Table 1. Molecular mass and composition of protein fractions obtained by gel chromatography of boar seminal plasma

Protein fraction	Molecular mass (kDa) (gel chromatography)	Main protein components	Protein yield (%)
I	>100	DQH, AQN 1, AQN 2, AQN 3, AWN 1, PSP IIa, PSP IIb, AWN 2	24
II	55	DQH, AQN 1, AQN 2, AQN 3, AWN 1, AWN 2	10
III	45	DQH, AQN 2, AQN 3, AWN 1	10
IV	30	PSP I, PSP Ia, PSP IIa, PSP IIb	51
V	5–15	AQN 1, SPI, X	5

DQH – DQH sperm surface protein ($\overset{1}{D}QHL\overset{5}{P}$...)
AQN 1, 2, 3 – AQN spermadhesins ($\overset{1}{A}QNK\overset{5}{G}$...)
AWN 1 – AWN spermadhesins ($\overset{1}{A}WNR\overset{5}{R}$...)
AWN 2 – N-terminus blocked
PSP I, Ia – porcine seminal plasma protein ($\overset{1}{L}DYH\overset{5}{A}$...)
PSP IIa, IIb – porcine seminal plasma protein ($\overset{1}{A}RIN\overset{5}{G}$...)
SPI – seminal proteinase inhibitor
X – not fully identified protein

Sperm-oocyte binding inhibition assay

About 30 oocytes freshly isolated from ovaries were suspended either in 100 µl PBS, pH 7.2, or in 100 µl of a 0.6 mg/ml solution of AQN 1 spermadhesin in PBS, and incubated for 1 h at 37°C in a 5% CO_2 atmosphere. The protein-incubated oocytes were then washed by transferring to 100 µl of PBS, pH 7.2. Protein-incubated and PBS-incubated oocytes were then mixed with 100 µl of the suspension of capacitated spermatozoa and incubated for 1 h at 37°C in a 5% CO_2 atmosphere. After washing the oocytes with PBS, an aliquot of each suspension was mounted on a slide and examined using a phase-contrast microscope.

Results

Protein aggregates in boar seminal plasma – isolation and characterization

Five protein fractions (with M_r >100, 55, 45, 30 and 5–15 kDa) were obtained by gel filtration chromatography of whole boar seminal plasma on Sephadex G-75 SF at pH 7.4 (Fig. 1). The percentage yield of obtained fractions is given in Table 1. Mild SDS-electrophoresis (non-reducing conditions, no sample boiling) of fractions I–IV resulted in partial dissociation of the high-molecular complex into low-molecular-mass monomers (Fig. 1). RP HPLC of protein fractions I–V (Fig. 2) and N-terminal sequencing of individual components (Table 1) revealed that the high-molecular-weight aggregates (fractions I–III, Fig. 1) consisted mainly of the DQH sperm surface protein (peak 2), AQN spermadhesins (peaks 3, 4, 5) and AWN spermadhesins (peaks 6, 7, 9) (Fig. 2). PSP II spermadhesin was present only in fraction I together with AWN 1 (Fig. 2 – peaks 6, 7). Fraction IV (Fig. 1) had about 51% of the total protein content and consisted of heterodimers of PSP I/PSP II spermadhesins only (Table 1, Fig. 2). A small amount of proteins (about 5%) was obtained in fraction V containing the AQN 1 protein, inhibitors and not fully identified protein X (M_r 14 kDa determined by SDS electrophoresis; protein with blocked N-terminal amino acid) (Fig. 1, Table 1). Under physiological conditions, aggregated forms of sperm surface proteins (AQN, AWN, PSP, DQH) strongly predominated over their monomeric forms in boar seminal plasma.

Binding properties of protein aggregates and of their separated components

Interactions of the protein fractions I–V as well as of their HPLC-separated components with phosphorylcholine, epididymal and ejaculated spermatozoa, acidic polysaccharides, cholesterol and zona pellucida glycoproteins were studied using ELBA with biotinylated derivatives of the examined ligands and proteins. The results are summarized in Tables 2 and 3.

Aggregated forms (fractions I–III) and their separated proteins bound to phosphorylcholine (Table 2). This might point to the interaction of these proteins with phospholipids on the plasma membrane of spermatozoa.

For the study of the binding properties of ejaculated and epididymal boar spermatozoa with biotinylated separated proteins and fractions I–V, the ELBA test using direct binding of sperm to microtiter wells without application of glutaraldehyde was used (Table 2). Biotinylated fractions I–IV containing AWN, AQN, DQH, PSP proteins were bound to boar epididymal and ejaculated spermatozoa with the same efficiency. The ability of protein aggregates to bind sperm was observed not only in those containing AWN, AQN and DQH proteins (fractions I–III), but also in the fraction IV containing the PSP I/PSP II heterodimer. Binding of protein fractions II–V to ejaculated sperm was confirmed by direct binding studies with biotinylated protein and FITC-avidin. Figure 3 shows the binding of fraction IV to ejaculated boar spermatozoa. The fluorescence was observed on the en-

