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Human Fertilization

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Spermatozoa Acrosin and Seminal Plasma Acrosin Inhibitors

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Properties of Acrosin Inhibitors

Acid-stable inhibitors of acrosin, the trypsinlike enzyme localized in the acrosome of the spermatozoon's head, are found in spermatozoa, in seminal plasma and in male sexual organs (Fritz et al. 1975). These inhibitors are polypeptides with molecular weights in the range of 6,000 to 7,000 and consist of approximately 60 amino acids. They can form an equimolar complex with acrosin by binding to the active site of the enzyme, thus blocking its hydrolytic activity towards natural and synthetic substrates. All known acrosin inhibitors are also inhibitors for trypsin (Table 1). Table 2 summarizes for some species the trypsin inhibiting activities found in the sex organs and in seminal plasma. The glands which contribute most to the inhibitory content of seminal plasma are the seminal vesicles.

Table 1 Inhibition properties of inhibitors of the male genital tract and porcine pancreatic secretory inhibitor

Source		Inhibition of Acrosin	Trypsin	Plasmin	Chymotrypsin
Human seminal plasma	I	–	+	–	+
	II	+	+	–	–
Boar seminal plasma	I	+	+	+	–
	II	+	+	+	–
Guinea pig seminal	I	+	+	–	–
vesicles	II	+	+	+	–
Porcine pancreas		+	+	–	–

Table 2 Trypsin inhibiting activities in male sex organs and seminal plasma

Species	mIU[+] per g tissue or ml plasm Testes	Epididymis	Seminal vesicles	Seminal plasma
Man	70–100	50–80	50–100	150–330
Pig	90–120	70–110	500–1,000	800–1,200
Cattle	40–70	50–80	900–1,500	2,400–3,100
Guinea pig	100–220	300–400	3,500–5,000	
Mouse	90–130	100–200	2,200–2,700	
Hamster	60–90	80–120	300–600	
Rat	100–200	90–130	1,400–1,600	

[+] 1 mIU inhibits the activity of approximately 1 μg trypsin Novo

Biochemically best characterized are the inhibitors from boar seminal plasma, guinea pig seminal vesicles and human seminal plasma. The amino acid sequence of the boar seminal plasma inhibitor (Fig. 1; Tschesche et al. 1976), is homologous to the sequences of pancreatic secretory inhibitors; the same holds true for one of the inhibitors of guinea pig seminal vesicles and very probably also for the acrosin inhibitor of human seminal plasma.

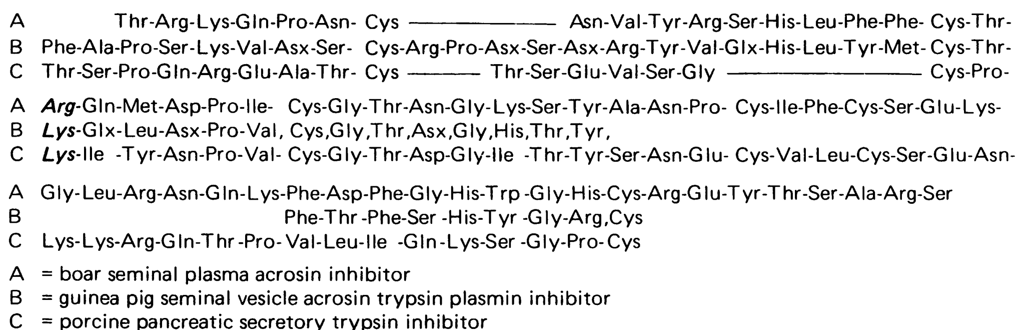

Fig. 1 Alignment of the homologous amino acid sequences of the boar seminal plasma acrosin inhibitor and the partial sequence of the guinea pig seminal vesicle acrosin trypsin plasmin inhibitor with the sequence of porcine pancreatic secretory trypsin inhibitor

Physiological Function of Acrosin Inhibitors

The physiological role of the acrosin inhibitors is not yet fully understood. But it is a reasonable assumption that the main function of the inhibitors is to protect spermatozoa as well as cell membranes and secretory proteins of the male and female genital tract against acrosin which might be liberated from disintegrating spermatozoa during maturation and after ejaculation.
The physiological function of the trypsin-chymotrypsin inhibitor present in human seminal plasma which has no effect on acrosin is discussed elsewhere in this volume (see Schiessler et al. this volume).

