,3(GalNAcβ1,6)GalNAc branched-core structure. It has recently been shown that plasma membrane glycoproteins from an ascites hepatoma, AH 66, also contain this core structure (43). In marked contrast, early branching of the oligosaccharide chains in rat colonic mucin glycoprotein (44) results in a core structure containing GlcNAcβ1,3(NeuAcα2,6)GalNAcO. Only the β-1,3-linked chain is extended in this oligosaccharide. Some of the oligosaccharides present in trachea mucin glycoprotein have been found in other glycoproteins. Oligosaccharide Fma has been found in human bronchial glycoproteins (45). Oligosaccharides Fn1 and Em1 are present in ovarian cyst fluid glycoproteins (46), human secretory IgA immunoglobulin (47), and plasma membranes of an ascites hepatoma, AH 66 (43). The acidic oligosaccharide Em3 has been found in many glycoproteins (30, 43, 47, 48).

Acknowledgment—We thank Dr. K. L. Matta (Roswell Park Memorial Institute, Buffalo, NY) for the generous gifts of synthetic trisaccharides, Galβ1,3(Galβ1,4GalNAc) and Galβ1,3(GalNAcβ1,6)GalNAc.

REFERENCES

Continued on next page.
Mucin Glycoproteins from Trachea and Cowper's Gland

Purification and Properties of Mucin Glycoproteins from Trachea and Cowper's Gland

Mucin Glycoproteins from Trachea and Cowper's Gland

Purification of Mucin Glycoproteins from Trachea and Cowper's Gland

The mucin glycoproteins were purified by chromatography on a DEAE-cellulose column. The column was equilibrated with 0.01 M potassium phosphate, pH 6.0, and a linear gradient of 0.01 M potassium phosphate was applied to the column. The column was washed with 0.01 M potassium phosphate, pH 6.0, and then the mucin glycoproteins were eluted in a single peak (0.01 M potassium phosphate, pH 6.0).

The mucin glycoproteins were further purified by chromatography on a Sepharose-4B column. The column was equilibrated with 0.01 M potassium phosphate, pH 6.0, and then the mucin glycoproteins were eluted with a linear gradient of 0.01 M potassium phosphate, pH 6.0.

The purified mucin glycoproteins were digested with pronase and then subjected to electrophoresis on a sodium dodecyl sulfate (SDS) gel. The SDS-gel electrophoresis was performed under reducing conditions.

Amino acid analysis of the purified mucin glycoproteins was performed on a Beckman amino acid analyzer. The amino acid composition of the purified mucin glycoproteins was compared with that of other mucin glycoproteins from different sources.

The purified mucin glycoproteins were further purified by size-exclusion chromatography on a Sepharose-4B column. The column was equilibrated with 0.01 M potassium phosphate, pH 6.0, and then the mucin glycoproteins were eluted with a linear gradient of 0.01 M potassium phosphate, pH 6.0.

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The purified mucin glycoproteins were digested with pronase and then subjected to electrophoresis on a sodium dodecyl sulfate (SDS) gel. The SDS-gel electrophoresis was performed under reducing conditions.
Mucin Glycoproteins from Trachea and Cowper's Gland

The number of oligosaccharide chains in glycoproteins were determined by two procedures which depend on the quantification of polar substances. The reduced oligosaccharides containing at least 0.15 mol of glucosamine and 0.1 mol of galactosamine were hydrolyzed in 2 N HCl at 100°C for 1 h and then in 4 M HCl for 4 h at 100°C for 2 h. The reaction was stopped with 10 N NaOH and then analyzed by the method described in Table 1. For analysis, the oligosaccharides were treated with 0.2 M NaOH at 100°C for 1 h to remove the sialic acid. The amount of sialic acid was determined by the method of Vigers [27] with slight modifications. The results are expressed as picomoles of sialic acid per milligram of sample.

Table 1

<table>
<thead>
<tr>
<th>Reaction Medium</th>
<th>Amount of Sialic Acid (μmol)</th>
<th>Amount of Glucosamine (μmol)</th>
<th>Amount of Galactosamine (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
<td>1.14</td>
<td>0.76</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>0.75</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>1.69</td>
<td>1.73</td>
</tr>
<tr>
<td>4</td>
<td>1.90</td>
<td>1.07</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Another independent procedure was also used to determine the number of chains in the mucin glycoprotein and reduced oligosaccharides. Mucins were hydrolyzed in 4 M HCl for 4 h at 100°C and then analyzed for sialic acid under the same condition as the tracheal mucin. These were then reanalyzed with 2 M NaOH, pH 11 [26].

Table 2

<table>
<thead>
<tr>
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<th>Amount of Glucosamine (μmol)</th>
<th>Amount of Galactosamine (μmol)</th>
</tr>
</thead>
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<tr>
<td>4</td>
<td>1.90</td>
<td>1.07</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Further, the number of sialic acid groups was determined by analysis of the free sialic acid recovered after digestion with Pronase. The results are expressed as picomoles of sialic acid per milligram of sample.

