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Heparin- and Zn^{2+}-binding proteins from boar seminal plasma

Dariusz Hołody✉ and Jerzy Strzeżek

Department of Animal Biochemistry, Olsztyn University of Agriculture and Technology, Oczapowski 5, 10-718 Olsztyn-Kortowo, Poland

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Low molecular mass, heparin-binding proteins from seminal plasma play an important role in gametes interaction whereas plasmatic Zn^{2+}-binding proteins stabilize chromatin and plasmalemma structures and protect spermatozoa in the female reproductive tract. By means of affinity chromatography the heparin- and Zn^{2+}-binding proteins were isolated from boar seminal plasma and both preparations were analyzed by reverse HPLC. Most of the proteins bound to heparine and Zn^{2+}-ions were classified as spermadhesins. Three fractions binding exclusively Zn^{2+} were isolated. They differ in amino-acid composition, content of glucosamine and content of protein components revealed by SDS/PAGE.

Seminal plasma from many mammalian species contains heparin-binding proteins which bind to the surface of spermatozoa during ejaculation. These proteins mediate sperm capacitation, modulated by glycosaminoglycans (GAG) which are present in the female reproductive tract during the follicular phase of estrous cycle. *In vitro* binding of heparin by specific receptors located on the surface of sperm head, participate in the acrosome reaction during the sperm-zona pellucida interaction (Calvete *et al.*, 1995; Töpfer-Petersen *et al.*, 1995). Most of boar seminal plasma proteins known as spermadhesins have affinity for heparin (Calvete *et al.*, 1994). On the other hand, plasmatic Zn^{2+} protein complexes stabilize chromatin and plasmalemma structures and also protect spermatozoa in the female reproductive tract (Silvestroni *et al.*, 1989; Björndahl & Kvist, 1990)

The compiled chromatographic purification procedure of Zn^{2+}-binding protein in our laboratory enabled isolation and characterization of 54 kDa glycoprotein (Gp 54) complex from boar seminal plasma (Hołody *et al.*, 1994).

✉Corresponding author: tel.: (48 89) 523 3509; fax.: (48 98) 524 0138; e-mail: dholody@art.olsztyn.pl

This isolated glycoprotein complex is electrophoretically heterogeneous (15 kDa, 16 kDa and 18 kDa) and shows strong precipitating (adhesion), hemagglutinating, immunosuppressive as well as a proteinase inhibiting properties (Strzeżek & Hołody, 1996).

The aim of this study was to identify boar seminal plasma proteins which possess affinity both for heparin and Zn^{2+}-ions, and to characterize protein fractions present exclusively in the zinc-ligand group.

MATERIALS AND METHODS

Heparin-binding proteins were isolated from boar seminal plasma on Heparin-Sepharose CL-6B according to Sanz *et al.* (1993). Zn^{2+}-binding proteins were separated from boar seminal plasma on Chelating Sepharose FF (column XK 50/30, Pharmacia LKB) loaded with Zn^{2+} ions, using the Bio-Pilot system (Pharmacia LKB). The column was equilibrated and the sample (100 ml) in 0.05 M Tris/acetate buffer (pH 3.0) containing 0.5 M NaCl was applied in and eluted with the same buffer. The separated fractions were concentrated in dialysis tubings using polyethylene glycol, dialyzed against water (Milli Q grade) and lyophilized.

The heparin- and Zn^{2+}-binding proteins were analyzed using reverse-phase HPLC (RP-HPLC). The lyophilized samples (100 μg) of heparin- or Zn^{2+}-binding proteins were solubilized in 25% acetonitrile/water (v/v) solution and then applied on a Lichrospher RP100 C18 column (Merck). The protein fractions were eluted at 1 ml/min with a mixture of 0.1% (v/v) trifluoroacetic acid (TFA) either in water or acetonitrile using the following gradients: 0–5 min (equilibration) – isocratically 25%; 5–10 min – 25–33%; 10–78 min – 33–50%; 78–83 min – 50–70%; 83–85 min – isocratically 70%; 85–88 min – 70–25%; 88–105 min (reequilibration) – isocratically 25%. Efflux from the column was registered at 220 nm. Fractions from each peak were collected and dried using a Speed-Vac (Savant).

