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Acrosomal Proteinase and Proteinase Inhibitor of Human Spermatozoa

Abstract. *The acrosomal proteinase of human spermatozoa was characterized and differs from other human proteinases. The enzyme has optimal activity at* p*H 8.0, is inactive below* p*H 5.0 or above* p*H 10.5, requires calcium for maximum activity, hydrolyzes fibrinogen, gelatin, and benzoylarginine ethyl ester, and is inhibited by various synthetic and natural proteinase inhibitors. The molecular weight was estimated to be 30,000. Human spermatozoa also possess a proteinase inhibitor that is similar to one of the proteinase inhibitors from human seminal plasma.*

Recently the proteinase of spermatozoa has received considerable attention. It was shown to be present in all mammalian sperm studied including those of the rabbit (*1–6*), rat (*5, 6*), guinea pig (*5*), mouse (*5, 6*), hamster (*6*), stallion (*5*), boar (*5, 7*), ram (*1, 5*), bull (*1, 5*), human (*3, 5, 6*), and rhesus (*3*) and squirrel monkeys (*5*). Gelatin slide experiments using whole sperm cells (*6*) substantiated previous evidence (*1, 2*) that the proteinase is located in the acrosome of the spermatozoon. Sperm acrosomal preparations dissolve the zona pellucida of rabbit ova (*1, 2*), a process that can be inhibited by proteinase inhibitors (*2*). Fertilization of rabbit ova is prevented in vitro by addition of soybean trypsin inhibitor to the sperm-egg medium (*8*) and in vivo if capacitated sperm—that is, sperm that resided in the female genital tract for at least 6 hours—are first incubated with the pancreatic trypsin inhibitor (Kunitz) or inhibitors isolated from rabbit seminal plasma before insemination into the oviducts of donor rabbits (*9*). Tosyllysine chloromethyl ketone (TLCK), a synthetic trypsin inhibitor, prevents fertilization when added to the rabbit vagina before coitus (*10*).

These experiments emphasize the important role of the sperm acrosomal proteinase in the reproductive process and show that the inhibition of this enzyme prevents fertilization. Although the rabbit sperm acrosomal proteinase has been studied (*11, 12*), no data are available concerning the acrosomal proteinase of human spermatozoa. The properties of this enzyme were determined, with special emphasis on the inhibitory effect of natural and synthetic proteinase inhibitors. During the initial study it became apparent that the acrosomal extracts of human spermatozoa contain an inhibitor of the acrosomal proteinase. The properties of this inhibitor were also evaluated.

Freshly collected human semen samples were centrifuged to remove the seminal plasma and washed three times with 0.15*M* NaCl. Acrosomal extracts were prepared by incubating the spermatozoa with 0.075 percent Hyamine 2389 (Rohm and Haas) and 0.075 percent Triton X-100 (Rohm and Haas) for 90 minutes at 37°C (*7*). The suspension was centrifuged, the supernatant solution was treated with ethanol (80 percent), and the precipitated protein was extensively dialyzed and lyophilized. The acrosomal preparations were tested for activity by dissolving in 0.1*M* borate buffer, *p*H 8.0, containing 0.05*M* $CaCl_2$ and subsequent addition to benzoylarginine ethyl ester (BAEE, 0.2 mg/ml) as substrate in the same buffer (*13*). One milliunit (mU) was defined as that amount of enzyme that caused a change in absorbance of 0.001 per minute at a wavelength of 253 nm. After Sephadex-gel filtration, acrosomal inhibitor activity was determined by incubating 0.1 ml of the inhibitor solution, adjusted to *p*H 8.0, with 0.1 ml bovine pancreatic trypsin (Worthington) (3 μg) for 5 minutes at room temperature before

Table 1. Comparative properties of various human proteinases. Inhibitor tests were made by incubating 0.1 ml of inhibitor in saline with 0.1 ml of the acrosomal proteinase in 0.1*M* borate buffer, *p*H 8.0, containing 0.05*M* $CaCl_2$, for 5 minutes at room temperature before addition of 0.1 ml of the mixture to BAEE. As control, the acrosomal proteinase was incubated with saline. Inhibition is shown as +, no inhibition as −. Inhibition is marked as progressive or immediate for those enzymes and inhibitors for which this has been determined.

Properties	Acrosomal proteinase	Pancreatic trypsin	Plasmin	Thrombin	Reference
Molecular weight	30,000	22,900	75,400	31,000	(*18, 19*)
Effect of inhibitors					
Tosyllysine chloromethyl ketone	+	+	+	+	(*18, 20, 21*)
Tosylamide phenylethyl chloromethyl ketone	−	−	−	−	(*18, 20, 21*)
Diisopropyl fluorophosphate	+	+	+	+	(*18, 21–23*)
Soybean trypsin inhibitor	+	+ (weak)	+	−	(*18, 24*)
Trypsin-kallikrein inhibitor (Kunitz)	Progressive	Immediate	+	−	(*18, 24*)
Potato trypsin inhibitor	+	−	−	−	(*24*)
Ovomucoid trypsin inhibitor	+ (weak)	−	−	−	(*24*)
Inter-α-trypsin inhibitor	+	+*	−	−*	(*25*)
α_1-Antitrypsin	Progressive	Immediate*	Progressive	−*	(*25*)
Antithrombin III	+	+*	+ (weak)	+*	(*25*)
α_{1x}-Antichymotrypsin	−	−*	−	−*	(*25*)

* Results are available for species other than the human only.

addition to BAEE. As a control, pancreatic trypsin was incubated with 0.1 ml of neutralized buffer.