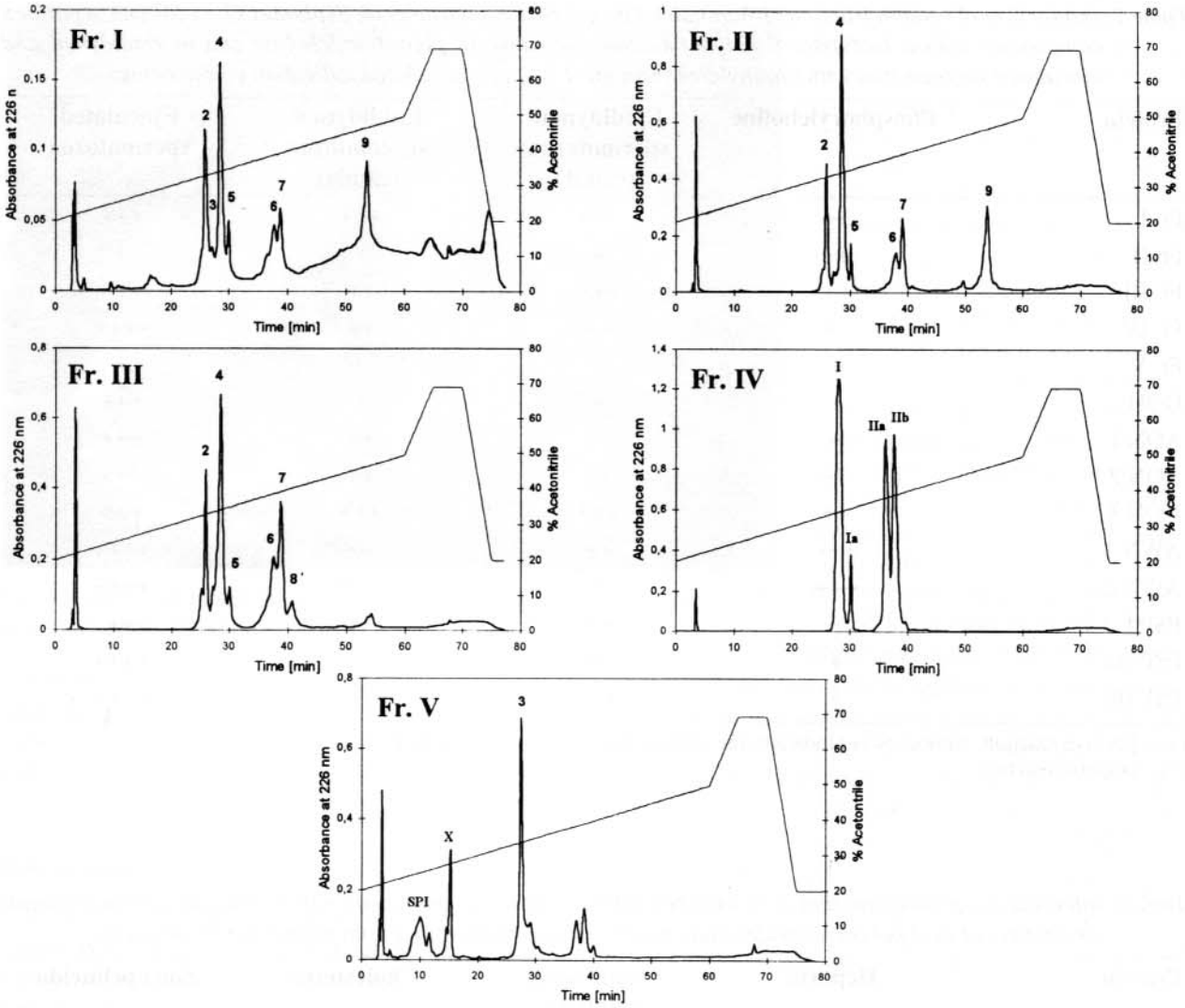

Fig. 2. RP HPLC of protein fractions I–V obtained by gel chromatography. Fraction I (Fr. I), fraction II (Fr. II), fraction III (Fr. III), fraction IV (Fr. IV), fraction V (Fr. V). Peak 2 - DQH sperm surface protein, peaks 3, 4, 5 - AQN 1, AQN 2, AQN 3 spermadhesins, peaks 6, 7 and 9 - AWN 1 and AWN 2 spermadhesins (Fr. I: peaks 6, 7 - AWN 1 and PSP II spermadhesins), peaks I, Ia, IIa, IIb - PSP I, Ia, IIa, IIb spermadhesins, peak SPI - seminal proteinase inhibitor, peak X - not identified protein.

tire head of sperm, on the middle piece of the flagellum and in some cases on the sperm as a whole.

Aggregates containing AWN spermadhesins and DQH protein (fractions I–III) interacted with acidic polysaccharides tested. As to the HPLC-separated components of these aggregates, the strongest interaction was observed with DQH, AWN 1 and PSP II proteins. In boar sperm capacitation, these proteins might act as counterparts to polysaccharide moieties of the proteoglycans secreted by epithelium of the female reproductive tract. Proteoglycans with heparin- and chondroitin sulphate-like glycosaminoglycan side chains stimulate capacitation in bull sperm (Chandonnet et al., 1990; Lane et al., 1999). In agreement with recently published data (Calvete et al., 1995), the fraction IV corresponding to the heterodimer PSP I/PSP II did not show any binding activity to acidic polysaccharides.

AWN 1 interacted strongly not only with acidic polysaccharides, but also with zona pellucida. Its aggregated forms (I–III) interacted more weakly with glycoproteins of zona pellucida (Table 3). The ability of the aggregates of DQH, AQN and AWN proteins (fractions I–III) to interact with cholesterol (Table 3) might be significant at capacitation of boar sperm. The aggregates may become acceptors of cholesterol molecules released from the sperm membrane during this process (cholesterol efflux). Recently, the presence of hydrophobic cavities in the aggregates from bovine seminal plasma (BSP) proteins (60–150 kDa), in which cholesterol could be trapped, have been described (Thérien et al., 1998).