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ENZYMATIC UNPACKING OF BULL SPERM CHROMATIN

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Summary

When isolated bull sperm chromatin is incubated with 0.1 M 2-mercaptoethanol at pH 8, an extensive proteolytic degradation of sperm histone occurs, being accompanied by a marked swelling of the chromatin masses. The degradation of sperm histone is strongly inhibited by monovalent or divalent metal ions. The protease found in isolated bull sperm chromatin possesses properties indistinguishable from those of an acrosomal protease of trypsin-type, acrosin (EC 3.4.21.10), and requires a combination of NaCl, urea and 2-mercaptoethanol for its extraction. Evidence suggests that the protease travels along chromatin strands and hydrolyzes essentially all the sperm histone molecules within the chromatin masses.

Introduction

Chromosomal DNA is tightly packaged in the head of spermatozoa. In eutherian mammals, the packaging of DNA is achieved as a result of replacement of somatic-type histones by arginine-, cysteine-rich sperm histone [1—5] during formation of spermatozoa in the testis [6]. The packaging becomes further tightened by cross-linking of sperm histone through formation of disulfides during maturation of spermatozoa in the epididymis [6]. During fertilization tightly packaged sperm chromatin is dispersed after incorporation of the sperm head into the egg cytoplasm as the sperm nucleus transforms into the male pronucleus. This process, a reverse of the DNA packaging during spermiogenesis, probably involves replacement of sperm histone by somatic-type his-

Abbreviations: Bz-Arg-OEt, *N*-benzoyl-L-arginine ethyl ester · HCl; Tos-Arg-OMe, *p*-tosyl-L-arginine methyl ester · HCl; Bz-Tyr-OEt, *N*-benzoyl-L-tyrosine ethyl ester; Bz-Arg-NNap, α-*N*-benzoyl-DL-arginine-β-naphthylamide · HCl.

tones. Because of technical difficulties in performing biochemical studies of zygotes at this phase of development, the mechanism of the dispersal of sperm chromatin has remained unelucidated. We report here that when isolated bull sperm chromatin is incubated under appropriate conditions, sperm histone is extensively degraded. It is possible that proteolysis of sperm histone may be key events during transformation of the sperm nucleus into the male pronucleus in the egg cytoplasm.

Materials and Methods

All the experiments described below were performed in plastic containers or in siliconized glassware.

Preparation of sperm chromatin

Frozen bull semen was obtained from Eastern Artificial Insemination Cooperative Inc. (Ithaca, N.Y.). Spermatozoa were sedimented from semen by centrifugation (1500 × *g*, 10 min) and washed three times with 0.01 M Tris · HCl (pH 8). Spermatozoa were sheared in the same buffer with a VirTis-45 homogenizer at 85 V for 15 min and the sperm head fraction was then separated from the tail fraction by centrifugation (1500 × *g*, 20 min) through 0.6 M sucrose. Sperm chromatin was prepared from the sperm heads by successive washings with 1% Triton X-100 and 0.01 M sodium deoxycholate as previously described [5].

Proteolysis of sperm chromatin

Bull sperm chromatin was incubated under various conditions. Degradation of sperm histone was examined by column chromatography and polyacrylamide gel electrophoresis. The standard reaction mixture (1 ml) contained 0.02 M Tris · HCl (pH 8.0), 0.1 M 2-mercaptoethanol and bull sperm chromatin equivalent to 1.3 mg of DNA. Incubation was carried out at 37 °C for 4 h unless otherwise specified. After incubation, the mixture was chilled, and centrifuged in a horizontal head at 1500 × *g* for 10 min. The sediment, in which essentially all the DNA was recovered, was next incubated in a mixture containing 1.1 M NaCl, 0.1 M 2-mercaptoethanol, 6 M urea and 0.15 M Tris · HCl (pH 8.0) at 37 °C for 90 min in order to dissociate sperm histone from DNA. To the resulting viscous solution iodoacetamide was added to a final concentration of 0.2 M, and the incubation was continued for another 60 min in the dark in order to block released sulfhydryls of sperm histone. The solution was treated (0 °C, 30 min) with four times its volume of 0.2 M HCl, followed by centrifugation at 10 000 × *g* for 20 min.