Table 3

<table>
<thead>
<tr>
<th>Reaction Medium</th>
<th>Amount of Sialic Acid (μmol)</th>
<th>Amount of Glucosamine (μmol)</th>
<th>Amount of Galactosamine (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.8</td>
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<td>4</td>
<td>1.90</td>
<td>1.07</td>
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Materials and Methods

The composition of all oligosaccharides was determined by the method of Vigers [27] with slight modifications. The results are expressed as picomoles of sialic acid per millgram of sample.
Equilibration was obtained after centrifugation at 45,000 x g for 15 h followed by centrifugation for 12 h at 46,000 x g. The density of the osmotic chloride gradient at this time dropped from 1.44 to 1.46 g/ml. Single symmetrical bands were obtained with both samples. The tracheal mucin glycoproteins loaded at a density of 1.51 g/ml and Cooper’s gland mucin glycoproteins showed a peak at 1.53 g/ml. These data are consistent with the high molecular weight of these glycoproteins observed in gel filtration studies.

### Table II

<table>
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<tr>
<th>Amino Acid</th>
<th>Mol %</th>
<th>Sialic Acid or Sulfate Residue</th>
<th>P1</th>
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<th>P3</th>
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<tr>
<td>N-acetylglucosamine</td>
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<tr>
<td>Galactose</td>
<td>19</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td></td>
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<tr>
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<td>20</td>
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<tr>
<td>Sialic Acid</td>
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<tr>
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<tr>
<td>Glucose</td>
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<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
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<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
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</tr>
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### References

1. Mucin Glycoproteins from Trachea and Cooper’s Gland

2. Equilibration and isolation of reduced oligosaccharides - The mucin glycoproteins were treated with alkali borohydride by the method described in "Experimental Procedures" to release O-glycosidically bound oligosaccharides. The rate of release was assessed by determining the formation of galactose after passing aliquots of the reaction mixture through AG1-X12 resin columns. The maximum yield of reduced oligosaccharides after further purification of tracheal glycoproteins was about 15% based on the recovery of carbohydrate assayed by the anthrone procedure. The total percent of neutral, sialic acid and sialic acid oligosaccharide mixture was nearly the same as the intact glycoprotein except for Galactose. About 33.2 mol% of Galactose was recovered as Galactose in the isolated oligosaccharides. This nearly 70% (13.2 / 19) of the Galactose in preparation P1 (Table II) is linked to sialic acid residues in the protein. Similar values, 70% and 60%, were obtained with preparations P2 and P3. The P2 reduced oligosaccharide mixture still contained 5 mol% Galactose. This amino sugar is present as a terminal residue in some of the oligosaccharide chains, since the mucin glycoprotein has blood group A activity.

3. Alkaline borohydride treatment of Cooper’s gland mucin glycoproteins under identical conditions resulted in the release of more than 90% of the oligosaccharide chains based on the recovery of anhydrous positive material, acidic and neutral. Essentially all, 95%, of the Galactose in the native glycoprotein was converted to Galactose after O-deamination of the oligosaccharide chains. Two highly substituted oligosaccharide chains, which comprised over 90% of the carbohydrate in Cooper’s gland mucin glycoproteins were isolated by chromatography on Bio-Gel P-4 columns (12).

4. The reduced oligosaccharides released from tracheal mucin glycoproteins were also separated on Bio-Gel P-4 columns. The dry samples obtained after the removal of borate as described in "Experimental Procedures" were dissolved in 10 ml of 0.1 M potassium acetate, pH 5.5, and 1 M borate were applied to this column (12). The column was eluted with the same solution and four broad fractions, 1 (200-300 ml), 2 (300-400 ml), 3 (400-500 ml) and 4 (500-600 ml), were collected under the colors shown at the top of Fig. 5 (Frame A). These fractions concentrated and each fraction was chromatographed on a second Bio-Gel P-4 column.

5. Six major fractions, A to F, were collected from these runs as shown in Fig. 5 (Frame B). The neutral and amino oligosaccharides in Fractions E (Fig. 5, Frame B) and F (Fig. 5, Frame A) were separated by
Mucin Glycoproteins from Trachea and Cowper's Gland

Fig. 6. Separations of reduced and unmodified mucin glycoproteins present in fractions A and B by chromatography on DEAE-cellulose. About 20 ml, in 1.5 ml, of partially deaggregated reduced glycoprotein from fraction A was applied to the DEAE-cellulose column (3.0 by 25 cm). The column was prewashed with 0.9 M sodium acetate, pH 5.0, and then washed extensively with water. The glycoproteins were eluted with a linear gradient made up of 300 ml of water in the mobile phase and 300 ml of 0.1 M sodium acetate, pH 5.0, in the reservoir. Fractions of 2 ml were collected and aliquots were taken for analysis and were added to the different samples. Peak 1, the glycoproteins in this fraction contained 0.17 ml of water and per 100 ml of buffer. Peak 2, the glycoproteins contained 0.5 ml of water and per 100 ml of buffer. Peak 3, the column was washed with 0.1 M sodium acetate, pH 5.0, and the wash was collected in Peak 3.