The obtained reverse HPLC elution profiles of heparin- and Zn^{2+}-binding fractions were compared, and protein peaks which were present only in Zn^{2+}-binding fractions were subjected for further analysis: i) amino-acid composition including amino sugars was determined using an Alpha Plus amino acid analyzer (Pharmacia LKB) after sample hydrolysis in 6 M HCl for 24 h at 110°C in evacuated and sealed ampoules, and ii) electrophoretic mobilities using SDS/PAGE (Laemmli, 1970) at 15% polyacrylamide concentration. Proteins bands on the gel were stained using silver method. Molecular masses were estimated using the following standard proteins: phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

RESULTS AND DISCUSSION

The reverse HPLC chromatographic profiles of the heparin and Zn^{2+}-binding proteins revealed the presence of DQH (pB1), AQN-1, AQN-2, AQN-3, AWN-1 and AWN-2 either among the heparin-binding proteins (Fig. 1a) or Zn^{2+}-binding proteins (Fig. 1b), belonging to the spermadhesin family (Hadjisavas *et al.*, 1994; Calvete *et al.*,1994; Strzeżek *et al.*, unpublished). However the content of DQH (pB1) and AQN-2 was higher and that of AWN-2 much lower in the heparin-binding proteins than in the Zn^{2+}-binding proteins. Interestingly, three additional protein fractions marked A, B and C were detected exclusively in the Zn^{2+}-binding protein fraction.

Fraction A (Fig. 1b) is highly hydrophilic and shows high content of glycine, glutamate, aspartate, serine and threonine (Table 1). A high content of aspartate, hydroxy amino acids as well as the presence of glucosamine (4.76 mol/100 mol amino acids) suggest