Acid extracts thus obtained were treated (0 °C, 20 min) with 20% trichloroacetic acid. The precipitates formed were collected by centrifugation (17 000 × *g*, 20 min), dissolved in 0.2 M HCl containing 20% sucrose, and subjected to disc electrophoresis in 15% polyacrylamide gels containing 6 M urea (pH 4.3) as previously described [7]. Electrophoresis was carried out at 5 mA per tube for 70 min. In some experiments, acid extracts were desalted by chromatography on a Sephadex G-10 column (2 × 20 cm) using 0.01 M HCl as eluant, and lyophilized. The lyophilized samples were then assayed for protein by the

method of Lowry et al. [8] and for arginine according to Satake and Luck [9]. Further characterization of the lyophilized samples were made by chromatography on a Sephadex G-50 column (1.2 × 95 cm) using 0.01 M HCl as eluant, and on a CM-cellulose column (2 × 7 cm; Bio-Rad) using a linearly increasing concentration of guanidinium chloride (pH 5) as previously described [5], and by polyacrylamide gel electrophoresis.

Dissociation of protease from sperm chromatin

Bull sperm chromatin equivalent to 1.3 mg DNA was incubated (0°C, 60 min) in 0.02 M Tris · HCl, pH 8.0 (1 ml) containing different combinations of NaCl (0.45 M), 2-mercaptoethanol (0.02 M) and urea (2 M). The mixtures were then centrifuged at 1500 × *g* for 10 min in a horizontal head using conical tubes and the proteolytic activities of the sediments and the supernatants were examined. The chromatin sediment was washed once with 0.02 M Tris · HCl (pH 8.0) by centrifugation (1500 × *g*, 10 min), resuspended in 1 ml of 0.02 M Tris · HCl (pH 8.0) containing 0.1 M 2-mercaptoethanol, and incubated at 37°C for 4 h. Degradation of sperm histone was then examined by polyacrylamide gel electrophoresis as described above. The supernatant was dialyzed overnight against 0.02 M Tris · HCl (pH 8.0) containing 0.45 M NaCl in an ice bath. To an aliquot (0.9 ml) of the dialyzed extract 0.1 ml of bull sperm histone (100 μg) prepared according to Marushige and Marushige [5] was added. This was then incubated at 37°C for 60 min. After incubation, the mixture was diluted with 3 ml of 0.2 M HCl, treated with 20% trichloroacetic acid and centrifuged at 17 000 × *g* for 20 min. The precipitate thus obtained was dissolved in 0.2 M HCl containing 20% sucrose (0.3 ml), and an aliquot (0.03 ml) was then subjected to gel electrophoresis.

Characterization of protease extracted from sperm chromatin

Bull sperm chromatin was incubated (0°C, 60 min) in a mixture containing 0.45 M NaCl, 0.02 M 2-mercaptoethanol, 2 M urea and 0.02 M Tris · HCl (pH 8) at the chromatin concentration equivalent to 4.3 mg DNA per ml. This was followed by centrifugation at 1500 × *g* for 10 min. The supernatant was dialyzed overnight against two changes of 500 volumes of 0.01 M HCl at 4°C. Insoluble materials formed during dialysis were removed by centrifugation (10 000 × *g*, 10 min).

The protease activity of the extract thus obtained was characterized using bull sperm histone as substrate. The complete reaction mixture (1 ml) contained 0.05 M Tris · HCl (pH 8.0), 0.45 M NaCl, 100 μg bull sperm histone and 0.15 ml of the extract. Incubation was carried out at 37°C for 60 min, and degradation of sperm histone was analyzed electrophoretically as described above.

The esterase activity of the extract was studied according to Schwert and Takenaka [10]. The complete reaction mixture (1 ml) contained 0.05 M Tris · HCl (pH 8.0), 0.05 M $CaCl_2$, 0.05 ml of the extract, and 0.5 mM of Bz-Arg-OEt, Tos-Arg-OMe or Bz-Tyr-OEt. Reaction was allowed at room temperature in a Beckman DB-GT spectrophotometer coupled with a potentiometric recorder. The hydrolysis of Bz-Arg-OEt was followed at 253 nm with the use of a molar absorption difference of 1150 $M^{-1} \cdot cm^{-1}$ and Tos-Arg-OMe at 247 nm

with 533 $M^{-1} \cdot cm^{-1}$. The hydrolysis of Bz-Tyr-OEt was monitored at 257 nm in 25% methanol.

