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Demonstration of acrosin in mouse spermatozoa

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Summary. Gelatinolytic activity of whole mouse spermatozoa was demonstrated by the gelatin film test. The presence of a trypsin-like protease (acrosin) in acidic extracts of mouse spermatozoa was shown by an electrophoretic method to separate the enzyme from a putative inhibitor.

Introduction

It is generally accepted that the physiological function of acrosin is to assist the spermatozoon in the penetration of the zona pellucida of the ovum and it would be expected that acrosin is present in the spermatozoa of all mammalian species. Erickson & Martin (1974) concluded that mouse spermatozoa do not have significant amounts of acrosin, but Brown & Hartree (1976) have described its occurrence in the spermatozoa of this species. We now present further evidence confirming the existence of mouse acrosin which can be extracted from the spermatozoa by acidic solutions.

Materials and Methods

Mouse spermatozoa, obtained from the epididymides of 24 NMRI mice were suspended in Tris diluent (Wendt, Leidl & Fritz, 1975). The suspension was centrifuged (15 min, 600 *g*) and the spermatozoa were washed once with 2 ml Tris diluent.

The standard version of the gelatine film test described by Wendt *et al.* (1975) was used to demonstrate proteolytic activity of whole spermatozoa.

To extract acrosin the spermatozoa were suspended in 3 ml 2% (v/v) acetic acid, pH 2·7 (adjusted with HCl), incubated at 4°C for 20 min, and the suspension centrifuged (15 min, 5000 *g*) (Fink, Schiessler, Arnhold & Fritz, 1972). Disc electrophoresis was performed in 1·5 mm-thick polyacrylamide slab gels containing 0·1% gelatin (running pH 4·5) (Schleuning, Hell, Schiessler & Fritz, 1975). Samples, corresponding to 0·02 ml of the acidic extract, were loaded into slots of the slab gel. After electrophoresis (4 h, 13 V/cm) the gel was cut into 1·5-cm wide strips and incubated under the conditions described in the legend of Pl. 1, Fig. 2. After staining and destaining, zones of proteolytic (gelatinolytic) activity were visible as colourless bands. The inhibitor used was that from guinea-pig seminal vesicles which inhibits acrosin, trypsin and plasmin (Fink & Fritz, 1976).

The assays for protease and inhibitor were performed by the methods described by Schleuning & Fritz (1976) and Fink & Fritz (1976) with benzoyl-L-arginine ethylester (BAEE) or *N*-α-benzoyl-L-arginine *p*-nitroanilide (L-BAPA) as substrates.

Results and Discussion

When the gelatin film test was applied to whole epididymal spermatozoa, digestion areas developed along the convex side of the sperm head (Pl. 1, Fig. 1). These areas were much smaller than those observed in this test for the spermatozoa of other species, indicating that the content of enzyme responsible for the gelatinolysis is low.

We were unable to demonstrate by photometric assays any neutral protease activity in the acidic extract and there was no indication of the presence of proacrosin (incubation of the extract at pH 8, 25°C for 30 min and re-acidification before assay). However, the extract contained trypsin inhibitory activity (52 mi.u./ml) and we assumed that the failure to detect acrosin was caused by formation of an enzyme–inhibitor complex.

To demonstrate acrosin activity in the acidic extract, even in the presence of an inhibitor, without preparative separation we chose an electrophoretic system (Schleuning *et al.*, 1975) in which the acid pH 4·5 causes dissociation of the acrosin–inhibitor. Incubation of the gel under various conditions (Pl. 1, Fig. 2) showed that mouse spermatozoa contain an enzyme which is a neutral protease (active at pH 8·0, Gel 1; inactive at pH 4·5, Gel 3) and is not inactivated at pH 4·5 (Gel 2). Inhibition studies carried out with a typical acrosin inhibitor (Gels 4 and 5) indicated that the enzyme is the trypsin-like protease, acrosin.

