Boar Proacrosin

PURIFICATION AND PRELIMINARY ACTIVATION STUDIES OF PROACROSIN ISOLATED FROM EJACULATED BOAR SPERM*

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Two forms of proacrosin have been purified from ejaculated boar spermatozoa. The isolation method utilized benzamidine to inhibit the premature activation of the zymogen and included pH precipitation, ammonium sulfate fractionation, and sodium chloride precipitation. Further purification was achieved by Sephadex G-200 filtration of the preparation after it was treated with 8 M urea. The overall proacrosin yield was 58% with a specific activity of 253 units/mg of protein. The molecular weights of the proacrosins determined by sodium dodecyl sulfate disc gel electrophoresis were 55,000 and 53,000. Proacrosin autoactivation followed the classical S-shaped activation curve and calcium was not required to obtain full activation. Time course activation studies in 0.1 M Tris/HCl, pH 8.4, at 0°C were monitored with sodium dodecyl sulfate-disc gel electrophoresis and analytical gel electrophoresis with staining techniques for protein and enzymatic activity. Under the conditions used, the zymogens were sequentially degraded to three different active species of acrosin (α, β, and γ). The approximate molecular weights of the acrosins were 49,000, 34,000, and 25,000 for the α, β, and γ forms, respectively. The autoactivation is concentration-dependent and can be proteolytically stimulated with either α- or β-acrosin and trypsin, indicating the activation of proacrosin can occur via a bimolecular process.

Acrosin (EC 3.4.21.10) is a unique proteinase present in mammalian sperm acrosomes which enables the sperm to penetrate the zona pellucida of the ovum. Attention to this process has been stimulated by the findings that natural and synthetic acrosin inhibitors prevent both in vivo (1, 2) and in vitro (3, 4) fertilization. Several reviews of this field have appeared in the last few years (5, 6).

Besides trying to understand the physiological process of ovum penetration by sperm, we have focused our investigations on the study of the inhibition and specificity of acrosin to determine the feasibility of developing active site-directed reagents which are specific for acrosin and could possibly be used for contraceptive purposes (7, 8). The major problem which has arisen from these studies is that several different acrosins are found in the acrosomal extracts. The fact that acrosin may exist as more than one molecular species has been inferred from several studies. These include the finding of several bands of acrosin activity by gel filtration (9-11), the observation of several BzArgNNab activities in analytical polyacrylamide gel electrophoresis (12, 13), and the finding of numerous proteins on sodium dodecyl sulfate-gel electrophoresis of the partially purified extracts (14, 15). However, it has not been shown if these differences are the result of aggregation, noncovalent binding of acrosin to other proteins in the extracts, or possibly proteolytic degradation.

Meizel and his co-workers (15-18) have found a zymogen form of acrosin in the testis and epididymal sperm from a number of species and have proposed that activation of this zymogen occurs during the sperm's capacitation process in the female reproductive tract (17). This proposal seems reasonable, for proacrosin accounts for greater than 95% of the acrosin in the ejaculated sperm from the bull, boar, ram, and man.2

The following studies were undertaken to purify the proacrosin from ejaculated boar sperm and to possibly elucidate the mechanism by which proacrosin is activated to acrosin. The procedures used allowed for a good recovery of a highly purified preparation containing two forms of proacrosin. This preparation was autoactivated in the absence of calcium ions and resulted in the sequential formation of three enzymatically active acrosins. Evidence is also presented which indicates that proacrosin can be activated by a bimolecular process. Preliminary data of some of this work have been previously presented (19, 20).

EXPERIMENTAL PROCEDURES

Materials—Sephadex G-200 and aldolase were obtained from Pharmacia. Tris (ultrapure), sucrose (ultrapure), and urea (ultrapure) were obtained from Schwarz/Mann. Black K salt was a product of ICN and K and K Laboratories, Inc. and the electrophoresis supplies were purchased from Bio-Rad Laboratories. Lysozyme, acetylated trypsin, BzArgNNab, and BzArgOEt were Sigma products. Trypsin was from Worthington and the miracloth was obtained from Calbiochem. Benzamidine was from Aldrich Chemical Co., Inc. Solutions used were made with water which was glass distilled, then deionized. Unless otherwise specified, all other chemicals were of reagent grade.

