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Characterization of human seminal plasma proteins homologous to boar AQN spermadhesins

Marek Kraus[a,*], Marie Tichá[b], Blanka Železná[a], Jana Pěknicová[a], Věra Jonáková[a]

[a] *Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 37 Prague 6, Czech Republic*
[b] *Department of Biochemistry, Charles University, Prague, Czech Republic*

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Abstract

Spermadhesins, proteins secreted by the boar sexual accessory glands, are believed to play an important role in sperm capacitation and primary contact of sperm and egg. We have previously found human seminal plasma proteins immunobiochemically related to boar AQN and AWN spermadhesins. In this study, we characterized further the AQN spermadhesin-related proteins, here designated as hSA (human spermadhesin-like) proteins. On Western blot, we immunodetected 14, 16 and 18 kDa forms of hSA proteins (hSA-14, hSA-16 and hSA-18, respectively) cross-reacting with rabbit antibody against AQN spermadhesins. Each relative molecular-mass form of hSA comprised three isoelectric isoforms (6.0, 6.8 and 8.4) as shown by 2D-PAGE. Glycoprotein analysis revealed that all hSA-16 and hSA-18 isoforms were *N*-glycosylated, and those of hSA-14 were non-glycosylated. Two isoforms of hSA-14 (pI 6.0 and 8.4) had affinity to heparin. Size-exclusion chromatography of human seminal plasma indicated that hSA proteins formed high molecular-mass complexes either with other hSA proteins or with seminal plasma lactoferrin and/or its fragments. Similarity of biochemical properties (relative molecular masses, isoelectric points and existence of non- and *N*-glycosylated forms) of hSA proteins and those of boar AQN spermadhesins, together with a previously described N-terminal amino acid sequence of one hSA protein identical to AQN spermadhesins, imply that hSA proteins are structurally related to boar AQN spermadhesins. However, localization of hSA proteins on the

* Corresponding author. Tel.: +420 220 183 530; fax: +420 233 331 274.
E-mail address: kraus@img.cas.cz (M. Kraus).

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sperm tail and neck suggests that their biological role differs from that of boar AQN spermadhesins located on the sperm head.

Keywords: Human seminal plasma; AQN spermadhesins; Lactoferrin; Heparin-binding proteins; Two-dimensional polyacrylamide gel electrophoresis

1. Introduction

Mammalian seminal plasma is a complex mixture of secretions originated from epididymis and the male accessory sex glands. It contains a variety of both inorganic and organic components, among which proteins are a major part of the high molecular-mass substances. Upon ejaculation, a portion of the seminal plasma proteins binds to the sperm cell membrane. The ability of some of the sperm-bound proteins to interact with saccharide moieties of glycoproteins and with acid polysaccharides of the heparin and hyaluronan type suggests that they play a role in formation of the oviductal sperm reservoir, sperm capacitation and sperm–ovum binding (reviewed in Visconti et al., 1998; Jansen et al., 2001; Suarez, 2001).

Boar spermadhesins are a group of 12–16 kDa proteins found in the seminal plasma and they are major secretory products of the seminal vesicle epithelium. These proteins are composed of 109–133 amino acids, contain two conserved disulfide bridges, and have 40–60% amino acid sequence identity (reviewed in Töpfer-Petersen et al., 1995, 1998). Both non- and *N*-glycosylated forms of spermadhesins have been described (Calvete et al., 1993). Two spermadhesin subfamilies AQN (AQN 1, AQN 2, and AQN 3) and AWN (AWN 1, and AWN 2) – named according to their first three N-terminal amino acids – were shown to bind to the acrosomal cap of boar epididymal spermatozoa, which classified them as sperm surface proteins (Jonáková et al., 1998). The affinity of sperm-bound spermadhesins for acid polysaccharides pointed to their interaction with heparin-like glycosaminoglycans secreted by the epithelium of the female reproductive tract (Tienthai et al., 2000). This hypothesis led to the idea that these proteins are involved in sperm capacitation. Besides that, their ability to bind to zona pellucida glycoproteins suggested their role in sperm–egg interactions (Sanz et al., 1993; Jonáková et al., 1998, 2000; Jonáková and Tichá, 2004; Tichá et al., 1998; Maňásková et al., 1999, 2000).

Proteins homologous to boar spermadhesins were also found in stallion and bull seminal plasma. Stallion seminal plasma protein HSP-7 differs only in three amino acids from boar AWN spermadhesins and its affinity to zona pellucida makes it a putative sperm–egg adhesion molecule (Reinert et al., 1996). Bull seminal plasma protein aSFP is also homologous to boar spermadhesins (Einspanier et al., 1994). However, its biological role differs from that of boar spermadhesins – it stimulates cell division and progesterone secretion of bovine granulosa cells in vitro (Einspanier et al., 1991).

Regarding human, molecules cross-reacting with antibody against boar AWN 1 have been detected on the equatorial segment of human ejaculated sperm (Töpfer-Petersen et al., 1995). In our previous communication, we reported the presence of low molecular-