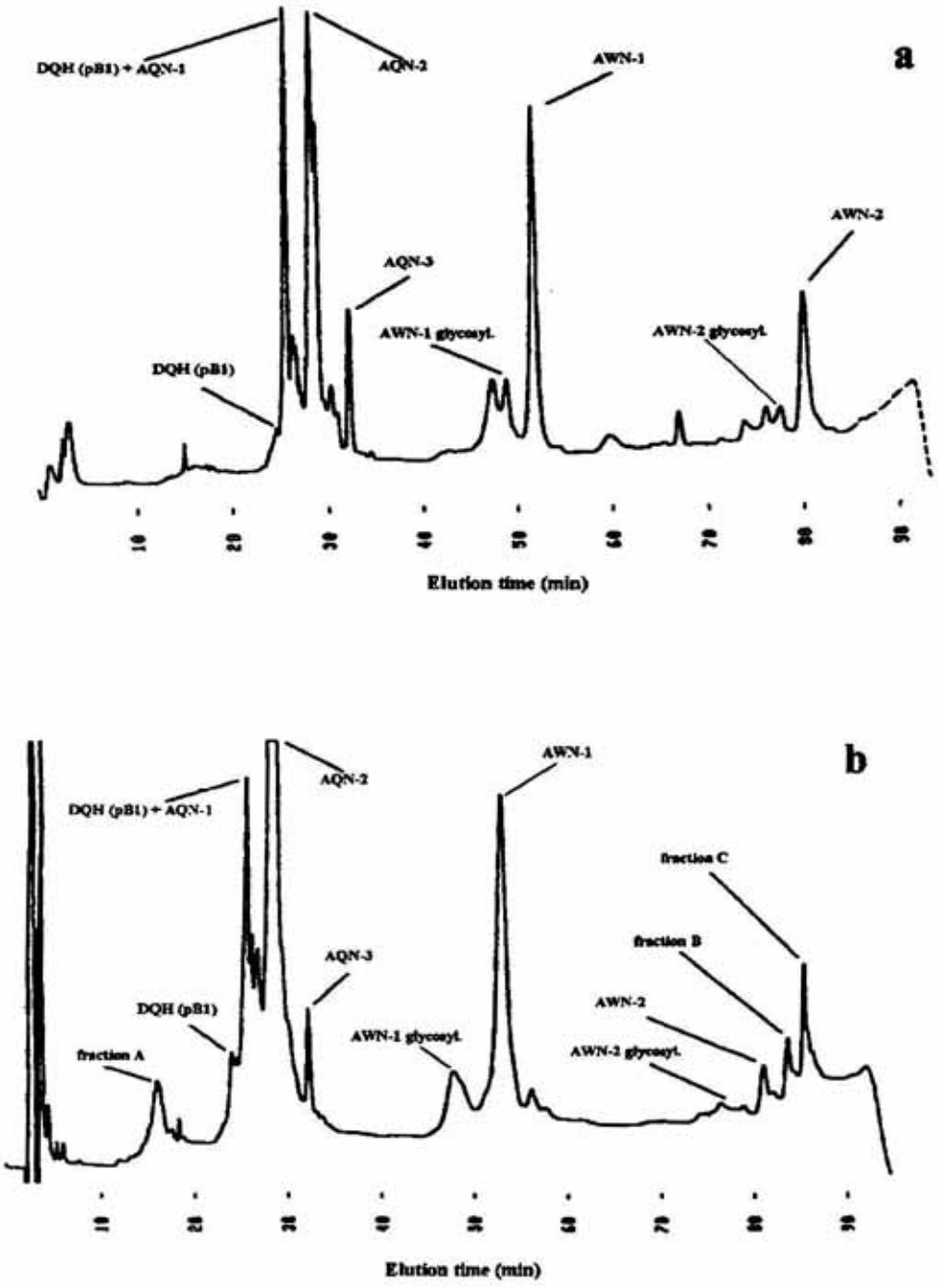

Figure 1. RP-HPLC elution profiles of (a) boar seminal plasma heparin-binding proteins separated on Sepharose CL-6B, and (b) Zn^{2+}-binding proteins on Sepharose FF. DQH (pB1), AQN, AWN, proteins from the spermadhesin family were used for identification purpose.

glycosylation probably of asparagine residue of the aforementioned protein.

The two other fractions B and C eluted during RP-HPLC at high concentration of acetonitrile show strong hydrophobic properties. SDS/PAGE analysis of the isolated A, B and C fractions showed heterogenity of all these fractions and different intensity of protein electrophoretic bands (Fig. 2). The presence of higher molecular mass proteins (60–70 kDa) may suggest their strong aggregation and formation of oligomeric structures, the properties characteristic for spermadhesins and components of 54 kDa

Table 1. Amino-acid and amino sugars composition of the Zn^{2+}-binding protein fractions. The composition was determined as described in Materials and Methods

Amino acid and amino sugars	mol/100 mol amino acids and amino sugars		
	Fraction A	Fraction B	Fraction C
Aspartic acid+asparagine	10.94	12.24	7.65
Threonine	8.27	3.73	2.46
Serine	7.57	15.22	6.83
Glutamic acid+glutamine	12.97	14.48	37.16
Proline	0.13	6.72	12.30
Glycine	14.11	23.28	24.04
Alanine	3.76	0.90	0.55
Cysteine	1.51	1.34	0.27
Valine	3.64	5.22	0.55
Methionine	0.79	0.30	0.5
Isoleucine	5.97	1.19	2.19
Leucine	5.17	1.34	1.91
Tyrosine	4.17	4.93	0.82
Phenylalanine	2.64	0.45	0.82
Histidine	2.89	1.79	1.09
Lysine	5.49	5.37	0.82
Arginine	5.23	1.34	0.00
Tryptophan	n.a.*	n.a.*	n.a.*
Glucosamine	4.76	0.15	0.00

*n.a. – not analyzed

glycoprotein complex (Calvete *et al.*, 1997; Strzeżek *et al.*, unpublished).

Three protein fractions (A, B, C in Fig. 1) among Zn^{2+}-binding proteins of boar seminal

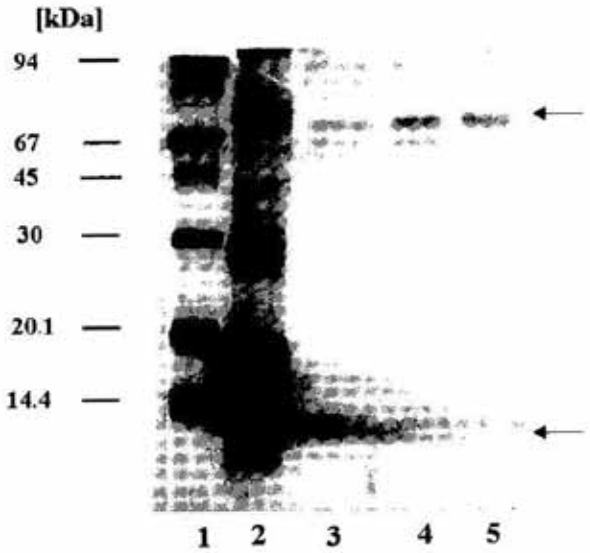

Figure 2. SDS/PAGE of Zn^{2+}-binding proteins (lane 2) and protein fractions: A (lane 3), B (lane 4) and C (lane 5) obtained after RP-HPLC.

The arrows on the right side indicates the protein bands of the analyzed fractions. Lane 1 contains molecular mass standards.

plasma, lacking affinity for heparin require further investigation.

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