* This research was supported by National Institutes of Health Grant 09422 and by a grant from the Rockefeller Foundation.
Acrosomal Extract—Freshly ejaculated boar semen was adjusted with 1 M benzamidine to a final concentration of 50 mM and filtered through miracloth. Twenty-milliliter aliquots were carefully placed upon a mixture of sucrose, 50 mM sodium chloride, 0.02% sodium azide, and were then centrifuged for 30 min at full speed in a model HNS clinical IEC centrifuge at room temperature. The sperm pellet was resuspended in 10% glycerol, which contained 0.02% sodium azide through miracloth. Twenty-milliliter aliquots were carefully placed with 1 M benzamidine to a final concentration of 50 mM Tris/HCl containing 50 mM calcium chloride and 500 μM BzArgOEt at pH 8.0. The reaction was monitored at 253 nm at 10-s intervals using the Gilford model 240 spectrophotometer equipped with a Gilford 410 digital absorbance meter, a Gilford 4009 data lister, and a constant temperature bath set at 30° ± 0.1°. A molar absorption difference of 1150 M⁻¹ cm⁻¹ was used to convert the data lister.

Purification of Proacrosin—Acrosin activity was measured spectrophotometrically by following the hydrolysis of BzArgOEt (21). The assays were performed in 3.0-ml volumes utilizing a substrate mixture of 50 mM Tris/HCl containing 50 mM calcium chloride and 500 μM BzArgOEt at pH 8.0. The reaction was monitored at 253 nm at 10-s intervals using the Gilford model 240 spectrophotometer equipped with a Gilford 410 digital absorbance meter, a Gilford 4009 data lister, and a constant temperature bath set at 30° ± 0.1°. A molar absorption difference of 1150 M⁻¹ cm⁻¹ was used to convert the data lister.

Proacrosin was assayed by determining the amount of acrosin activity which was produced from the zymogen after activation. The purified proacrosin was autoactivated by incubating the zymogen at pH 8.0 to 8.5, in a final concentration of 0.1 M Tris, 0.1 M calcium chloride, 0.02% sodium azide, and 2.0 mg/ml of bovine serum albumin. At various time intervals, aliquots were removed and assayed for acrosin activity as previously described. One proacrosin unit was equal to one acrosin unit after the proacrosin had been activated. The crude acrosomal extracts contained several acrosin inhibitors (4) which masked the activation at these pH values. These extracts were activated to acrosin when incubated at pH 5.3, in 100 mM sodium acetate, 100 mM calcium chloride, 0.02% sodium azide, and 5 mg/ml of bovine serum albumin. These incubations were run at 37° for 24 h. The extracts were then adjusted to pH 3.0 with 1 M HCl, incubated for 10 min at ambient temperature, and assayed for acrosin at pH 8.0, as previously described. An in depth description of these procedures is found elsewhere (22).

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RESULTS

Purification of Proacrosin—An aliquot of the acrosomal extract prepared as described under “Experimental Procedures” was thawed and diluted to a protein concentration of 2 mg/ml with cold 50 mM benzamidine. Unless specified, all subsequent operations were performed at 0–4°. The pH of the solution was adjusted to pH 5.8 with 1 M NaOH. After a 20-min incubation, the mixture was centrifuged at 27,000 × g for 30 min. The clear supernatant fluid was adjusted to pH 3.0 with 1 M HCl and brought to 30% saturation with solid ammonium sulfate (16.4 g/100 ml). The fluid was readjusted to pH 3.0, and incubated in an ice bath for at least 6 h to allow for complete precipitation of the zymogen. The pellet obtained after centrifugation for 30 min at 27,000 × g was resuspended in approximately 2.0 ml of 1 M HCl, pH 3.0, containing 5 mM benzamidine. A clear solution was obtained and dialyzed overnight against 2 liters of 500 mM NaCl, pH 3.0, containing 5 mM benzamidine. This step removed any active acrosin that was present in the preparations (less than 1% in most of the extracts) as well as several contaminating proteins of greater than 60,000 molecular weight. At this point, sodium dodecyl sulfate gels indicated the presence of the zymogen and another lower molecular weight protein which could not be separated by simple gel chromatography. Preliminary experiments demonstrated that proacrosin was reversibly denatured in urea and that the removal of the urea by dialysis caused no loss in recovered acrosin activity after activation. This was incorporated as the step to separate the low molecular weight protein from the proacrosin. One milliliter of the zymogen preparation was dialyzed for at least 10 h against fresh 8 M urea which had been adjusted to pH 3.0. Two milliliters of the urea solution was applied to a Sephadex G-200 column followed by the zymogen preparation and an additional 10 ml of the urea solution. The column was then developed with 1 M HCl (Fig. 1). A typical protocol for the combined purification procedures is presented in Table I.

