THE GLYCOPROTEIN NATURE OF FORSSMAN ANTIGEN IN DOG GASTRIC MUCUS

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1. Introduction

Forssman substance is a heterophile antigen that specifically inhibits the reaction between sheep erythrocytes and anti-sheep erythrocyte antibody derived from different species [1]. This substance has been shown to be a glycosphingolipid [2,3], and like ABH antigens associated with glycolipids occurs in multiple structural forms [4–6]. Three such variants, all bearing terminal GalNAc α(1 → 3)GalNAc structure which determines the immunological specificity of Forssman antigen, occur in the dog gastric mucosa [4,5].

Since blood group antigens of gastric mucosa are of glycosphingolipid nature, while those of mucous secretions are associated with glycoproteins [7], we investigated the presence and the nature of Forssman antigen in the gastric mucus. These results show for the first time that the carbohydrate chains bearing Forssman antigenic determinant are associated with the mucus glycoproteins.

2. Experimental

2.1. *Materials*

Dog stomachs, dissected immediately prior to mucus isolation, were obtained from the Hematology Section of our school. Heterophile Forssman antiserum was from Difco (Detroit MI). IDF cells for microimmunodiffusion and sheep red cells were purchased from Cordis (Miami FL). Forssman glycolipid (globopentaglycosylceramide) used here was prepared as in [4]. Limpet α-*N*-acetylgalactosaminidase [8] was donated by Drs S. C. Li and Y. T. Li (Tulane University LA). Human red cells ABO types, human blood grouping anti-A and anti-B serum, and anti-H lectin (*Ulex europeus* agglutinin I) were from Biological Corp. America (Port Reading NJ). Bio-Gel A–50m (50–100 mesh) was supplied by Bio-Rad (Richmond CA) and non-ionic detergent (Tergitol NP-40) by Sigma (St Louis MO).

2.2. *Isolation of mucus glycoprotein*

The surface mucus lining and preformed intracellular mucus were obtained by instillation of the ligated stomach (antrum and fundus) with 2 M NaCl [9]. The instillates were dialyzed against distilled water, lyophilized and dissolved, at 5 mg/ml solution, in 6 M guanidine hydrochloride. The samples (10 ml portions) were introduced onto a Bio-Gel A-50 column (2.5 × 170 cm) and eluted with 0.5 M NaCl containing 0.1% of Tergitol. The eluted fractions (5 ml) were assayed for glycoprotein as in [10]. Fractions containing mucus glycoprotein were pooled, dialyzed and lyophilized. The dry samples were dissolved in a small volume of 0.5 M NaCl and separated from the residual Tergitol by gel filtration on a Bio-Gel P-100 column (2 × 170 cm) eluted with 0.5 M NaCl, dialyzed and lyophilized. Delipidation of mucus glycoproteins was performed with chloroform–methanol as in [11].

2.3. *Analytical methods*

The carbohydrate components of the isolated glycoproteins and the lipid extracts were determined by gas–liquid chromatography following methanolysis, re-*N*-acetylation, and derivatization with silylating reagent using 3% SE-30 columns [11]. The protein content was measured as in [12] and sulfate as in [13]. Thin-layer chromatography of the lipid extracts was performed on silica gel HL plates developed in chloroform–methanol–water (65/35/8, by vol.) and the glycolipids were visualized with orcinol reagent. Removal of fucose from glycoproteins was accom-

plished by hydrolysis in 0.1 M trichloroacetic acid [14]. Enzymatic digestion of glycoprotein fractions with α-*N*-acetylgalactosaminidase was done as in [8]. Reactions were carried out in 0.05 M sodium citrate buffer, pH 4.0 at 37°C for 24 h. Following heat inactivation of enzyme, the liberated sugar recovered by dialysis was reduced, acetylated and quantified by gas–liquid chromatography [15]. Alkaline degradation of the studied glycoproteins and Forssman globopentaglycosylceramide was performed with 0.5 M NaOH at room temperature for 60 h [7]. The alkali-treated samples were exhaustively dialyzed against distilled water and lyophilized.

Sedimentation velocity and equilibrium studies were performed on a Beckman model E analytical ultracentrifuge at 20°C using double sector 12 mm interference centerpiece with Schliren and Rayleigh optics, and a diaphragm angle of 60° and 90°, respectively. The samples were dialyzed for 48 h against 4 M guanidine hydrochloride containing 0.5 M NaCl. Sedimentation coefficient measurements were performed at 50 740 rev./min using samples at 5 mg/ml. Sedimentation equilibrium studies were carried out at 6995 rev./min over 48 h at 0.4 mg/ml samples. A partial specific volume of 0.633 was used for the calculation of M_r [16].

2.4. *Immunological assays*

Hemagglutination and hemagglutination inhibition assays for ABH bloodgroup and Forssman activity were performed with the Takatsy microtitrator using 0.025 ml loops, and 2% suspension of human and sheep red cells, respectively. The anti-A and anti-B sera were diluted to 4 units/0.025 ml, anti-H to 2 units/0.025 ml and anti-Forssman serum to 8 units/0.025 ml. Forssman activity was also tested by a double diffusion micromethod [17]. The wells were filled with 10 μl of undiluted antiserum and 10 μl 2 mg/ml solutions of the glycoproteins in 0.2 M phosphate buffer (pH 7.0) containing 0.15 M NaCl.

