Isolation, Purification and Partial Characterization of a Glycoprotein from Plasma Secretion

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Summary: The antigenic polymers present in seminal plasma play a key role in the processes of reproduction, survival of cells and provide genetic markers. A high molecular-weight glycoprotein component has been isolated from seminal plasma, purified and its partial structure has been investigated by lectin affinity and haemagglutination inhibition. A possible function of this glycoprotein has been discussed.

Introduction

The antigenic macromolecules present in seminal plasma as well as on the sperm surface critically influence the morbidity and mortality of the sperm and the process of reproduction. A number of macromolecules, most of which originate from seminal plasma, adhere to the surface of spermatozoa. Human seminal plasma contains various proteins, amino acids, lipids and ions in high concentration [1]. In addition, secretion from the accessory glands which have different effects on sperm motility have also been detected in seminal plasma [1]. The initial portion of the secretion contains prostatic and Cowper's gland fluid whereas additional component arise from seminal vesicles. Human seminal plasma also contains lgG, lgA and fragments of β-globulin in addition to different antigenic glycoproteins. A water-soluble glycoprotein has been isolated from seminal plasma and was shown by immunodiffusion studies to be secretory product of seminal vesicles. It contained sialic acid as well as galactose residues as nonreducing terminal sugars [2].

Although glycoproteins, which are related to autoimmune interference in male fertility [3] and are of diagnostic value, are known to occur in seminal plasma [4], the relationship between the composition and structure of glycoprotein and function of seminal plasma has not been studied. The present study pertaining to the isolation, purification and partial characterization of seminal plasma glycoprotein was undertaken in an attempt to comprehend the role of macromolecules in the secretion.

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Materials and Methods

Secretion was collected from sexually mature and normal type-A secretor donor, and was normal in terms of sperm count and morphology. The semen (2 ml) was diluted with phosphate buffer-saline (PBS) (Dulbecco, 5 ml). The suspension was mildly centrifuged (750 g) for 30 minutes at 4°C in order to avoid injury to sperm. The sperm was washed four times with PBS to remove adhering materials. Finally the supernatants were combined and centrifuged at 2500g for 20 minutes at 4°C. The supernatant was dialysed and lyophilized to give the seminal plasma macromolecules.

The lyophilized material (7 mg) in PBS was treated with galactose oxidase (250 units, Worthington) at 37°C for 8 hours. The mixture was treated with sodium borotritide (0.5 ml solution in 0.005-M sodium hydroxide containing 10 mCi) for 2 hours followed by treatment with sodium borohydride (25 mg) at 4°C for 8 hours. The mixture was treated with 4 ml acetic acid to pH 5.5 and then dialysed against distilled water at 4°C. The retentate was lyophilized to give the tritium-labeled seminal plasma glycoprotein.

The lyophilized seminal plasma glycoprotein was applied to a column (0.8 x 32 cm) of Bio-Gel P-200 in 0.05-M pyridine-acetic acid (0.5 ml) pH 5.4. Fractions containing carbohydrates and tritium were combined and lyophilized. The carbohydrate and amino acid composition of this glycoprotein is given in Table 1. The glycoprotein was further purified by gel filtration on a column of Sepharose 4B under conditions similar to those described for the P-200 column. Fractions containing tritium, amino acids and carbohydrates were combined (Table 1 and Fig.1) to give a glycoprotein component. The glycoprotein was examined for homogeneity in agarose

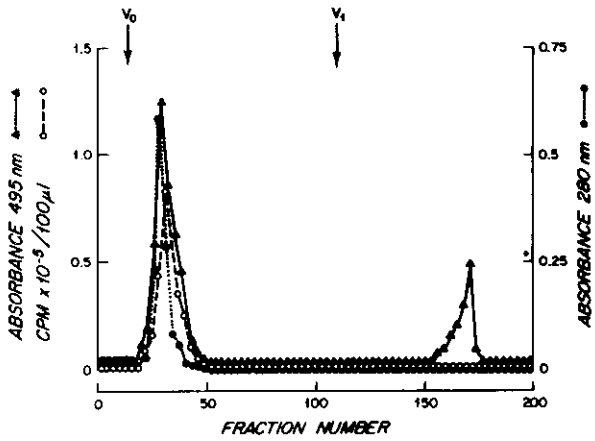

Fig.1: Fraction of Bio-Gel p-200 purified glucoprotein from human seminal plasma on a column of Sepharose 4B. Fractions of 2 ml were collected, and every third fraction was examined for the presence of tritium, hexose [18] and for amino acids (absorption at 280 nm). The column was calibrated with Blue Dextran (VO, M_r 2000000) and apoferritin (VI, M_r 480000).

[5] and SDS-polyacrylamide gel electrophoresis (PAGE) [6]. Ricinus communis haemagglutinin (120, Boehringer), Triticum vulgaris and Concanavalin A (Sigma) were used as such. The inhibition assays were performed according to the procedure of Matsumoto and Osawa [7] using type-O erythrocytes. The glycoprotein was treated with neuraminidase [5] and the sialic acid free glycoprotein was examined for inhibition of haemagglutination.

The affinity assays in double diffusion were performed in microscope slides in 1% agarose gel in Veronal buffer (pH 8.2). The outer wells were filled with 10 µl solution of lectin (500 µg per ml) for Ricinus communis 120 and 300 µg per ml for Triticum vulgaris, Concanavalin A and Helix pomatia, from Sigma) and the center well was filled with 10 µl of a solution of seminal plasma glycoprotein(100 µg per 100 µl). The slides were incubated in a humid chamber at 4°C.

