ACROLYSIN, THE AMINOPROTEINASE CATALYZING THE INITIAL CONVERSION OF PROACROSIN TO ACROSIN IN MAMMALIAN FERTILIZATION

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SUMMARY: Extracts of mammalian sperm acrosomes and testes contain a proteinase, acrolysin, that hydrolyzes the aminopeptide bonds of hydrophobic aminoacyl residues. Acrolysin has hydrolytic specificity and properties similar to the proteinase, thermolysin (E.C. 3.4.24.4), that also catalyzes the conversion of proacrosin to acrosin (E.C. 3.4.21.10). Acrolysin is the apparent initiator of proacrosin activation in mammalian fertilization.

Mammalian sperm acrosomes contain several proteinases as determined by hydrolysis of synthetic substrates. Acrosin, an enzyme hydrolyzing primarily Arg-X bonds (1), was initially detected in rabbit acrosomal extracts using Bz-Arg-O-Et as substrate (2). An enzyme hydrolyzing the Leu-Gly bond of the chromophoric collagen substrate 4-phenylazobenzyloxycarbonyl-L-Pro-L-Leu-Gly-L-Pro-D-Arg exists in bull, human and rat acrosomal extracts (3), an arylamidase has been observed in bull acrosomal extracts that hydrolyzes the naphthylamides of Met>>Ile>Leu, Val>Phe>α-Asp (4). Acrosin dissolves the zona pellucida of ova *in vitro* and is apparently involved in sperm penetration of this layer in normal fertilization (5). No role in fertilization has been assigned to the other proteinases.

Protein digestion by ram acrosomal extracts indicates the presence of multiple proteinases based on activity at varying pH optima (6). An acidic proteinase in extracts of the sperm acrosomes of the rooster and five mammalian species has been briefly described (7).

More systematic study of the hydrolysis of protein and peptide substrates

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of known sequence has permitted detailed characterization of the proteolytic activity in mammalian sperm acrosomes and testes. Acrosin is the major proteinase in the sperm acrosome. Acrolysin, present in much smaller amounts, is a proteinase that initiates the conversion of proacrosin to acrosin by cleaving the aminopeptide bond of hydrophobic aminoacyl residues.

MATERIALS AND METHODS

Phosphoramidon was kindly supplied by Dr. T. Aoyagi, Institute of Microbial Chemistry, Tokyo. Other materials were from commercial sources: thermolysin (Calbiochem), RCM-Insulin A and B chains (Ins-A, Ins-B, Schwarz/Mann) and Concanavalin A Sepharose (Con A, Pharmacia).

Proacrosin was purified from 0.15 M NaCl, 0.01 M HCl extracts of testes by chromatography on 2 ml Con A columns after dilution with equal volumes of 2 M NaCl in 0.2 M acetate buffer, pH 6.0, containing 2.5 mM, Ca^{++}, Mg^{++} and Mn^{++}. Elution was accomplished with ion-free buffer containing 30 mg/ml α-methyl-mannoside as shown in Fig. 1.

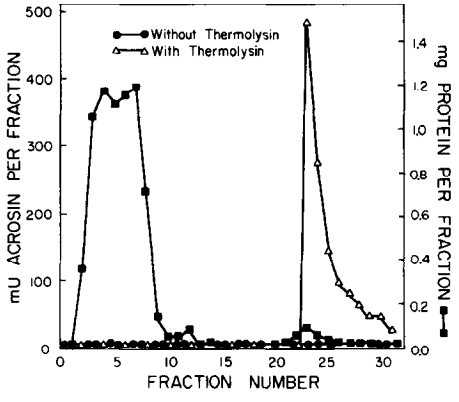


Figure 1. Purification of Proacrosin or Concanavalin A Sepharose 2 ml ConA, 1.8 ml fractions, 7 min activation with thermolysin.