The proteinase activity of the acrosomal extracts varied between 50 to 150 mU per milligram of protein. The activity increased six- to sevenfold when the *p*H of the preparations was lowered to below 3.0 before addition to the substrate-buffer solution (*p*H 8.0) and decreased again when the *p*H was raised before testing for activity. This could be repeated various times with the same solution. Similarly, if untreated ejaculated human spermatozoa were incubated in 0.1*M* tris buffer, *p*H 6.9, for 30 minutes at room temperature and centrifuged, the activity of the supernatant solution increased sevenfold when the *p*H of this solution was lowered to 3.0 before addition to BAEE. These results indicate (*7*) that most of the proteinase that is released from the human sperm acrosome is reversibly bound to a proteinase inhibitor.

The acrosomal extracts were dissolved in 0.1*M* KCl–HCl buffer, *p*H 2.2, for 2 hours at room temperature to cause complete dissociation of the proteinase–proteinase inhibitor complex. The solution was centrifuged and the supernatant added to a Sephadex G-50 column with the same buffer as eluent. The acrosomal proteinase was eluted with the unabsorbed material, whereas the inhibitor was retarded. The highly active proteinase fractions did not vary in activity when the *p*H of the solutions was raised or lowered before the BAEE test, an indication that all inhibitor was removed. These fractions were used for the characterization of the enzyme. The inhibitor fractions were pooled and concentrated by lyophilization.

Human acrosomal proteinase hydrolyzes fibrinogen and gelatin as well as BAEE. The enzyme does not hydrolyze benzoyltyrosine ethyl ester, a synthetic chymotrypsin substrate. The proteinase is stable at *p*H 2.2 for at least 7 days, has an optimum *p*H of 8.0, is inactive below *p*H 5.0 or above *p*H 10.5 and requires calcium for maximum activity. The molecular weight of the acrosomal enzyme was estimated to be 30,000 by gel filtration with a Sephadex G-100 column with the use of bovine serum albumin, horseradish peroxidase, pancreatic trypsin (bovine), cytochrome c, and highly purified trypsin-kallikrein inhibitor from bovine lung (*14*) as standards and 0.1*M* acetate buffer, *p*H 6.0, containing 0.15*M* NaCl as buffer (*15*).

Human acrosomal proteinase is inhibited by (i) the synthetic proteinase inhibitors: diisopropyl fluorophosphate (DFP), tosyllysine chloromethyl ketone, but not tosylamide phenylethyl chloromethyl ketone, a chymotrypsin inhibitor; (ii) the natural proteinase inhibitors: trypsin-kallikrein inhibitor from bovine lung (*14*), soybean trypsin inhibitor, potato trypsin inhibitor (*14*), and less effectively by ovomucoid trypsin inhibitor; and (iii) the serum proteinase inhibitors that are also present in human cervical mucus except at mid-cycle (*16*): inter-α-trypsin inhibitor (*14*), α_1-antitrypsin (*14*), and antithrombin III (*14*), but not α_{1x}-antichymotrypsin (*14*). α_1-Antitrypsin and the trypsin-kallikrein inhibitor from bovine lung are progressive inhibitors of the human acrosomal proteinase, but are immediate inhibitors of pancreatic trypsin. Table 1 shows that the inhibitor spectrum of the human acrosomal proteinase differs from that of trypsin, plasmin, and thrombin. The other mammalian proteinases—such as chymotrypsin, elastase, collagenase, the cathepsins, and the kallikreins—differ from the acrosomal proteinase not only in inhibitor spectrum but also in various other respects, for instance, substrate specificity, *p*H optimum, molecular weight, or cofactor requirement. The acrosomal proteinase of human spermatozoa therefore appears to be a unique enzyme.

The human acrosomal proteinase inhibitor has a molecular weight of 5600 as estimated by Sephadex G-100 gel filtration and inhibits trypsin as well as the acrosomal proteinase when either BAEE or fibrinogen are used as enzyme substrates. The acrosomal proteinase inhibitor does not inhibit chymotrypsin. These characteristics are similar to those of the low molecular weight inhibitor (fraction II) of human seminal plasma (*17*), and these two inhibitors are most likely identical. At present it is not known whether this inhibitor is located inside the outer acrosomal envelope and in complex to the acrosomal proteinase, or is situated on the outside of the acrosome and forms a complex with the acrosomal proteinase as this enzyme is released from the spermatozoon. The inhibitor is most likely removed from the spermatozoon in the female genital tract so that sperm penetration through the zona pellucida can occur.

Further studies on the proteinase–proteinase inhibitor system of human semen and uterine secretions that may be involved in the fertilization process as well as in the mechanism of sperm migration could open new avenues to fertility control by means of biochemical or immunological interference with the action of this apparently unique acrosomal proteinase.

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