The failure of Erickson & Martin (1974) to detect mouse acrosin, even with a highly sensitive radioassay, was probably due to the presence of the acrosin inhibitor. Our present report of the occurrence of acrosin in mouse spermatozoa confirms the finding of Brown & Hartree (1976), who used a different acidic extraction procedure.

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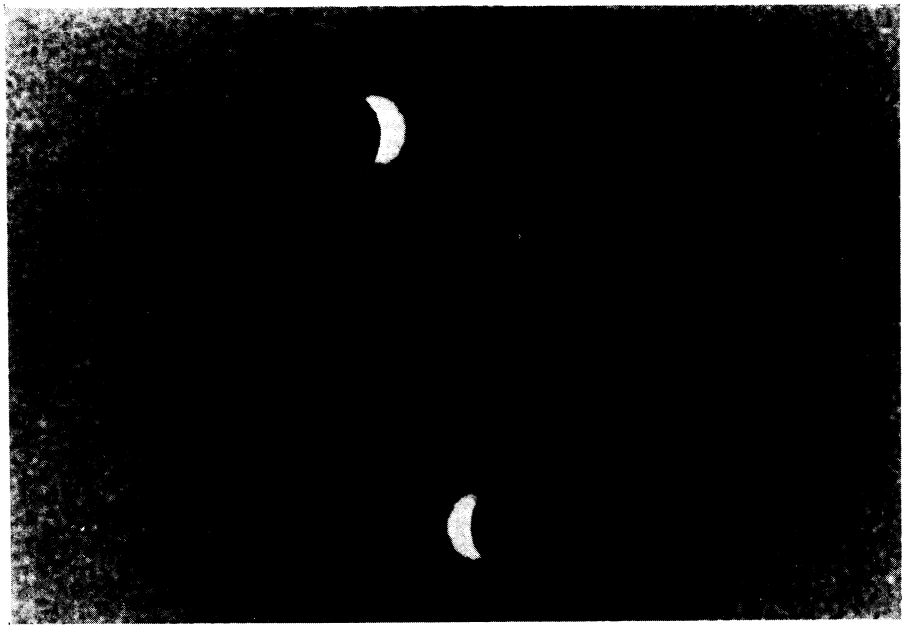

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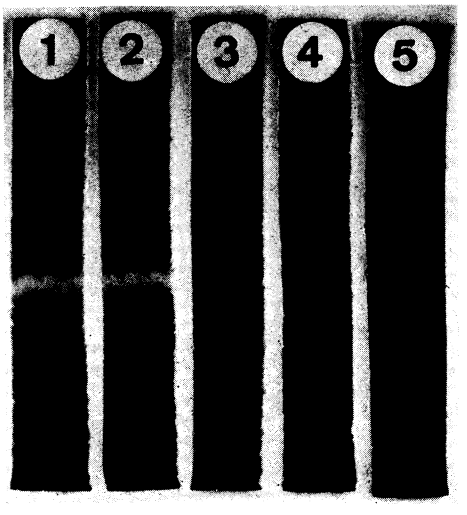

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Fig. 1. Gelatinolytic effect of washed epididymal mouse spermatozoa. For details of the method see Wendt *et al.* (1975).

Fig. 2. Electrophoresis of an acid extract of epididymal mouse spermatozoa with subsequent incubation of the gels under various conditions. The light bands represent zones of proteolytic activity. Gel 1, incubation at pH 8·0; Gel 2, first incubation at pH 4·5, second incubation at pH 8·0; Gel 3, incubation at pH 4·5; Gel 4, first incubation at pH 4·5 with inhibitor added, second incubation at pH 8·0; Gel 5, incubation at pH 8·0 with inhibitor added. All incubations were carried out at 37°C for 1 h; the incubation buffers were 0·2 M-sodium acetate pH 4·5 or 0·1 M-sodium phosphate, pH 8·0.

(*Facing p.* 76)