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PARTIAL CHARACTERIZATION OF THE ACIDIC OLIGOSACCHARIDES FROM RAT SUBLINGUAL GLYCOPROTEIN

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SUMMARY - Five sialic acid-containing oligosaccharides composed of nine, ten, twelve, thirteen and fifteen sugar residues, respectively, have been isolated from rat sublingual glycoprotein. Each oligosaccharide contained sialic acid, N-acetylglucosamine, N-acetylgalactosaminitol and galactose. The partial structures of desialyzed oligosaccharides, as determined by sequential degradation with specific glycosidases, are proposed to be: GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$GalNAc-ol (oligosaccharide I), GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$GalNAc-ol (oligosaccharides II and III) and GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$Gal$\beta\rightarrow$GlcNAc$\beta\rightarrow$GalNAc-ol (oligosaccharides IV and V).

INTRODUCTION - The mucous secretions of the salivary glands of higher animals consist of a high molecular weight glycoproteins called mucins. The physical properties and biological functions of mucins are determined by their high carbohydrate content, which in most species exceeds 65% of the total weight of glycoprotein (1-5). In rat the sialic acid-containing mucus glycoproteins are mainly derived from sublingual glands (1,6,7), while the submaxillary glands contain very little of sialic acid (6). Although the immunological properties, chemical composition and the nature of carbohydrate to protein attachment in rat sublingual glycoprotein have been studied in great detail (1,8), the structure of carbohydrate prostetic groups was never elucidated. In this report we describe the isolation and partial characterization of the sialic acid-containing oligosaccharides from the major glycoprotein of rat sublingual gland.

MATERIALS AND METHODS - Rat sublingual glands (50), dissected from the submaxillary glands, were provided by Dr. J. Moschera of our school. β-Galactosidase and β-N-acetylhexosaminidase were kindly supplied by Drs. Y.T. Li and S.C. Li (Tulane University, La.). The V. cholerae neuraminidase was obtained from Worthington Biochemical Corporation (Freehold, N.J.).

The major rat sublingual glycoprotein, which was not absorbed onto hydroxyapatite, was isolated according to the procedure of Tettamanti and Pigman (9) as modified by Moschera and Pigman (1). The major glycoprotein, isolated by this procedure, was obtained in a yield of 40.2 mg per 2.1 g of wet weight of the tissue.

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Alkaline borohydride degradative hydrolysis of the isolated glycoprotein was performed according to the procedure of Carlson (10). The glycoprotein (30 mg) was incubated in 0.05 M KOH and 1 M $NaBH_4$ for 15 h at 45°C in a final volume of 30 ml. After destruction of the excess of borohydride by tritration to pH 5 with dilute acetic acid, the solution was dialyzed against distilled water, dialysable material was concentrated and boric acid was removed as methyl borate. Separation of neutral oligosaccharides from the acidic was accomplished on a column (1.2 x 10 cm) of Dowex 1-X2 (Cl^-, 50-100 mesh). The neutral oligosaccharides were eluted with water, and the sialic acid-containing oligosaccharides were eluted from the column with 0.5 M NaCl. The latter fraction was desalted by passing through Bio-Gel P-2, lyophilized and acetylated (11). Acetylated oligosaccharides, recovered from the acetylation mixture by evaporation with toluene (11), were dissolved in a small volume of chloroform/methanol (4/1, v/v) and purified to homogeneity by thin-layer chromatography in chloroform/methanol (9/1, v/v), and chloroform/methanol (85/15, v/v). The purified individual oligosaccharides were deacylated with sodium methoxide (11) and desalted on a P-2 column.

Removal of sialic acid from the isolated deacylated oligosaccharides was accomplished with neuraminidase (1). Enzymatic degradation of the desialyzed oligosaccharides was performed by sequential treatment of the oligosaccharides with β-galactosidase and β-hexosaminidase (12). The degraded oligosaccharides were isolated by gel filtration of the boiled digest on a P-2 column (13). The liberated monosaccharides were monitored by paper chromatography and quantitated by gas-liquid chromatography (14).

The protein content of the isolated glycoprotein was measured by the method of Lowry et al (15). The carbohydrate components in glycoprotein and in the isolated oligosaccharides were determined by gas-liquid chromatography following methanolysis and derivatization with silylating reagent (16).

RESULTS - The chemical composition of the major sublingual glycoprotein is given in Table I. The principal carbohydrate components were sialic acid, N-acetyl glucosamine, N-acetylgalactosamine and galactose. Lesser quantities of mannose and fucose were also present. Analyses of the carbohydrate components of this glycoprotein after alkaline borohydride reductive cleavage, prior to dialysis, indicated a complete loss of N-acetylgalactosamine, which was accompanied by a corresponding production (98.7%) of N-acetylgalactosaminitol. No significant destruction of N-acetylglucosamine (1.2%) and other sugars was observed. Sialic acid, N-acetylglucosamine, N-acetylgalactosaminitol, galactose and fucose, but not mannose, were also found in the dialyzable fraction. These results suggest that all of the N-acetylgalactosamine residues in this glycoprotein are involved in O-glycosyl linkage to protein, and that mannose is not a constituent of the alkali-lablile fraction.

The sialic acid-containing oligosaccharides, separated from the neutral components on a Dowex 1-Cl^- column, represented about two-third of the dialyzable

Table I. Chemical composition of the major glycoprotein of rat sublingual gland.

