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THE IDENTIFICATION OF A HEPARIN-BINDING PROTEIN ON THE SURFACE OF BOVINE SPERM

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We report the identification of a sperm surface protein which binds tightly to heparin. The protein was isolated by affinity chromatography on heparin agarose, and its affinity for heparin was confirmed by electrophoresis in the presence of heparin under non-denaturing conditions. The protein consists of a single polypeptide chain with a molecular weight of 45,000, as determined by electrophoresis under denaturing conditions. The protein may bind glycosaminoglycans in vivo and play a part in initiating the capacitation/acrosome reaction.

Freshly ejaculated sperm are unable to fertilize ova; the ability is acquired only after the sperm undergo the biochemically ill-defined processes of capacitation and the acrosome reaction in the female tract (1). The agents in the female tract which induce these processes are essentially unknown, and relatively little is known concerning the nature of the biochemical events involved within the sperm. Recent evidence from several laboratories has suggested that glycosaminoglycans within the female tract may play a role in inducing sperm function. Incubation of sperm with glycosaminoglycans in vitro (2-6), including glycosaminoglycans or proteoglycans isolated from the female tract (7, 8), is capable of producing functional sperm, and the female tract contains glycosaminoglycans at concentrations which are capable of producing the changes in vitro (9-11). Sperm also have binding sites with a high affinity for heparin (12), thereby suggesting that a specific interaction between sperm and heparin-

ABBREVIATIONS

CHAPS: 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate
MOPSO: 3-(N-Morpholino)-2-hydroxypropane sulfonic acid
THAM: tris-(hydroxymethyl)aminomethane

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like glycosaminoglycans may be a significant factor in producing sperm function.

In this communication we have investigated whether any sperm surface proteins have the requisite high affinity for heparin to serve as a glycosaminoglycan receptor. To this end, we first used affinity chromatography to determine that one or more such proteins exist. Secondly, electrophoresis in the presence of heparin was used to show that this interaction was strong and specific.

MATERIALS AND METHODS

Bovine testes were collected at a local abattoir and were transported immediately to the laboratory. The vas deferens and cauda epididymus were excised and placed into sterile physiological saline and refrigerated overnight. Short (0.5 mm) incisions were made in the caudae, and sperm were expressed into a beaker containing Biggers, Whitten, and Whittingham's balanced salt solution (BWW)(13). Sperm were washed by 2 cycles of centrifugation at 600X g with BWW. The final pellet (7 ml) was extracted for 1.5 h in 50 ml of CHAPS-benzamidine buffer to release sperm surface proteins. This buffer consisted of 2 mg/ml CHAPS (Sigma Chemical Co., St. Louis, MO), 10 mM benzamidine (Sigma), 10 mM MOPSO (Sigma), pH 6.8.

The sperm surface protein preparation (30 ml containing 3.3 g protein) was batch adsorbed onto 10 ml of heparin-agarose. The gel was resuspended and poured into a 1.5 x 13 cm column. Protein fractions were eluted with 4 column volumes (15 ml each) of 0.05M, 1.0M, and 3.0M NaCl/0.01M Na_2HPO_4, pH 7.8 buffers and labeled as low, medium and high heparin-affinity protein respectively. The protein fractions were dialyzed against 0.05M buffer, lyophilyzed, dissolved in deionized water to a concentration of approximately 0.5 - 1 mg/ml and the protein concentration determined (14). To test the adequacy of elution, a separate experiment was performed in which the first four column volumes were separately dialyzed, lyophilized, and redissolved in 1 ml of deionized water and 0.5 ml analyzed for protein. The proteins (approximately 10 ug/well) were separated by electrophoresis under denaturing and non-denaturing conditions. The denaturing gel electrophoresis was performed in a 10 % polyacrylamide gel, 1.5 mm thickness, with a running buffer of 0.025 M THAM, 0.192 M glycine, pH 8.3 containing 10 % sodium dodecyl sulfate. The samples were electrophoresed at 30 mA constant current for approximately 4 hr, until the tracking dye (Bromphenol Blue) had migrated to 90 % of the length of the gel. The nondenaturing electrophoresis was performed in the same buffer as above, but without sodium dodecyl sulfate. To further confirm that some proteins had a high affinity for heparin, the protein fractions were mixed with 50 ug heparin per well prior to electrophoresis.

RESULTS

Table 1 shows the elution of protein from the heparin-agarose column. Approximately 1 % of the retained (1M and 3 M fractions) protein was recovered in the high affinity fraction.

TABLE 1 Recovery of sperm surface proteins with different heparin affinities from heparin agarose

Fraction (Mol/L NaCl)	Protein eluted (micrograms)
0.05 M	4,900
1.0 M	2,800
3.0 M	30

The check of elution adequacy showed 99.7 % of the protein eluted in the first 15 ml, 0.7 % in the second, and protein was undetectable in the third and fourth volumes.