The final preparation appeared to be homogeneous, for the
zymogen appeared in a single symmetrical band of constant specific activity; however, two closely migrating protein bands were observed in both the sodium dodecyl sulfate-gel electrophoresis (first gel in Fig. 5) and in the analytical gel electrophoresis (first gel in Fig. 6). To obtain an accurate molecular weight estimation, the purified preparation was applied to an 11-cm sodium dodecyl sulfate disc gel with the following reference proteins: bovine serum albumin, aldolase, trypsin, and lysozyme (Fig. 2). A comparison of the zymogen bands in the sodium dodecyl sulfate gel to the reference proteins gave indicated molecular weights of 55,000 and 53,000 (Fig. 3).

**Autoactivation of Boar Proacrosin**—Acrosin activity was not observed if the purified preparations were refrigerated at pH 3.0, for up to 4 weeks, although rapid activation occurred when the pH was adjusted to a pH above 7.0. The time course of the activation of proacrosin in 0.1 M Tris, pH 8.4, at 0° was not linear with time, but the appearance of acrosin activity followed a sigmoidal curve (Fig. 4). Aliquots were removed at various time intervals and subjected to sodium dodecyl sulfate-gel electrophoresis (Fig. 5). Examination of the entire activation time course demonstrated that both of the proacrosin bands were converted to three major components. Within minutes of the activation, the \( M_0 = 55,000 \) protein band disappeared and apparently was converted to the \( M_0 = 53,000 \) protein, for no new protein bands appeared. This was then converted to the \( M_0 = 49,000 \) species which hydrolyzed Bz-ArgOEt. After approximately 23 min of activation, a second protein band appeared which had a molecular weight of 57,000.

### Table I

**Purification of boar proacrosin**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrosomal extract</td>
<td>22.229</td>
<td>835</td>
<td>38</td>
<td>100</td>
</tr>
<tr>
<td>pH 5.8 supernatant</td>
<td>12.276</td>
<td>701</td>
<td>57</td>
<td>84</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>6.578</td>
<td>673</td>
<td>102</td>
<td>81</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.438</td>
<td>551</td>
<td>124</td>
<td>66</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>1.868</td>
<td>472</td>
<td>253</td>
<td>57</td>
</tr>
</tbody>
</table>

* One unit of proacrosin is equal to one unit of acrosin after activation.

![Fig. 2 (left). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proacrosin preparation in combination with the reference proteins. Electrophoresis and staining with Coomassie brilliant blue R-250 were carried out as described in the text. Ten micrograms of each of the proteins was applied to the gel.

![Fig. 3 (right). Graphic determination of the molecular weight of the purified proacrosin obtained from sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. The molecular weights used for the reference proteins were: bovine serum albumin, 68,000; aldolase, 40,000; trypsin, 23,300; and lysozyme, 14,300.

![Fig. 4. Time course of proacrosin activation followed with Bz-ArgOEt hydrolysis. Proacrosin (100 \( \mu \)g/ml) was activated in 0.1 M Tris/HCl, pH 8.4, at 0° containing 0.02% sodium azide. At specified time intervals, 10-\( \mu \)l aliquots were removed and spectrophotometrically assayed for Bz-ArgOEt hydrolysis at 30° as described in the text.

![Fig. 5. Time course of proacrosin activation followed by sodium dodecyl sulfate-gel electrophoresis. Proacrosin (100 \( \mu \)g/ml) was activated in 0.1 M Tris/HCl, pH 8.4, at 0°. At the time intervals given, 10-\( \mu \)l aliquots were removed from the activation mixture and added to 50 \( \mu \)l of 2% sodium dodecyl sulfate, 2% mercaptoethanol, pH 2.8, at 100° and incubated for 2 min. Sodium dodecyl sulfate-gel electrophoresis was run at 5 mA/tube at room temperature until the tracking dye migrated 7 cm into the gel. Staining with Coomassie brilliant blue R-250 and destaining were carried out as described in the text.
Boar Proacrosin

34,000. This was partially converted to a \( M_r = 25,000 \) species after a 14-h incubation on ice. One can also observe several lower molecular weight products accumulating. The most predominant one migrated ahead of lysozyme, the lowest molecular weight reference protein, indicating the molecular weight of this protein was less than 14,300.