Protein was estimated according to Bradford (8). Acrosin (and activated proacrosin) activity was measured by hydrolysis of Bz-Arg-O-Et at 253 nm (9). Amino acid analyses were conducted on a Beckman 120C amino acid analyzer. Acrosomal extracts were prepared according to Srivastava et al. (10).

RESULTS AND DISCUSSION

RCM-lysozyme hydrolyzed at 37°C by 0.05 M $MgCl_2$ extracts of rabbit epididymal sperm in 0.2 M N-ethyl morpholine buffer, pH 8.6 for 12-18 hours yielded large amounts of arginine and much smaller amounts of lysine on

analysis due to hydrolysis of Arg-X (and Lys-Lys) bonds by acrosin and apparently an acrosomal carboxypeptidase B. In addition, the release of the remaining 18 residues of the RCM-lysozyme molecule indicated the presence of an amino peptidase and the possibility of other endoproteinase activity in the extracts. Varying enzyme concentrations, incubation times and pH failed to provide further clues to the existance of other proteinases since acrosin was the major proteinase present.

Ins A (containing no basic aminoacyl residues) was selected for further study to eliminate complications from acrosin hydrolysis. Digests of Ins A (0.33 μM) under the same conditions gave the following amino acid analysis, clearly establishing the presence of an aminopeptidase hydrolyzing (/) bonds and a proteinase hydrolyzing the amino peptide bond of leucine residues (//).

	1	2	3
	H_2N-Gly /	Ile /	Val /
μM Found	.010	.007	.006

	12	13	14	15	16	17	18	19	20
	Ser //	Leu /	Tyr /	Gln //	Leu /	Glu /	Asn /	Tyr -	Cys -
μM Found	0	.275	.22	.22	.275	.25	.10	0	0

Ins B chain, known to give Ala and peptides with N-terminal Leu, Phe, Tyr and Val on thermolysin digestion (11), digested with similar acrosomal extracts yielded only these 5 amino acids plus Gly arising from cleavage of the ^{22}Arg-^{23}Gly bond by acrosin and subsequent aminopeptidase hydrolysis.

Thermolysin (12), the proteinase from Bacillus thermoproteolyticus is competitively inhibited by phosphoramidon (13). Phosphoramidon also reduced acrosomal aminoproteinase activity by 80% in similar experiments with Ins A. The aminopeptidase was not inhibited.

Thermolysin is a zinc-enzyme (1 Zn; 37,500 gm) that is stabilized by Ca^{++} and contains large but variable amounts of Ca^{++} after rigorous purification (14). The chelating agents, EDTA and 1,10-phenanthroline inhibit both enzymes (Table 1).

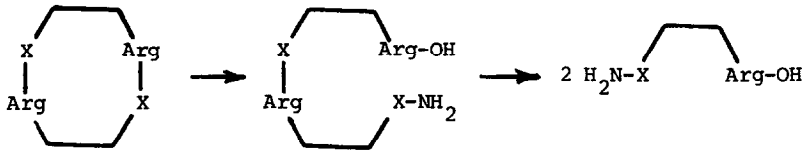
Acrosin occurs as the proenzyme in testicular extracts (15) and in

TABLE 1

Comparison of Properties of Aminoproteinases

Characteristics on Test Substances	Thermolysin	Acrolysin
Specificity, Ins B	X-leu,X-ilu, X-val,X-ala,X-phe (11)	X-leu,X-ilu X-val,X-ala,X-phe
EDTA	Inhibits (11)	Inhibits
1,10-phenanthroline	Inhibits (11)	Inhibits
Phosphoramidon	Inhibits (13)	Inhibits
Proacrosin	Activates	Activates

acrosomal extracts of epididymal (16) and ejaculated (17) sperm. Proacrosin is activated only by enzymes hydrolyzing Arg-X bonds: trypsin, acrosin, plasmin and kallikrein (18). "Autoactivation" follows a sigmoid activity curve and as activity appears a molecular weight of 73,000 is maintained. As optimum activity is approached the molecular weight of acrosin (38,000) is achieved (16). The proenzyme apparently contains two enzyme units rather than one enzyme and an activation peptide of similar molecular weight since the specific activity of the totally activated proenzyme is the same as that of acrosin (19). A model based on current data for proacrosin activation is as follows:



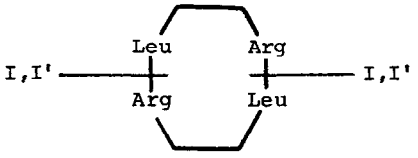
73,000 inactive Proacrosin

73,000 partially active Proacrosin'

38,000 active Acrosin

If the Arg-X activation linkage contains a leucine, isoleucine, valine

or other hydrophobic X residue hydrolyzed by acrolysin and thermolysin, the proacrosin model becomes:



Where (I) would be the initiating reaction by aminoproteinase and (I') would be subsequent autoactivation by acrosin.

To test this hypothesis the effect of thermolysin in proacrosin activation was studied in the presence of Bz-Arg-O-Et, which competes (as does Bz-Arg after hydrolysis) with proacrosin for any activated acrosin molecules. The results are presented in Figure 2. Thermolysin activates all rabbit proacrosin in 5 min. Autoactivation by acrosin is suppressed as shown by the linear hydrolysis of substrate in the absence of thermolysin. These results clearly demonstrate that aminoproteinases can specifically activate proacrosin. The reaction is not unique to the rabbit since we have observed similar activation of proacrosin from the cat, hamster, opossum and pig.

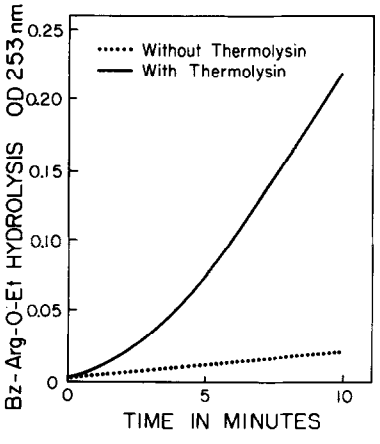


Figure 2. Enzymatic Activation of Proacrosin

Purified Proacrosin (85X), Bz-Arg-O-Et (17 mg/100 ml) in 0.05 M Tris buffer, pH 8.6, 0.05 M $CaCl_2$, 25°C.

Complete purification of proacrosin has not been reported. Activation of the purified fractions appears to be the result of contamination by acrolysin since "autoactivation" in the rabbit (16) and hamster (20) is inhibited by Zn^{2+} and activated by Ca^{2+}. Acrolysin is also inhibited by Zn^{2+} and activated by Ca^{2+} in the Ins A digestion system.

On the basis of occurrence and similarity to thermolysin (Table 1) we recommend the trivial name, acrolysin, for the acrosomal aminoproteinase and the systematic name, proacrosinase. As the initiator of proacrosin activation acrolysin may be a major factor in the regulation of mammalian fertilization.

Combinations of inhibitors of both acrosin and acrolysin could be possible contraceptive agents since a block of both enzymes would prevent penetration of the zona pellucida.

The roles of acrosomal carboxypeptidase B and aminopeptidase in fertilization remain unclear. The aryl amidase of Meizel and Cotham (4) is apparently analogous to acrosomal aminopeptidase. The N-terminal residue of boar acrosin has been reported as either alanine (21) or valine (22) both of which could arise from acrolysin activation of proacrosin. The traces of N-terminal alanine and methionine observed (22) could be the result of subsequent aminopeptidase action. The collagenase previously reported (4) could also hydrolyze at the aminopeptide, rather than the carboxypeptide bond of leucine, in the synthetic substrate.

The C-terminal residues of acrosin have not been determined. Either or both exopeptidases could function in the production of the multiple forms of acrosin observed in many species (18,23,24). Similar cleavages could also follow the hydrolytic function of acrosin during penetration of the zona pellucida by sperm.

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