The extract was also characterized by electrophoretic fractionation. The sample in 0.01 M HCl was electrophoresed in a 15% polyacrylamide gel under the same conditions as described for electrophoresis of sperm histone except that urea was omitted from the gel. After electrophoresis (5 mA/tube, 75 min) the amidase activity in the gel was visualized using the chromogenic substrate, Bz-Arg-NNap coupled with Fast Garnet GBC (*O*-aminoazotoluene, diazonium salt; Sigma) as previously described [11].

Results

Proteolysis of bull sperm chromatin

Isolated bull sperm chromatin, which is composed mainly of sperm histone and DNA, retains the oval shape characteristic of the bull sperm head [5]. When isolated bull sperm chromatin is incubated (37°C) in 0.02 M Tris · HCl (pH 8.0) containing 0.1 M 2-mercaptoethanol, the oval chromatin masses become highly swollen and their capacities to bind methylene blue increase. Following incubation of the sperm chromatin for various lengths of time under such conditions, sperm histone fractions isolated therefrom have been assayed for protein and arginine (Fig. 1), and characterized by column chromatography (Fig. 2) and by polyacrylamide gel electrophoresis (Fig. 3). As seen in Fig. 1, during the first 4—8 h the amount of protein remaining bound to the chromatin decreases to approx. 40% of the original amount, while the arginine content of the sperm histone fraction decreases to approx. 65% of the original during the same period of incubation. No further decrease of protein and arginine content has been observed during prolonged incubation up to 48 h. Bull sperm histone contains 24 arginine residues. 19 of these residues are known to be arranged in the middle of the molecule in three clusters of respectively 7, 6, and 6 residues [2]. It would thus appear that less basic portions of sperm histone molecules are preferentially removed from the DNA during the incubation. As clearly seen in profiles of sperm histone fractions after chromatography on Sephadex G-50 columns (Fig. 2A), bull sperm histone is degraded into

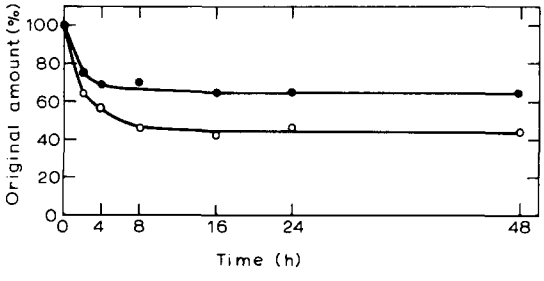

Fig. 1. Changes of protein and arginine contents of sperm histone fractions during incubation of bull sperm chromatin. Sperm histone fractions obtained from bull sperm chromatin which had been incubated for various times (0—48 h) were desalted by Sephadex G-10 chromatography, lyophilized, and assayed for protein and arginine.

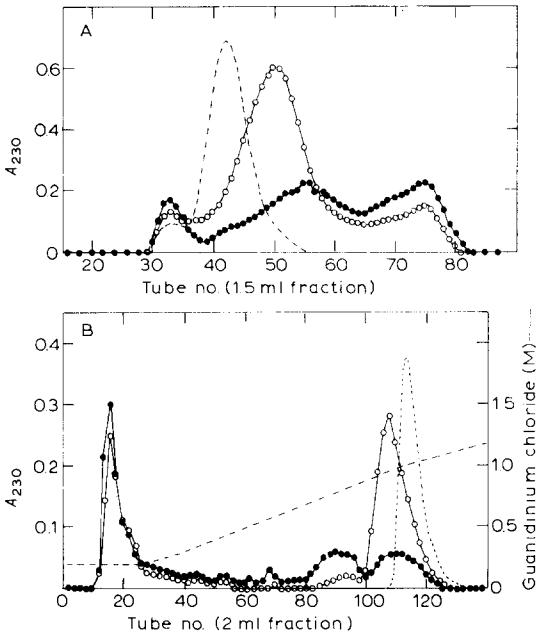

Fig. 2. Changes of chromatographic profiles of sperm histone fractions during incubation of bull sperm chromatin. (A) Sperm histone fractions obtained from bull sperm chromatin which had been incubated for 0 (— — —), 4 (○———○) and 16 h (●———●), were desalted by Sephadex G-10 chromatography, lyophilized, and subjected to chromatography on a Sephadex G-50 column. (B) Chromatographic fractions (tubes 38—80) of A were combined, lyophilized, and analyzed by chromatography on a CM-cellulose column.