Isolation and Properties of Acrosin

Acrosin was demonstrated to be present in the spermatozoa of all species investigated. There was only one report claiming that mouse spermatozoa did not contain acrosin (Erickson and Martin 1974). However, recently Brown and Hartree (1976) published results showing that acrosin is present also in mouse spermatozoa. These results were confirmed in our laboratory by an experimentally different approach (Fink and Wendt, in press).

Acrosin can be extracted from spermatozoa by neutral detergent-containing buffers or by acidic solutions. We usually use the acidic extraction procedure (Fink et al. 1972) and isolate the acrosin from the extract by gel filtration followed by affinity chromatogrophy on p-aminobenzamidine aminoalkyl cellulose (Schleuning et al. 1975).
Boar acrosin isolated by this method from ejaculated spermatozoa showed only one substantial protein band in acrylamide gel electrophoresis at pH 4.8 and one sharp digestion zone when gelatin was incorporated in the gel (Fig. 2). In SDS electrophoresis the molecular weight of the main protein band was calculated as 38,000. Two additional minor fractions had molecular weights of 37,000 and 34,000. These two bands may represent "degraded" molecules with one or more peptide bonds hydrolyzed either by acrosin itself or by other proteinases.
Human acrosin was isolated by the same procedure as boar acrosin. By electrophoretic analysis (Schleuning et al. 1976) of the preparations four forms of acrosin were distinguish-

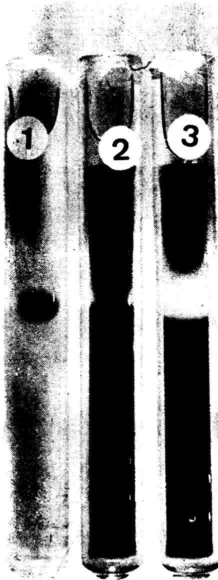

Fig. 2 Polyacrylamide gel electrophoresis of boar acrosin preparations
Gel 1: pure acrosin, Coomassie blue stained;
Gel 2: acidic sperm extract;
Gel 3: pure acrosin.
The unstained bands in Gel 2 and 3 represent zones of gelatinolytic activity

able. The ratio of the four forms in the acrosin preparations differed from batch to batch. By SDS electrophoresis of the four forms protein bands with the apparent molecular weights of 64,000, 38,000, 25,000, 15,000 and 12,000 were observed. The bands with 15,000 and 12,000 daltons can be interpreted as two peptides originating from an acrosin form with a cleaved peptide bond.
The low molecular weight acrosin forms can be produced from the high molecular weight form by incubation at pH 8. During this incubation the enzymic activity increases twofold. We cannot yet discriminate whether the high molecular weight form is already enzymatically active or, at least partly, represents the inactive precursor of acrosin, called proacrosin. Molecular forms of different molecular weights for acrosins of various species have been observed by several authors, the occurrence of an active acrosin form with similar molecular weight as proacrosin was recently shown for rabbit testis acrosin by Mukerji and Meizel (1975). Inactive proacrosin was demonstrated by these authors first in testes and later on in epididymal spermatozoa (Meizel and Mukerji 1975). Proacrosin has also been found in ejaculated spermatozoa by other groups (Zaneveld et al. 1975) and in our laboratory (Fritz et al. 1975a). Presumably, the activation of proacrosin takes place via limited proteolysis, a mechanism which is well established for several proteases involved in the blood clotting system. During such an activation process intermediate enzyme forms are produced with molecular weights between those of the proenzyme and the fully activated enzyme.

The enzymic properties of boar and human acrosin and most probably of the acrosins from other species are very similar to those of trypsin (Table 3); to our knowledge, only quantitative but thus far no qualitative differences have been observed. There are striking similarities also in the amino acid compositions of some forms of acrosin and trypsin (Schleuning et al. 1976, Stambaugh and Smith 1975), but in contrast to trypsin acrosin is a glycoprotein.

