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Isolation and partial characterization of a basic protein from bovine sperm heads

In fish spermatozoa, protamines are associated with DNA. Little is known, however, about the basic protein that is associated with DNA in mammalian spermatozoa. This is largely due to the fact that no basic protein can be extracted from mammalian spermatozoa by the usual methods. Some authors have described the presence in mammalian spermatozoa of a basic protein that is rich in arginine and cysteine[1-3]. They were unable, however, to characterize this protein to an appreciable extent. This communication describes the isolation and partial characterization of a basic protein from the heads of bull spermatozoa.

The heads of bull spermatozoa were separated from the tails by shearing a suspension of spermatozoa in water (3 g/10 ml) by means of a Bühler homogenizer for 15 min at maximum speed. The suspension was then layered on an equal volume of 20% (w/v) sucrose in water and centrifuged for 15 min at 1000 × *g*. The resulting sediment consisted of two layers: a white firmly packed bottom layer which consisted mainly of heads and a yellowish loosely packed upper layer which consisted of tails. By shaking the sediment with water the upper layer was removed. The bottom layer was resuspended in water and again centrifuged through a sucrose layer. After removal of a small loosely packed upper layer, the sediment now consisted almost exclusively of heads. The contamination with tails was less than 1%.

The isolated heads were suspended (15 mg/10 ml) in a solution containing 5 M guanidinium chloride, 0.28 M 2-mercaptoethanol, 0.005 M EDTA and 0.5 M Tris–HCl (pH 8.5). Upon incubation for 60 min at 37° the heads dissolved completely. Released sulfhydryl groups were then blocked by the addition of ethylenimine to a final concentration of 0.56 M and further incubation for 90 min at 37°. The solution was then cooled and acidified by the addition of $^1/_5$ volume of a solution containing 5 M HCl and 2.5 M

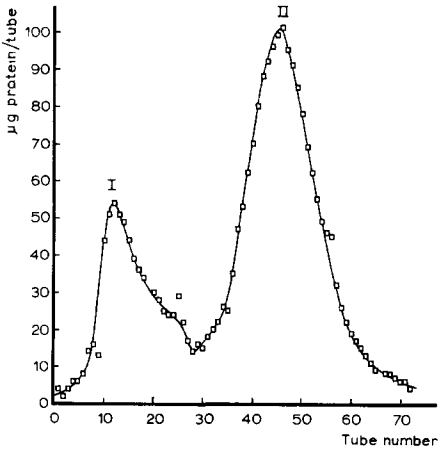

Fig. 1. Chromatography of 2.6 mg of the extracted head proteins on a Sephadex G-75 column (1.8 cm × 40 cm) in 0.01 M HCl–0.025 M NaCl. Fractions of 0.6 ml were collected.

TABLE I

AMINO ACID COMPOSITION OF BOVINE SPERM HEAD PROTEINS

Values are given in molar ratios.

Amino acid	*Fraction I*	*Fraction II*	*Fraction II after purification on Amberlite IRC-50*
Arg	3.2	23.6	24.3
AEt Cys	1.4	5.7	5.9
Thr	3.4	3.0	3.0
Tyr	1.4	2.0	2.0
Ser	4.8	2.0	1.9
Val	3.0	2.0	2.0
Gly	3.8	2.1	2.1
Ile	2.1	1.0	1.0
Leu	4.6	1.0	1.0
Ala	3.9	1.0	1.0
His	1.3	1.0	1.0
Phe	1.8	1.0	1.0
Glu	6.1	1.1	1.0
Asp	4.3	0.1	0
Pro	3.5	0.1	0
Lys	3.2	0.1	0
Met	1.0	0	0

guanidinium chloride in water. Subsequently the solution was dialyzed against 7 changes of 0.25 M HCl at 4° for 2 days. Virtually all of the original protein was recovered after dialysis indicating the absence of proteolysis. A precipitate containing all of the DNA and 5–10% of the protein originally present in the sperm heads was removed by centrifugation. The supernatant solution, containing 90–95% of the original sperm head protein, was dialyzed against 4 changes of distilled water at 4° for 40 h. The dialyzed solution was concentrated in a rotary evaporator at 30° and subsequently applied to a Sephadex G-75 column (1.8 cm × 40 cm) equilibrated with 0.01 M HCl–0.025 M NaCl. The column was developed with the same solvent. As depicted in Fig. 1 two fractions were obtained. The first fraction comprised 25% of the protein, the second fraction 75%. Amino acid analyses (Table I) indicated that the second fraction was a strongly basic protein rich in arginine and cysteine. This second fraction was submitted to a final purification by means of chromatography on an Amberlite IRC-50 column (2 cm × 10 cm)[4]. A small amount (2%) of contaminating material was eluted with 0.2 M acetic acid, while the main fraction (98%) was eluted with 0.05 M HCl. The amino acid composition of this fraction is shown in Table I.