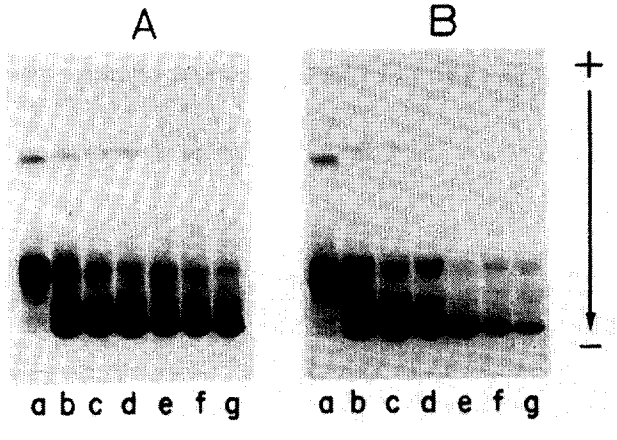

Fig. 3. Changes of electrophoretic profiles of sperm histone fractions during incubation of bull sperm chromatin. Sperm histone fractions obtained from bull sperm chromatin which had been incubated for 0 (a), 2 (b), 4 (c), 8 (d), 16 (e), 24 (f), and 48 h (g) were either (A) desalted and lyophilized or (B) precipitated with 20% trichloroacetic acid, and analyzed by polyacrylamide electrophoresis. Samples obtained from the chromatin equivalent to 30 μg of DNA were applied to each gel.

smaller and more heterogeneous components during the incubation. Analyses by chromatography on CM-cellulose columns (Fig. 2B) reveal that considerable amounts of such components are eluted between 0.7 and 1.0 M guanidinium chloride even in the sperm histone fractions obtained from the chromatin which had been incubated for 16 h. Electrophoretic analyses (Fig. 3A) show that upon incubation of the chromatin, the histone gives rise to faster migrating materials which accumulate at the solvent front as a sharp band. Data of Fig. 3A also show that the amount of materials accumulated at the solvent front remains relatively constant during prolonged incubation. Further degradation of sperm histone is, however, detectable by electrophoretic analyses of sperm histone fractions prepared by trichloroacetic acid precipitation (Fig. 3B) instead of Sephadex G-10 chromatography and lyophilization (Fig. 3A). As can be seen in Fig. 3B (d–g), the amount of electrophoretic components at the solvent front decreases markedly during prolonged incubation. The faintly stained material with a slow mobility (R_F = 0.35) is an unknown minor component of bull sperm chromatin.

It seems thus clear that sperm histone is extensively degraded during incubation of bull sperm chromatin under these conditions. Isolated bull sperm chromatin can be stored in 0.01 M Tris · HCl (pH 8.0) for at least 6 months without appreciable loss of its proteolytic activity. The proteolytic activity is, however, completely lost by preheating of bull sperm chromatin at 80 °C for 10 min. The proteolysis of bull sperm chromatin exhibits a pH optimum at around 8 (Fig. 4A) and is strongly inhibited by $CaCl_2$ (Fig. 4B). An inhibition of the proteolysis similar to that observed with 50 mM $CaCl_2$ (Fig. 4B, d) has been found to occur also at 0.15—0.3 M NaCl. A high concentration (0.2 M) of 2-mercaptoethanol is essential for proteolysis in the absence of urea (Fig. 4C, a—c). Although a combination of urea and 0.2 M 2-mercaptoethanol is clearly inhibitory (compare Fig. 4C, a with d or g), the concentrations of 2-mercaptoethanol required for proteolysis appears to be lowered by the presence of urea. At 0.02 M 2-mercaptoethanol, more degradation of sperm histone thus occurs in the presence of urea than in its absence (compare Fig. 4C, d—e or g—h with a—b). Bull sperm histone contains six cysteine residues [2]. Essentially all the cysteine residues are present in the chromatin as disulfides and these disulfides are between sperm histone molecules [5]. The requirement of the thiol for proteolysis of bull sperm chromatin may in part be attributed to an increased susceptibility of sperm histone as a result of cleavages of these disulfide bonds.