Localization of Acrosin

The subcellular localization of acrosin is not yet definitively known. Several groups could demonstrate a relatively strong binding of acrosin to acrosomal membranes, probably the inner acrosomal membrane and/or the equatorial segment (for further references, see Fritz

Table 3 Properties of acrosin and trypsin

	Acrosin	Trypsin
Specific activity U/mg		
BAPA[a]	18.8[e]	1.2
BAEE[b]	214	36
Equal casein digestion rate is caused by	4.5 mg	1 mg
Bimolecular rate constant of inhibition, l/mole x min		
DFP[c]	630	300
TLCK[d]	23	34
Dissociation constants K_i (mole/l) of complexes with:		
leupeptin and antipain	7×10^{-8}	2×10^{-7}
seminal acrosin inhibitors	$< 1 \times 10^{-9}$	$< 1 \times 10^{-9}$
kinin liberation from kininogen	+	+

a N-α-benzoyl-DL-arginine p-nitroanilide
b N-α-benzoyl-L-arginine ethyl ester
c Diisopropylfluorophosphate
d N-α-tosyl-L-lysyl-chloromethane
e Protein was estimated by the biuret method

et al. 1975a and Zaneveld et al. 1975). This assumption is especially supported by the following observations.

1. Denuded, i.e. acrosome-free ram spermatozoa contain nearly as much acrosin activity as can be extracted from intact spermatozoa (Brown and Hartree 1974)
2. In spermatozoa of frozen-thawed semen samples usually a significant increase in extractable acrosin activity is observed. By this treatment the plasma membrane and the outer acrosomal membrane are extensively disrupted and removed (Schill and Wolff 1974)
3. Immunochemical studies (Morton 1975, Schill and Wolff 1974) give strong evidence that acrosin is bound to the inner acrosomal membrane.

Physiological Role of Acrosin (Fig. 3)

It is generally accepted that acrosin is involved in the penetration of sperm through the zona pellucida of the egg (Zaneveld et al. 1975, Fritz et al. 1975a, Stambaugh and Smith 1975). In the presence of acrosin inhibitors the zona layer is not dissolved by acrosin and penetration of spermatozoa through the zona is prevented.

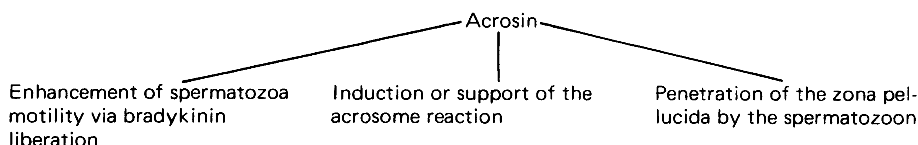

Fig. 3 Possible effects of acrosin

Very recently Meizel and Lui (1976) presented evidence for a possible involvement of acrosin in the acrosome reaction. The acrosome reaction is a process during which fusion of the outer acrosomal membrane and the adjacent plasma membrane occurs followed by vesiculation and consequently loss of the acrosomal content. This sequence of events is essential to mammalian spermatozoa penetration of the zona pellucida and takes place in spermatozoa that are within or close to the cumulus oophorous cell layer and have under-

gone capacitation. Meizel and Lui (1976) demonstrated that the acrosome reaction in capacitated hamster spermatozoa is drastically inhibited by low molecular weight trypsin inhibitors (α-N-tosyl-L-lysine chloromethylketone and p-nitrophenyl-p-guanidinobenzoate). The authors conclude that a trypsinlike enzyme of the spermatozoa, possibly acrosin, is involved in the acrosome reaction.

Another function of acrosin may be the enhancement of spermatozoa motility by the release of kinins since acrosin is a potent bradykinin liberating protease. This is discussed in more detail in this volume (see Schill et al.).

Summarizing the present knowledge one can draw the following speculative conclusions on the role of acrosin (Fig. 3) and acrosin inhibitors in fertilization:

Acrosin is synthesized as proacrosin and bound to the inner acrosomal membrane of the spermatozoa. To prevent premature autoactivation and liberation of acrosin, there are inhibitors present in the acrosome and in the adjacent fluids. After ejaculation, further acrosin inhibitors are picked up from the seminal plasma and are adsorbed to the outer surface membranes of the acrosome. These adsorbed inhibitors protect intact spermatozoa against acrosin released from disintegrated spermatozoa. During migration of the spermatozoa through the female genital tract or during capacitation, the inhibitor (s) is removed from the spermatozoa surface and proacrosin is converted to acrosin. The acrosome reaction, in which acrosin plays a role, takes place within or close to the cumulus oophorous of the egg and then the spermatozoon penetrates the zona pellucida by the assistance of acrosin.

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