3. Results

Application of the solubilized antral and fundic mucus to Bio-Gel A-50 columns yielded, in each case, 2 peaks. The excluded M_r peak contained mucus glycoprotein, while the included fraction represented ~12% of the glycosubstance originally present in the solubilized mucus and consisted mainly of protein.

Table 1
Chemical composition of dog antral and fundic mucus glycoprotein

Component	Relative weight (%)	
	Antrum	Fundus
Fucose	8.72	5.56
Galactose	12.73	13.54
N-Acetylgalactosamine	17.11	15.09
N-Acetylglucosamine	9.97	10.80
Sialic acid[a]	3.55	5.53
Sulfate	2.20	2.64
Protein	38.10	36.80

[a] Expressed as *N*-acetylneuraminic acid

On sedimentation velocity analysis the mucus glycoprotein derived from antrum and fundus gave respectively single, broadening with time, peaks with corrected values of 6.9 S and 10.3 S. The M_r determinations by sedimentation equilibrium gave apparent value of 3.4×10^5 for antrum glycoprotein and 4.3×10^5 for fundus glycoprotein.

The chemical composition of the antral and fundic mucus glycoproteins is given in table 1. Carbohydrates constituted over 50% of the dry weight of each glycoprotein and consisted mainly of *N*-acetylgalactosamine, galatose, *N*-acetylglucosamine, and fucose. Both glycoproteins exhibited higher content of *N*-acetylgalactosamine than *N*-acetylglucosamine. The ratios of the 2 were found to be 1.7:1 for antral glycoprotein and 1.4:1 for fundic glycoprotein. Neither glycoprotein contained glycosphingolipids, as judged by the results of thin-layer and gas chromatography analyses of their lipid extracts. Treatment of the antral glycoprotein with α-*N*-acetylgalactosaminidase resulted in the loss of 26.4% of *N*-acetylgalactosamine, while similar treatment of the fundic glycoprotein released 23.1% of *N*-acetylgalactosamine. These data indicate that both glycoproteins contain oligosaccharide chains terminated with α-*N*-acetylgalactosamine.

In the double diffusion micromethod, both glycoproteins gave a single precipitation lines with anti-Forrsman antiserum which were fused with that of Forssman globopentaglycosylceramide lipid. In hemagglutination-inhibition assays the native and delipidated glycoproteins completely inhibited agglutination of sheep red cells by anti-Forssman antiserum, and also showed a weak activity in human A

Table 2
Hemagglutination-inhibition assays of the native and modified dog gastric mucus glycoproteins

Sample	Antigenic activity[a]		
	Forssman	A	H
Antral glycoprotein	+	+	+
Fundic glycoprotein	+	+	+
Forssman globopentaglycosylceramide	+	+	–
Delipidated glycoproteins of antrum and fundus	+	+	+
Defucosylated glycoproteins of antrum and fundus	+	+	–
Antral and fundic glycoproteins treated with α-*N*-acetylgalactosaminidase	–	–	+
Alkali-degraded glycoproteins of antrum and fundus	–	–	–
Alkali-degraded Forssman globopentaglycosylceramide	+	+	–

[a] The extent of Forssman activity of the native glycoproteins against 8 units of anti-Forssman antiserum was 6.2 –12.5 μg/0.1 ml and of Forssman globopentaglycosylceramide, 1.8–3.6 μg/0.1 ml. The A activity of glycoproteins against 4 units of anti-A serum was 0.25 mg/0.1 ml and H activity, against 2 units of anti-H lectin, 0.5 mg/0.1 ml

(+) signifies inhibition of agglutination; (–) indicates agglutination

anti-A and H anti-H system (table 2). Removal of fucose from glycoproteins resulted in the loss of H activity, but had no effect on their activity in human A anti-A system. Treatment of glycoproteins with α-*N*-acetylgalactosaminidase resulted in the loss of both the Forssman and A antigenic activities. The Forssman and AH activities were also completely destroyed by the treatment of glycoproteins with alkali. The alkaline degradation, however, had no effect on Forssman globopentaglycosylceramide lipid. The above results suggest that dog gastric mucus glycoproteins contain *O*-glycosidically linked carbohydrate chains which bear the Forssman antigenic determinant.

4. Discussion

Our previous studies on ABH antigens of gastric mucosa and gastric secretion established that in the mucosa these antigens are associated with glycosphingolipids, while those present in gastric secretion are glycoproteins [7,18]. These data, together with [4,5,14], clearly indicate that Forssman antigen in dog stomach is associated with both glycoproteins and glycosphingolipids. The glycosphingolipids bearing Forssman antigen are the integral part of the mucosa cell membranes, whereas glycoprotein antigen belongs to the secretion. Although the structural aspects of glycoprotein-associated Forssman antigen remains to be elucidated the results of enzymatic and immunological assays suggest that these glycoproteins, like Forssman glycosphingolipids, contain GalNAcα(1 → 3)GalNAc structure which determines the immunological specificity of Forssman antigen. This structure apparently resides on the non-reducing termini of oligosaccharide chains linked *O*-glycosidically to the protein core.

Since the observed A-activity of the glycoproteins was not affected by the removal of fucose, it is apparent that this activity derived from Forssman determinant and not from A antigen. The H-activity of the glycoproteins indicates that some of the oligosaccharide chains of dog gastric mucus glycoproteins also carry α-L-fucosyl-(1 → 2)β-galactosyl residues. Whether these residues are the part of branched oligosaccharide chains which also bear Forssman determinants remains to be determined.

Acknowledgements

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