Effects on proteolysis of varying concentrations of bull sperm chromatin during incubation were next investigated. As seen in Fig. 4D (a—c), dilution of the chromatin does not inhibit, but rather enhances the degradation of sperm histone. This absence of inhibition suggests that the proteolysis takes place within the confines of the sperm chromatin masses with little or no protease diffusing into the medium during incubation. This enhancement of proteolysis by dilution of the chromatin implies that the degradation of sperm histone may be inhibited by products diffusing into the incubation medium. In fact, when fresh (unincubated) sperm chromatin is incubated with the supernatant obtained after incubation (37 °C, 4 h in 0.02 M Tris · HCl, pH 8, and 0.1 M 2-mercaptoethanol) of the sperm chromatin, the degradation of sperm histone is significantly inhibited (compare Fig. 4D, a with d).

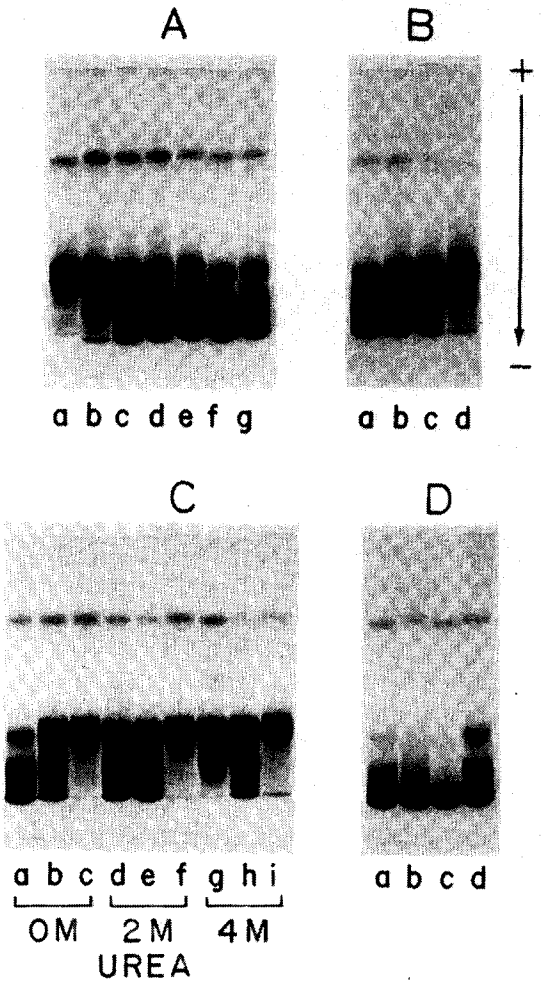

Fig. 4. Incubation conditions for proteolysis of bull sperm chromatin. The sperm chromatin was incubated under standard conditions except for variations as indicated below. Sperm histone fractions were precipitated with 20% trichloroacetic acid, and analyzed by electrophoresis. (A) pH dependence; a, unincubated control; b, sodium acetate, pH 5.0; c, Tris · HCl, pH 6.2; d, pH 7.2; e, pH 7.6; f, pH 8.0; and g, pH 8.2. (B) Addition of $CaCl_2$; a, 0; b, 0.5 mM; c, 5 mM; and d, 50 mM. (C) Effects of 2-mercaptoethanol and urea; 2-mercaptoethanol, 0.2 M (a,d,g), 20 mM (b,e,h) and 2 mM (c,f,i) in the presence of 0 (a–c), 2 (d—f) and 4 M (g—i) urea. (D) Dilution of chromatin; sperm chromatin equivalent to 1.3 mg DNA in 1 ml (a), 4 ml (b) and 16 ml (c) of the standard incubation mixture, and 1 ml (d) of the supernatant (1500 × *g*, 10 min) obtained from (a). Samples obtained from sperm chromatin equivalent to 30 μg of DNA were applied to each gel.