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Fig. 1: Isolation of reduced oligosaccharides from fractions E and F by gel filtration of Bio Gel P-6. The oligosaccharides were applied to a Bio Gel P-6 column (1.4 x 60 cm) and eluted with pyridine-acetic acid water pH 5.5. Fraction 5 was concentrated into three fractions, E₁, E₂, E₃. Fraction 5 was also concentrated into three fractions, F₁, F₂, and F₃. These peaks were collected as indicated in the figure. Each fraction was concentrated to 1 ml and rechromatographed on another Bio Gel P-6 column (2.2 x 200 cm).

Fig. 2: Isolation of nonreduced oligosaccharides from fraction E by gel filtration. The oligosaccharides were applied to a Bio Gel P-6 column (2.2 x 200 cm) and they were eluted with pyridine-acetic acid water pH 5.5. Fraction 5 was concentrated to two peaks designated E₅ and E₆.

Fig. 3: Separation of partially purified mucin by preparative column chromatography of pyridine-acetic acid water pH 5.5. The column was equilibrated with pyridine-acetic acid water pH 5.5. The sample was applied to the column and eluted with pyridine-acetic acid water pH 5.5. The eluted fractions were collected and analyzed for anthrone and reducing sugar content.

Fig. 4: Detection of partially purified mucin by preparative column chromatography of pyridine-acetic acid water pH 5.5. The column was equilibrated with pyridine-acetic acid water pH 5.5. The sample was applied to the column and eluted with pyridine-acetic acid water pH 5.5. The eluted fractions were collected and analyzed for anthrone and reducing sugar content.

Glycoprotein DE₅ - Analytical analysis yielded equal amounts of 3,4,6-tri-O-methylglucuronic acid and 4,5,6-tri-O-methylglucuronic acid. Based on this data the structure of glycoprotein DE₅ is shown in Figure 5.

Glycoprotein DE₈ - The rechromatography showed that glycoprotein DE₈ contained nearly equal amounts of fucose, galactose, sialic acid, and N-acetylglucosamine. The rechromatography yielded equal amounts of 3,4,6-tri-O-methylglucuronic acid and 4,5,6-tri-O-methylglucuronic acid. Based on this data the structure of glycoprotein DE₈ is shown in Figure 6.
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Fig. 10. Mass spectra of partially methylated acetolysis products from glycoprotein isolated from Trachea and Cowper's Gland. A Fourier transform mass spectrometer equipped with a computer system was used in these experiments. The relative abundance of mass ions is shown in Table 7. From the 1,4,6-tri-O-acetylgalactosamine (MeOCH\(\text{HCOMe}\)) and 1,4,6-tri-O-acetylglucosamine (MeOCH\(\text{MeOCH}\)) spectra, it can be seen that the glycoproteins isolated from these sources contain the same structure of 1,4,6-tri-O-acetylgalactosamine (MeOCH\(\text{HCOMe}\)) and 1,4,6-tri-O-acetylglucosamine (MeOCH\(\text{MeOCH}\)).

Table 7: Carbohydrate decomposition products of glycoproteins

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>Partial methyl</th>
<th>Galactosamine</th>
<th>Glucosamine</th>
<th>Molar Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein 1</td>
<td>1,4,6-Tri-O-acetylgalactosamine</td>
<td>1,4,6-Tri-O-acetylglucosamine</td>
<td>1.45:1.00</td>
<td>1.45:1.00</td>
</tr>
<tr>
<td>Glycoprotein 2</td>
<td>1,4,6-Tri-O-acetylgalactosamine</td>
<td>1,4,6-Tri-O-acetylglucosamine</td>
<td>1.43:1.00</td>
<td>1.43:1.00</td>
</tr>
<tr>
<td>Glycoprotein 3</td>
<td>1,4,6-Tri-O-acetylgalactosamine</td>
<td>1,4,6-Tri-O-acetylglucosamine</td>
<td>1.42:1.00</td>
<td>1.42:1.00</td>
</tr>
</tbody>
</table>

The peaks marked A and B in the Fourier transform mass spectrometry (FTMS) spectra of the glycoproteins isolated from Trachea and Cowper's Gland were identified as methylated 1,4,6-tri-O-acetylgalactosamine (MeOCH\(\text{HCOMe}\)) and 1,4,6-tri-O-acetylglucosamine (MeOCH\(\text{MeOCH}\)) respectively. The results indicate that the glycoproteins isolated from these sources contain the same structure of 1,4,6-tri-O-acetylgalactosamine (MeOCH\(\text{HCOMe}\)) and 1,4,6-tri-O-acetylglucosamine (MeOCH\(\text{MeOCH}\)).