AN ORGANIZED DISTRIBUTION OF ACROSOMAL PROTEINASE IN RABBIT SPERM ACROSOMES (1)

RICHARD STAMBAUGH, MONICA SMITH AND SAMIHA FALTAS
Division of Reproductive Biology, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174

ABSTRACT Acrosomal proteinase was found to be present in a highly organized distribution within the acrosomes of rabbit spermatozoa by cytochemistry. This distribution consists of at least six linear loops of evenly spaced proteinase granules, which run diagonally across the flat side of the sperm head in a criss-crossing pattern with the two most anterior loops surrounding the region of the acrosome reaction. This is the first description of an organized distribution of enzymes within a lysosome-like organelle.

Acrosomal proteinase or acrosin is present in the acrosomes of spermatozoa, and its lytic activity is essential for fertilization (Stambaugh and Buckley, '68,'69,'72; Stambaugh et al., '69; Zaneveld et al., '70; Polakoski et al., '71; Multamäki and Niemi, '72; Pederson, '72; Srivastava et al., '64). Initially we demonstrated the subcellular localization of this proteinase in the acrosome by subcellular fractionation using sucrose density gradient centrifugation (Stambaugh and Buckley, '68, '69), and confirmed this subcellular localization by histochemistry using fluorescein-labeled trypsin inhibitors (Stambaugh and Buckley, '72). Fluorescein-labeled lima bean and soybean inhibitors were specifically bound by the acrosomes of epididymal, ejaculated, or capacitated rabbit sperm, indicating that the enzyme exists in an active form in the acrosome both before and after capacitation. This article describes a highly organized distribution of acrosomal proteinase within acrosomes using a modification of the silver-proteinate staining procedure of Yanagimachi and Teichman ('72).
MATERIALS AND METHODS Ejaculated spermatozoa were collected from New Zealand White rabbits with an artificial vagina and epididymal spermatozoa were flushed from the caudal end of the epididymides with isotonic sodium chloride. Thin smears were made from these washed sperm (3X), air-dried, and fixed in 100% ethanol at -20°C for 30 minutes. The slides were rinsed with 50% ethanol followed by distilled water for five minutes.

Staining of the slides for proteolytic activity was carried out for 4-6 hours in the dark at 37°C with 47 ml of 0.21% silver proteinate in 0.094M Tris-maleate buffer, pH 7.5, containing 0.085% potassium bromide. The stained slides were washed five times with distilled water and transferred to Kodak D-76 developer for 5 minutes at room temperature. Immediately after developing, they were treated with 2.0% sodium thiosulfate for 5 minutes, rinsed once with distilled water, and dehydrated in ethanol. The slides were cleared with xylene, mounted, and examined by phase-contrast microscopy. The sites of proteinase activity are indicated by a dark brown-black precipitate of silver. Silver proteinate for staining was obtained from Ebisu Pharmaceutical Co. (Japan).

RESULTS Figures 1a and 1b show a highly organized distribution of acrosomal proteinase in the acrosomes of rabbit spermatozoa. At least six linear loops of evenly spaced proteinase granules are evident in each acrosome, which criss-cross on the flat side of the sperm head. The loops seem to expand with the outer acrosomal membrane in the hypotonic silver proteinate staining medium. In the rabbit an additional loop is usually present encircling the equatorial region of the head. No differences were evident between ejaculated and epididymal spermatozoa, and the proteinase is obviously enzymically active in both types of spermatozoa.
From the observation of numerous stained acrosomes it seems possible that all of these linear strands of proteinase activity may be one continuous linear array of enzyme granules, which wind back and forth to form these distribution patterns.

DISCUSSION Staining with silver proteinate has given us more detailed information concerning the subcellular distribution of acrosomal proteinase, and it demonstrates for the first time an organized enzyme distribution within a lysosomal-like body. It seems likely that the region of cross-over of the anterior loops closely surrounds the acrosome reaction region, immediately exposing the zona pellucida to a high concentration of proteolytic activity. The appearance and distribution of these sites of proteinase activity in intact and disrupted acrosomes suggest that these loops may all be situated in one continuous strand in this organelle, and the observation that these loops expand with the outer acrosomal membrane in hypotonic staining medium suggest that the enzyme is not bound to the inner acrosomal membrane as we previously believed (Stambaugh and Buckley, '72). It is tempting to speculate that additional membrane bound acrosomal enzymes will be found on the same strand between the proteinase granules. It will also be interesting to determine if lysosomes from other cells contain similar organized enzyme distributions, since the acrosome originates like a lysosome during spermiogenesis and is apparently a highly modified lysosome (Allison and Hartree, '70).

LITERATURE CITED


REFERENCES

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FIGURE LEGENDS

1 Rabbit spermatozoa stained with silver proteinate for acrosomal proteinase activity: (a) epididymal, (b) ejaculated