Dissociation and properties of protease of bull sperm chromatin

Bull sperm chromatin has been extracted with various combinations of NaCl (0.45 M), urea (2 M) and 2-mercaptoethanol (0.02 M). Analyses of the chromatin sediments and the extracts for their proteolytic activities show that no or little extraction of proteolytic activity from the chromatin is achieved by either urea or 2-mercaptoethanol alone, or even by a combination of these two (Fig. 5A, a and Fig. 5B, a). As can be seen in Fig. 5A(b) and 5B(b), the

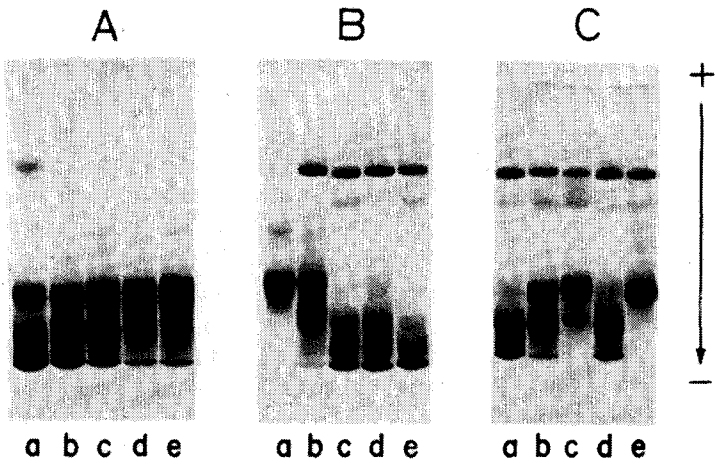

Fig. 5. Dissociation and properties of protease of bull sperm chromatin. In (A) and (B), bull sperm chromatin was treated in mixtures containing various combinations of NaCl (0.45 M), urea (2 M) and 2-mercaptoethanol (0.02 M): a, urea 2-mercaptoethanol; b, NaCl alone; c, NaCl, 2-mercaptoethanol; d, NaCl, urea; or e, NaCl, urea, 2-mercaptoethanol. The mixtures were centrifuged and proteolytic activities of the chromatin sediment (A) and the extract (B) were examined as described in Materials and Methods. In (C), the extract obtained with NaCl, urea, 2-mercaptoethanol was dialyzed against 0.01 M HCl, and incubated in 0.05 M Tris · HCl (pH 8.0) containing a, 0.45 M NaCl and 100 μg bull sperm histone; b, a plus 5 mM 2-mercaptoethanol; c, a plus 50 mM 2-mercaptoethanol; d, a plus 10 mM iodoacetamide; and e, a minus NaCl.

protease activity is partially extracted with NaCl alone. The addition of either 2-mercaptoethanol or urea to NaCl facilitates the extraction (c, d of Figs 5A and 5B), while most efficient dissociation of the protease activity from the chromatin occurs in the presence of all three (Fig. 5A, e and Fig. 5B, e). The concentrations of NaCl required for dissociation of the protease activity in the presence of 0.02 M 2-mercaptoethanol range from 0.3 to 0.45 M. There has been no dissociation of sperm histone from the chromatin under any of the conditions used in this experiment. Extracts obtained by treatment of the sperm chromatin with a mixture of 0.45 M NaCl, 2 M urea and 0.02 M 2-mercaptoethanol and then dialyzed against 0.01 M HCl, can be stored (0°C) for at least several days without appreciable loss of activity.

Some of the properties of the proteolytic reaction of the extract obtained from bull sperm chromatin are summarized in Fig. 5C. The complete incubation mixture consists of 0.05 M Tris · HCl (pH 8), 0.45 M NaCl, 100 μg of bull sperm histone, and the extract (Fig. 5C, a). In contrast to an absolute requirement of 2-mercaptoethanol for autolytic degradation of sperm histone in bull sperm chromatin (cf. Fig. 4C, a–c), degradation of sperm histone by the dissociated enzyme is strongly inhibited by the thiol (Fig. 5C, b and c). This would suggest that the enzyme may be protected in some way from inactivating effects of 2-mercaptoethanol during proteolysis of the sperm chromatin. The activity is not inhibited by iodoacetamide (Fig. 5C, d). An omission of 0.45 M NaCl from the reaction mixture completely abolishes proteolysis (Fig. 5C, e), nor does degradation of sperm histone occur at 0.15 M NaCl, although incubations at NaCl concentrations higher than 0.45 M have exhibited activity similar