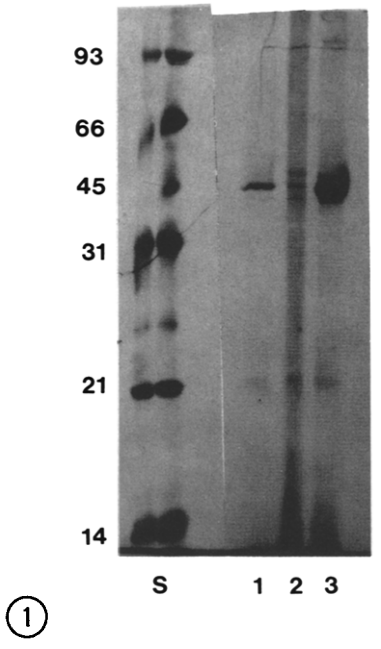
Fig 1 shows a photograph of a denaturing polyacrylamide gel of the protein fractions isolated by affinity chromatography on heparin agarose. The original sperm surface protein preparation appeared identical to the 0.05 M fraction and is not shown separately. The low affinity fraction (Lane 3) contains a major band at 45 K, with a number of other minor bands being discernible. The medium affinity fraction (Lane 2) contains bands at 95, 48, 45, and 42 K. The high affinity fraction (Lane 1) contains 2 bands at 45 and 42 K.

Confirmation that one of the proteins in the high-affinity fraction has a high affinity for heparin was obtained by electrophoresis of the protein in the presence of heparin. The high negative charge of heparin will grossly alter the charge of any protein to which it binds. The result of this experiment is shown in Fig. 2, which shows that one of the bands disappears in the presence of heparin under nondenaturing conditions while the mobility of the other is unaltered.

DISCUSSION

We have demonstrated that the sperm from the cauda contain a protein of approximately 45,000 molecular weight which binds tightly to heparin. This protein is seen most clearly in the fraction which elutes with 3 M NaCl, where it appears to be approximately 50 % pure. The remaining protein is slightly smaller at approximately 42,000 molecular weight.

The mammalian sperm surface contains a number of proteins either as integral or superficial membrane proteins. While the functions of few, if any, are known with certitude, the qualitative and quantitative composition of surface proteins changes



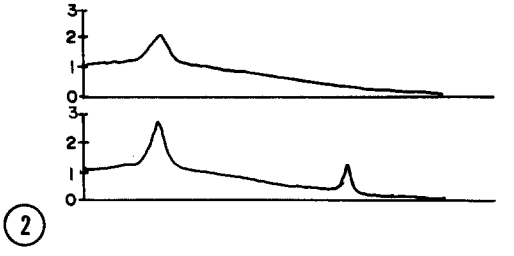


Fig. 1.--Electrophoretic separation of sperm surface proteins under denaturing conditions. Lane S: Molecular weight standards, Lane 1: 3 M NaCl fraction, Lane 2: 1 M NaCl fraction, Lane 3: 0.05 M NaCl fraction

Fig. 2.--Densitometric scans of sperm surface proteins separated by electrophoresis under non-denaturing conditions. The y-axis shows an arbitrary absorbance measured by densitometry, the X-axis shows migration. Bottom: No heparin present, Top: 0.1 mg/ml heparin added to proteins.

during maturation (15, 16) and varies with the site on the sperm (17). Although changes in at least some of these sperm proteins are likely to have important effects upon the fertilizing ability of sperm, the identities of these proteins and their functions have not been established.

Several surface proteins must have receptor functions. A fibronectin-like protein with a high affinity for collagen has been identified (18), as have receptors for proteins of the zona pellucida (19) and various carbohydrates (20), including heparin (12), but the nature of the protein(s) involved has not been demonstrated. In this communication we demonstrate for the first time that the surface membrane proteins of sperm contain a single protein with a high affinity for heparin. This protein, roughly 45 KD, binds tightly to heparin immobilized to agarose and to heparin in solution, as shown by the ability of heparin to alter the electrophoretic mobility of the protein. The interaction is strong; like antithrombin, the protein is eluted from heparin-agarose with 3 M NaCl but not 1 M NaCl. In the case of heparin-

antithrombin, the dissociation constant of the complex is of the order of 100 nM at physiological ionic conditions (21).

The existence of a surface protein with such a high affinity for heparin is of interest, given the reports that glycosaminoglycans can trigger the capacitation of sperm (1-6). This finding suggests that interactions of glycosaminoglycans with this single protein may initiate a complex series of events resulting in the eventual acquisition of the ability to fertilize ova.

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