Component	Relative weight (%)
Sialic acid [a]	25.2
GlcNAc	18.5
GalNAc	10.8
Gal	15.2
Man	2.0
Fuc	0.7
Protein	17.8

[a] Sialic acid is expressed as N-acetylneuraminic acid.

fraction and contained sialic acid, N-acetylglucosamine, N-acetylgalactosaminitol and galactose. Fucose was found among the carbohydrate components of the neutral fraction. Following acetylation, the major oligosaccharides of the acidic fraction were purified to homogeneity by thin-layer chromatography. After rigorous purification five individual oligosaccharides (I-V) were isolated. The thin-layer chromatogram of these compounds is given in Fig. 1. The purified oligosaccharides I,II, III,IV and V were obtained in a yield of 0.15, 0.26, 0.37, 0.27 and 0.51 μM, respectively, per 30 mg of glycoprotein. The composition and molar ratios of carbohydrates in the isolated oligosaccharides is given in Table II.

Complete removal of sialic acid residues from the deacylated oligosaccharides was accomplished after 24 h treatment with neuraminidase. The desialyzed oligosaccharides were resistant to the action of β-galactosidase, but each oligosaccharide lost one N-acetylglucosamine residue when treated with β-N-acetylhexosaminidase. One residue of galactose was lost from each compound when the recovered oligosaccharides were treated with β-galactosidase. Stepwise treatment of the

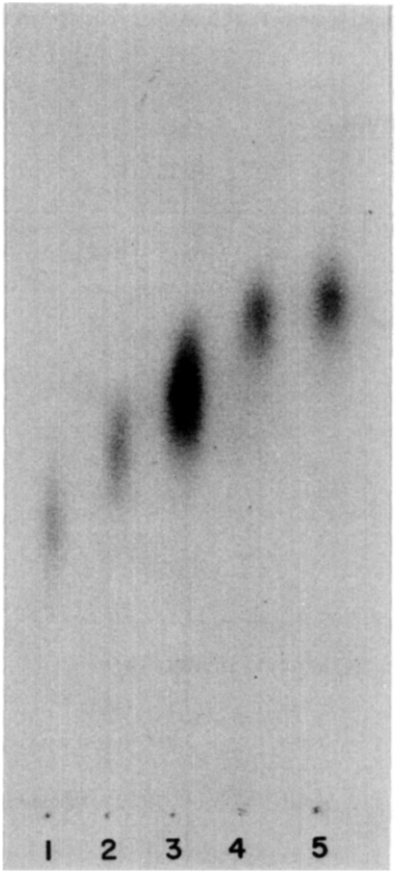

Fig. 1. Thin-layer chromatogram of the acetylated oligosaccharides purified from rat sublingual glycoprotein.

1, Oligosaccharide I; 2, oligosaccharide II; 3, oligosaccharide III; 4, oligosaccharide IV; 5, oligosaccharide V. Conditions: Silica gel HR 250 nm developed in chloroform/methanol (85/15, v/v). Visualization: orcinol reagent.

oligosaccharides with glycosidases, in sequence β-N-acetylhexosaminidase, β-galactosidase, resulted in conversion of all five compounds to a GlcNAc→GalNAc-ol disaccharide. This disaccharide was in turn converted to N-acetylglucosamine and N-acetylgalactosaminitol by β-N-acetylhexosaminidase. The results of enzymatic degradation presented above indicate that the carbohydrate chains of rat sublingual glycoprotein consist of a variable number of βGlcNAc→βGal disaccharide residues linked to the GlcNAc$\overset{\beta}{\rightarrow}$GalNAc disaccharide adjacent to the protein core.

Table II. The composition and molar ratios of carbohydrates in the isolated oligosaccharides.

Oligosaccharide	Molar ratios			
	Gal	GlcNAc	GalNAc[a]	NeuAc
I	3.85	4.93	1.0	4.91
II	2.97	3.86	1.0	4.90
III	3.03	3.90	1.0	3.87
IV	2.05	3.10	1.0	3.92
V	2.01	2.91	1.0	2.84

[a] Determined as N-acetylgalactosaminitol.

DISCUSSION - Studies of Moschera and Pigman (1) on glycoprotein of rat sublingual gland have established that the oligosaccharide chains are linked O-glycosidically to the protein core through N-acetylgalactosamine. This bond is alkali-labile, and in the presence of alkaline borohydride reduced oligosaccharide chains can be obtained (10,17).

Using optimal conditions for the release of intact oligosaccharides (10), we have isolated and purified to homogeneity five sialic acid-containing oligosaccharides (I-V) from the rat sublingual glycoprotein. These oligosaccharides were found to be composed of nine, ten, twelve, thirteen and fifteen sugar residues, respectively, and contained sialic acid, N-acetylglucosamine, N-acetylgalactosaminitol and galactose. The partial structures of these oligosaccharides were elucidated with the aid of specific glycosidases as:

I GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}$GalNAc-ol.

II and III GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}$GalNac-ol.

IV and V GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}Gal\overset{\beta}{\rightarrow}$GlcNAc$\overset{\beta}{\rightarrow}$GalNAc-ol.

Although the oligosaccharides II and III, and IV and V contained identical number of hexose and hexosamine residues, they differ from each other with respect to the number of sialic acid residues (Table II). It is not known, as

yet, which sugar residues in the oligosaccharide chains are substituted with sialic acid. In the salivary mucins of other animals, sialic acid was found linked to galactose, N-acetylgalactosamine and N-acetylglucosamine (10,17,18).

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