to that observed at 0.45 M NaCl. We suggest that a high salt concentration might be required to prevent non-specific aggregation between the enzyme and highly basic sperm histone. Addition of $CaCl_2$ (up to 50 mM) has been found to be neither inhibitory nor stimulatory.

Further characterization of protease extracted from bull sperm chromatin

The enzyme extracted from bull sperm chromatin as described above has been found to hydrolyze Bz-Arg-OEt. As shown in Fig. 6, when bull sperm chromatin is incubated with Bz-Arg-OEt, this ester inhibits the degradation of sperm histone, presumably, as a consequence of its competition with sperm histone for the enzyme.

A trypsin-like protease, acrosin, is known to be present in the acrosome of mammalian spermatozoa (cf. ref. 12). Therefore the extract obtained from bull sperm chromatin has been further characterized using synthetic substrates and has been compared with a crude extract of acrosomal fractions of bull spermatozoa prepared by the method of Polakoski et al. [13].

As shown in Table I, both the chromosomal and the acrosomal extract hydrolyze Bz-Arg-OEt at a higher rate than Tos-Arg-OMe, while hydrolysis of Bz-Tyr-OEt has been observed with neither extract. It has been reported that acrosin displays a greater hydrolytic activity on a molar basis toward Bz-Arg-OEt than towards Tos-Arg-OMe [14–16]. Table I shows that on the basis of sperm DNA, the esterase activity of the chromatin fraction is approximately one-seventh that of the acrosome fraction. The esterase activities of both ex-

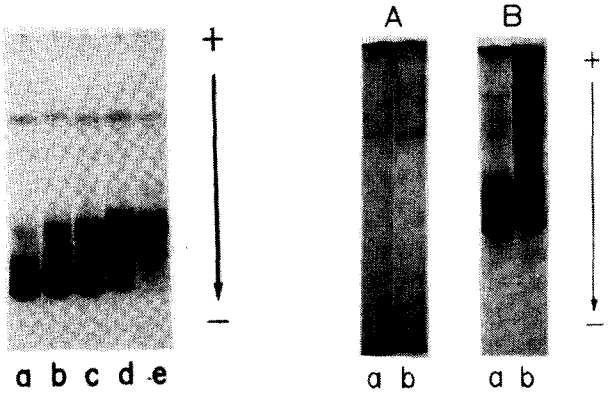

Fig. 6. Inhibition of proteolysis of bull sperm chromatin by Bz-Arg-OEt. Sperm histone fractions obtained from bull sperm chromatin incubated in the presence of 0 (a), 5 (b), 10 (c), 20 (d) and 40 mM (e) of Bz-Arg-OEt were precipitated with 20% trichloroacetic acid, and analyzed by electrophoresis. Samples obtained from the chromatin equivalent to 30 μg of DNA were applied to each gel.

Fig. 7. Electrophoretic fractionation of extracts obtained from the chromatin and the acrosome fractions of bull spermatozoa. The chromatin and the acrosome fractions (cf. text) of bull sperm were extracted with 0.01 M HCl. A sample (0.1 ml) obtained from bull sperm chromatin equivalent to 3.2 mg DNA (a) or that (0.1 ml) obtained from the acrosome fraction which was derived from bull spermatozoa equivalent to 0.5 mg DNA (b) were electrophoresed at 5 mA per tube for 75 min. The amidase activity was then detected by incubating the gels in 0.1 M sodium phosphate (pH 7.5) containing 2.8 mM Bz-Arg-NNap and 0.9 mg/ml Fast Garnet GBC at 37°C for 2 h (A). Another set of gels electrophoresed under the identical conditions was stained with buffalo black (B).