Table-1: Carbohydrate and amino acid composition of seminal plasma glycoproteins

	Biol-Gel P-200 purified		Sepharose 4B purified	
	(%)	Molar ratio[a]	(%)	Molar ratio[a]
Carbohydrates[b]				
L-Fucose	1.4	0.61	2.1	0.84
D-Galactose	4.6	1.84	6.1	2.21
D-Mannose	tr		tr	
N-Acetylglucosamine	3.1	1.00	3.4	1.00
N-Acetylgalactosamine	1.3	0.42	2.2	0.65
N-Acetylneuraminic acid	2.6	0.72	3.3	0.72
Amino acids[b,c]				
Alanine	39		60	
Valine	47		48	
Glycine	33		54	
Isoleucine	32		58	
Leucine	70		79	
Proline	119		86	
Threonine	182		210	
Serine	118		104	
Phenylalanine	51		51	
Aspartic acid	76		70	
Glutamic acid	142		110	
Lysine	91		90	

[a] Molar ratio related to N-acetylglucosamine.

[b] Determined by gas-liquid chromatography; carbohydrates according to the procedure of Reinhold[17].

[c] Residues per 1000 residues.

tr: Trace

Results

Mild treatment of plasma by repeated washing and centrifugations removed the maximum quantity of macromolecules as well as sperm coating antigens without injury to sperm. Galactose oxidase treatment followed by reduction of the resulting aldehyde residues with sodium borotritide resulted in a high yield of tritium incorporation into glycoprotein. The tritium-labelled component purified on Bio-Gel P-200 and fractionated on Sepharose 4B (Fig.1) gave a glycoprotein with a high amount of tritium incorporated into the galactose or N-acetylgalactosamine residues. The purified glycoprotein (Table 1)

showed a marked increase in the relative amount of sugar residues, in particular those of galactose, N-acetylglucosamine and N-acetylneuraminic acid. The high molecular-weight glycoprotein barely entered the gel and was homogeneous in agarose electrophoresis. In SDS-polyacrylamide (5%) electrophoresis the glycoprotein did not enter the gel.

Inhibition of haemagglutination of type-O erythrocytes with Ricinus communis, by the glycoprotein was observed, suggesting the presence of the terminal D-galactose residue linked, β(1→4),to N-acetylglucosamine. Treatment of the glycoprotein with neuraminidase resulted in a significant increase in the inhibition of haemagglutination of type-O erythrocytes with Ricinus communis, suggesting the linkage Neu Gal. The absence of inhibition against wheat germ agglutinin despite the significant amount of N-acetylglucosamine residue suggests that this sugar resides in the glycoprotein, either in a configuration or sequence which would be expected to be inactive. Weak inhibitory activity against Concanavalin A is in agreement with the compositional analysis of the glycoprotein. In double diffusion Ricinus communis showed a strong reaction whereas Concanavalin A, wheat germ and Helix pomatia agglutinins had a very weak precipitin reaction, confirming the results of haemagglutination inhibition.

Discussion

The present studies clearly demonstrated the presence of a high molecular weight glycoprotein in seminal plasma. The glycoprotein was homogeneous in agarose electrophoresis but did not enter the gel in SDS-PAGE, a feature common to high molecular weight secretory glycoproteins [8]. Due to limited amount of glycoprotein and the restricted procedures available to asses the purity of macromolecule, it was only possible to suggest that the glycoprotein was homogeneous, albeit polydisperse. The microheterogeneity of the carbohydrate chains, a common feature of secretory glycoproteins, may account for polydispersity of the macromolecules. Compositional analysis of the purified glycoprotein showed the presence of N-acetylglucosamine and N-acetylgalactosamine in a molar ratio appropriate for a secretory glycoprotein. Furthermore, the ratio of serine to threonine in the glycoprotein also suggested a secretory-type and homogeneous glycoprotein. Lectins were utilized to obtain structural information mainly becuase of minute quantity of the glycoprotein available. In addition some lectins may preferentially bind to mucin-type or serum-type carbohydrate chains of glycoproteins, and therefore may provide information as to the basic mode of structure. Wheat germ agglutinin has been reported to react with both types of glycoproteins [9]. The reaction of wheat germ agglutinin with the carbohydrate moiety of glycoprotein are complex. It may react with the inner sequence N-acetylglucosamine β(1→4) N-acetylglucosamine of N-linked glycoproteins if this sequence is accessible to the lectin [10,11]. It reacts also with N-acetylneuraminic acid and to a lesser extent with N-acetylgalactosamine [12,13]. However, it reacts intensely with terminal, non-reducing N-acetylglucosamine residues [12,13]. Helix pomatia lectin reacts with blood group A substances or molecules containing an -linked N-acetylgalactosamine residue [11,14]. Since the plasma glycoprotein was obtained from secretor type-A donors reaction with Helix pomatia was expected. The inability of the glycoprotein to exhibit a positive reaction could be attributed to the absence of the required sequence or anomery in the linkage of N-acetylgalactosamine. Ricinus communis 120 has shown to have high

(i) α-Neu-(2→?)-Gal-1→

(ii) β-Gal-(1→4)-NAcGlc-1→

(iii) β-Gal-1→

Fig.2 Partial structure of oligosaccharide chains in the glycoprotein.

affinity for -galactose, and to react with secretory and cell surface glycoproteins [11,15].

The reaction of the seminal plasma glycoprotein with lectins suggests the presence of structural features shown in Fig. 2 as well as N-acetylglucosamine linked in the inner core and N-acetylgalactosamine either -linked in the inner core or -linked in non-reducing terminal position in the glycoprotein. The partial structural information obtained clearly suggests that it is a secretory-type glycoprotein and might be similar to the uncharacterized seminal plasma glycoproteins reported by Li et al. [3] and Uhlenbruck et al.[2].

In addition to its usefulness in the regulation of fertilization a completely characterized glycoprotein may provide a genetic marker in human seminal plasma [16].

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