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Purification, structural and biophysical characterisation of the major seminal plasma protein from Texel rams

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ABSTRACT

Spermadhesins are a group of low molecular weight proteins present in seminal plasma. In Texel rams, they represent more than 70% of the seminal plasma proteins. Although their functions have not yet been fully clarified, there is much discussion about the role of these proteins in maintaining sperm viability during and after the semen freezing process. This work sought to isolate the major component of the seminal plasma from rams of the Texel breed (*O. aries* SPD2) and to evaluate its structural and biophysical characteristics in order to better understand its role in spermatic viability. The protein was isolated by centrifugation and ion exchange chromatography and its biophysical properties were evaluated by circular dichroism spectrometry. Molecular dynamics simulations of the modelled protein compared to the homologous bovine protein were also carried out. The results showed that *O. aries* SPD2 has a transition temperature (T_m) of 65 °C and a ΔH_m of 322.5 kJ mol^{-1}, similar to the results for other spermadhesins described in the literature. The estimated composition of the secondary structure elements for the native protein is in agreement with that observed for the theoretical model. Its structural characteristics were preserved in simulations at temperatures of 27 °C and 40 °C, as was the case for bull spermadhesin. Taken together, these results suggest that the major component of the spermadhesins of Texel rams (*O. aries* SPD2) may play an important role in maintaining the viability of spermatozoa in fresh semen as well as after thawing.

1. Introduction

Semen is composed of spermatozoa and seminal plasma, a complex mixture rich in organic and inorganic elements produced by the testicles, epididymides and adjoining glands. These substances are important in the maintenance and viability of sperm, and the proteins are the major component of the seminal plasma. The composition of the seminal plasma proteins (SPPs) varies in different species and may also vary among individuals of the same species (Cardozo et al., 2006; Caballero et al., 2008). The seminal plasma has factors that influence both the sperm function and also the female genital tract during spermatic transport. Many of these factors are related to the activity of the seminal plasma proteins (Bergeron et al., 2005). SPPs may be divided into three families in ungulate animals: cysteine-rich secreted proteins (CRISPs), proteins containing the fibronectin type II domain (FN II), and proteins of the spermadhesin family (Bergeron et al., 2005; Haase et al., 2005).

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Spermadhesins (SPDs) are low molecular weight proteins (12–16 KDa) secreted by the male genital tract and its associated glands. They are bonded to the surface of the spermatozoa and are the major proteins found in the seminal plasma of some species (Bergeron et al., 2005). Their structure presents a CUB domain (Romero et al., 1997), the acronym CUB originating from the first proteins identified with this pattern: C from complement subcomponents (C1r, C1s), U from sea urchin embryo protein (Uegf), and B from bone morphogenetic protein 1 (Bmp1) . Spermadhesins are considered members of a class of animal lectins and are, therefore, multifunctional proteins capable of binding carbohydrates, protease inhibitors and phospholipids. These characteristics suggest that they may function in several stages of fertilization, among them spermatic capacitation (Dostàlovà et al., 1994; Töpfer-Petersen et al.,2004).

There are literature reports of the structural or biophysical characterisation of these proteins only in pigs, bulls (Varela et al., 1997), horses (Hoshiba and Sinowatz, 1998) and goats (Nascimento et al., 2012). As a consequence, very little is known about the structural and functional diversity of the spermadhesin family. In addition, nothing is known about the properties of sheep spermadhesin; one of the most widespread livestock animals in the world. Studies of the properties of spermadhesins may increase understanding of the functional roles of this family of proteins in the maintenance of *in vivo* sperm activity, as well as its influence on the conservation of ovine semen *in vitro.* This may lead to improvements in cryopreservation techniques for this species, since the use of frozen ram semen presents reduced rates of fertility and conception compared with fresh semen (Langford et al., 1979; Maxwell and Watson, 1996). In this context, the objective of this work was to investigate the structural and biophysical properties of the major protein component of the ram seminal plasma spermadhesins from the Texel breed, in order to better understand their role in sperm viability.

2. Materials and methods

2.1. Semen collection

Semen was collected from four adult Texel rams by means of electro-ejaculation. Immediately after sample collection, the semen samples were centrifuged at 5000*g* for 15 min to separate the plasma, and mixed to obtain a homogeneous pooled sample. Thereafter, the plasma was immediately used in the assays to minimise possible changes in properties of interest.

2.2. Protein purification and identification

Protein purification was achieved by means of single step of ion (anion) exchange chromatography using an AKTA Pure M20 (GE Life sciences) device coupled to an HP HiTrap Q column (GE Life sciences) of 1 mL volume. A solution of 7% seminal plasma dissolved in buffer A (Tris-HCl 20 mM, pH 8.0) was applied to the column and the protein fractions were eluted with a linear gradient of buffer B (Tris-HCl 20 mM + NaCl 1.0 M, pH 8.0). Aliquots of 2 mL from the column eluate were collected with an automatic F9-R fraction collector (GE Life sciences). The tubes containing the target protein were identified by means of a silver-stained 15% SDS-PAGE gel (Laemmli, 1970) as seen in Fig. 2, which correspond to the red peak in the chromatogram of Fig. 1. After identification, the tubes containing the target protein were mixed and concentrated using a 10 KDa cut-off Amicon Ultra (Millipore) concentrator at 4 °C. Protein quantification was performed by the Biuret method (Nowotny, 1979) using the Gold Analisa Kit (MG, Brazil).

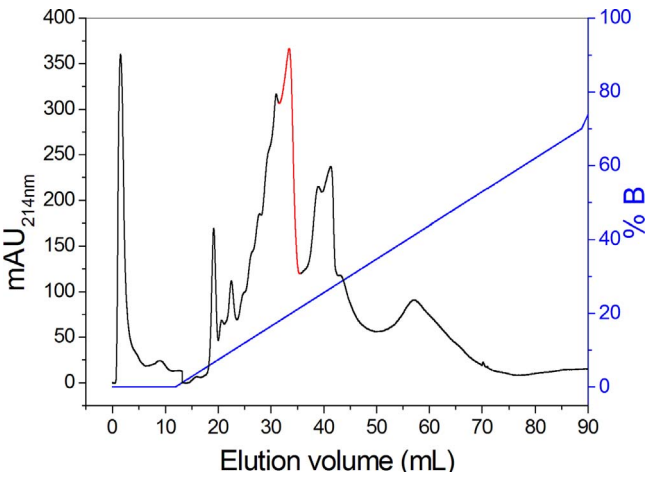

Fig. 1. Chromatographic elution profile of the Texel ram seminal plasma highlighting the peak representing the fraction containing spermadhesin (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)