TABLE I

ESTERASE ACTIVITIES IN EXTRACTS OBTAINED FROM CHROMATIN AND ACROSOME FRACTIONS OF BULL SPERMATOZOA

Bull sperm chromatin was treated with 0.45 M NaCl, 2 M urea, 0.02 M 2-mercaptoethanol, and the extract was dialyzed against 0.01 M HCl. Acrosome fractions of bull spermatozoa were extracted with 0.01 M HCl. The assay conditions were given in Materials and Methods. One unit is equivalent to the hydrolysis of one micromole of substrate per min.

Substrate	Activity (units/mg sperm DNA)	
	Chromosomal extract	Acrosomal extract
Bz-Arg-OEt	0.054	0.37
Tos-Arg-OMe	0.035	0.24
Bz-Tyr-OEt*	0.00	0.00

* Bz-Tyr-OEt was hydrolyzed by chymotrypsin under the conditions used.

tracts are inhibited (80—90%) by soybean trypsin inhibitor or lima bean trypsin inhibitor at an inhibitor concentration of 50 μg/ml. As has been shown in Fig. 5, the protease activity of bull sperm chromatin can be partially extracted with 0.45 M NaCl. By the esterase assay, this extract contains approx. 20% of the activity of the material extracted with a mixture of NaCl (0.45 M), urea (2 M), and 2-mercaptoethanol (0.02 M). It has also been found that approx. 50% of the esterase activity in bull sperm chromatin is extractable with 0.01 M HCl.

The chromosomal and the acrosomal extracts of bull spermatozoa were fractionated electrophoretically in polyacrylamide gels and their amidase activities detected in the gels using a chromogenic substrate, Bz-Arg-NNap. The electrophoretic profiles of total proteins in these extracts are shown in Fig. 7B. As seen in Fig. 7A, the chromosomal and the acrosomal extracts each give a single major band of activity with identical electrophoretic mobilities. The bands of amidase activity of the chromosomal extracts obtained with 0.45 M NaCl alone, 0.01 M HCl (shown in the figure), and NaCl, urea, 2-mercaptoethanol also possess identical electrophoretic mobilities, excluding a possibility that different enzymes are extracted from bull sperm chromatin under such conditions.

Discussion

When isolated bull sperm chromatin is incubated under appropriate conditions, essentially all the sperm histone molecules of the chromatin become degraded (Figs 1—3). Moreover, dilution of the chromatin in the incubation medium does not lessen the sperm histone degradation (Fig. 4D). These results indicate that a protease travels along the chromatin strands during incubation without an appreciable loss from the condensed chromatin to the medium. This protease found in isolated bull sperm chromatin is trypsin-type (Table I) and is electrophoretically indistinguishable from acrosin. This investigation has shown that it possesses properties essentially identical to those reported for acrosin [11,12,14—17]. Acrosin has been assigned a role in the penetration of

spermatozoa through the zona pellucida of the egg, implying that the mode of this proteolytic activity is extracellular. This is in contrast to the observed proteolysis of bull sperm chromatin, where the hydrolysis occurs intracellularly. The question as to whether the protease found in isolated chromatin is acrosin remains to be clarified on the basis of further studies in purified systems.

An examination by Giemsa staining according to the method of Hartree and Srivastava [18] has revealed that isolated bull sperm chromatin is essentially free from acrosomal materials, while the equatorial segment of the sperm has been clearly seen under these conditions. We have found that there is no increase of the proteolytic activity of the chromatin when isolated bull sperm chromatin is mixed with acrosomal fractions obtained from the equivalent amount (on the basis of DNA) of bull spermatozoa and carried through the isolation procedure. Furthermore, extensive degradation of sperm histone has also been observed upon incubation of saline-washed bull spermatozoa under the same conditions used for the chromatin. Therefore, the observed proteolysis of sperm chromatin seems unlikely to be merely an artefact of association of released acrosin or other proteases with the chromatin during its isolation. It remains, however, to be determined if the protease found in isolated sperm chromatin is of acrosomal origin, or dispersed amongst chromatin strands within the nuclear mass, or arranged along the surface of nucleus. The tightly packaged sperm chromatin is known to be dispersed immediately after the entry of the sperm nucleus into the egg cytoplasm [19]. Proteolysis of sperm histone could play a key role in the unpacking of sperm chromatin during fertilization.

Acknowledgments

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