When examined for purity by electrophoresis (pH 4.2) in a 10% polyacrylamide gel, the basic protein migrated as a single band. In sedimentation velocity studies with 0.75% solutions of the basic protein in 0.2 M KCl, 0.05 M Tris–HCl (pH 8.0) in a Spinco Model E ultracentrifuge, using a synthetic boundary cell, the material sedimented as a homogeneous substance at 59 780 rev./min (Fig. 2). Determination of NH_2-terminal amino acids by the cyanate method[5] revealed the presence of a single NH_2-terminal amino acid, alanine. The amount of NH_2-terminal alanine was at least

80% of the total amount of alanine found in the protein. This indicated that all of the alanine in the protein is probably NH_2-terminal. For the determination of the carboxyl-terminal amino acids the protein was digested with carboxypeptidase A (Worthington, DFP treated) at pH 8.0 according to AMBLER[6], using a molar ratio of enzyme to substrate of 1:100. The released amino acids were separated from the remaining protein by chromatography on an Amberlite IRC-50 column according to TOBITA *et al.*[4]. The released amino acids were eluted with 0.2 M acetic acid. To discriminate between glutamine and threonine, which run together in the Technicon analytical system, the following procedure was followed.

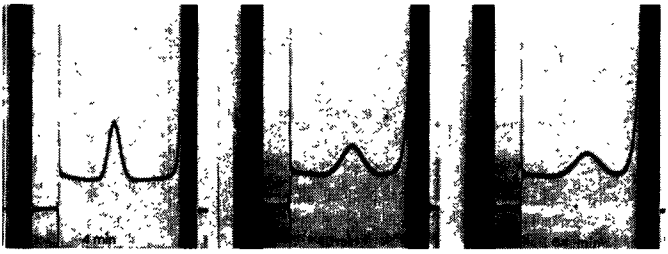

Fig. 2. Sedimentation velocity pattern of the basic head protein in a synthetic boundary cell, 4, 40 and 64 min after attaining full speed. Sedimentation was performed at 59 780 rev./min at 20°. The direction of sedimentation was from left to right. The solution contained 7.5 mg of protein per ml of 0.2 M KCl, 0.05 M Tris–HCl (pH 8.0).

The amino acid fraction was evaporated to dryness, hydrolyzed for 1 h in 1 M HCl at 100° and analyzed. Another portion of the amino acid fraction was analyzed without hydrolysis. An enzyme blank and a protein blank were determined in the same way. These analyses showed that a single amino acid, glutamine, was released upon carboxypeptidase A digestion. The amount of carboxyl-terminal glutamine equals the amount of glutamic acid in the protein, which indicates that all glutamic acid in the protein is present as carboxyl-terminal glutamine.

The data of the end group analyses, together with the results of the electrophoretic and sedimentation velocity studies indicate that the isolated basic protein fraction is probably homogeneous.

The amino acid analysis of the basic protein, together with the data of the end group analyses, suggest the following composition: Ala (Arg_{24}, Cys_6, Thr_3, Tyr_2, Ser_2, Gly_2, Val_2, Leu_1, Ile_1, Phe_1, His_1) Gln. The molecular weight calculated from this composition is 6200.

The basic protein which, apart from the presence of 6 cysteine residues, closely resembles the protamines of fish spermatozoa, originates most probably in the nuclei of the sperm heads. Our results thus support the hypothesis advanced earlier by BRIL-PETERSEN AND WESTENBRINK[1] according to which in the compact nucleus of bull spermatozoa the DNA is associated with a network of keratinoid threads which are rich in arginine. Our results suggest that such a network is composed of identical subunits of low molecular weight that are cross-linked by numerous disulfide bonds.

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