The proacrosin had a migration \( R_f \) of 0.36 and 0.38 in the analytical disc gel electrophoresis (Fig. 6). The three components formed upon activation, referred to as \( \alpha, \beta \) and \( \gamma \)-acrosin, had \( R_f \) values of 0.40, 0.53, and 0.65, respectively. Identical gels were assayed for BzArgNNab activity as described under “Experimental Procedures” and positive enzymatic activity was observed in the respective \( R_f \) positions (Fig. 7). These results demonstrate that not one, but at least three active acrosins can be produced from the purified zymogen preparations. By selectively stopping the activation of purified proacrosin with benzamidine and subjecting the mixture to Sephadex G-75 SF chromatography, we have been able to obtain homogeneous \( \alpha \) and \( \beta \)-acrosin. Each of these forms has the respective molecular weight and corresponding \( R_f \) values described above. Examination of the first gel in Fig. 6 indicated that there were no contaminating BzArgNNab-hydrolyzing enzymes in these extracts that could have been present in amounts not detectable by the protein staining procedure. In addition, no trace activity bands were formed, even when the gel was incubated in the BzArgNNab substrate for 24 h with several changes of substrate solution. It should be noted that the zymogen activity bands at \( R_f \) 0.36 and 0.38, shown in the first gel in Fig. 7, developed only after incubation in the BzArgNNab substrate for at least 1 h, whereas the activity bands corresponding to the active enzyme began to form within minutes of the addition of substrate. This delay in the appearance of activity in the zymogen-containing gel suggested that the zymogen in the gel was converted to the active enzyme by an autocatalytic process, since any contaminating enzyme should have been separated by the electrophoresis. A possible complication in this electrophoresis was that a trace amount of acrosin was complexed to the zymogen and was responsible for the initiation of the autoactivation process. To minimize this possibility, 0.1 ml of the zymogen preparation (100 \( \mu \)g/ml) was incubated at pH 8.0, in 0.1 ml Tris/HCl, containing 1.0 mg of TosLysChCl (an irreversible acrosin inhibitor) for 30 min. When this sample was subjected to electrophoresis, the results were identical to those presented in the first gel in Figs. 5 and 6. Also, no activity bands were observed when the TosLysChCl (1.0 mg/ml) was incubated in both the gel incubation buffer and in the substrate buffer. These results demonstrate that thezymogens themselves would not yield the observed activity.

To determine if the activation of proacrosin can be the result of an intermolecular or intramolecular mechanism, the following experiments were performed. At a constant concentration of proacrosin, the effects of added proteinases on the activation process were ascertained (Table II). In each case, typical S-shaped activation curves were observed and full acrosin activity resulted. The time required for one-half of the activation was shortened by \( \alpha \)- and \( \beta \)-acrosin as well as trypsin, indicating that the activation under these conditions proceed by an intermolecular mechanism. The trypsin effects are related to the amount of trypsin present and indicate that the process is not unique to acrosin itself. However, it is not presently known if the resulting acrosin activity from trypsin activation is the same as that of the autoactivation process. These results also suggest, based on the known similarities between acrosin and trypsin (7), the activation occurs via the hydrolysis of an arginyl or lysyl peptide bond(s). Chymotrypsin did not increase the activation process, but actually had a reverse effect, as has been reported for the activation of prococonase (25). An activation time course was determined as a function of the zymogen concentration (Table III). Again, typical S-shaped activation curves resulted. The difference found in the time required for one-half of the acrosin activity to appear was directly proportional to the concentration of the zymogen present. These results also indicate that under the conditions employed the activation was an intramolecular reaction. A similar concentration dependence has been found for trypsinogen activation and was interpreted as evidence for an intramolecular reaction (26).

Calcium was included in these last two sets of experiments to slow the activation process sufficiently to allow for accurate determination of the activation curves. Without calcium, the time for one-half of the acrosin to be formed, with the 42.0 \( \mu \)g/ml concentration of proacrosin, was 4.8 min.

Properties of Proacrosin—The proacrosin in the acrosomal extracts prepared as described under “Experimental Procedures” was stable for at least 1½ years below -70°C and could be repeatedly frozen and thawed without noticeable loss in activity. The highly purified preparations of proacrosin were stable for several weeks at or below pH 3.0 at 4°C, but a substantial loss (25 to 40%) in activity occurred when the samples were frozen in a dry ice/acetone bath and thawed at 20°C. However, these preparations can be stored for up to 6 months at -20°C in 50% glycerol at pH 3.0.

Proacrosin was not irreversibly denatured in urea, for 1 ml of the purified preparations (40 \( \mu \)g/ml) when dialyzed against 500 ml of 8 M urea, pH 3.0, for 8 h and followed by dialysis against 1800 ml of 1 mM HCl, pH 3.0, for 14 h, and yielded 100% of the expected acrosin activity after activation.

In 1 mM HCl, pH 3.0, proacrosin had a remarkable heat stability. The purified proacrosin solution (20 \( \mu \)g/ml) was heated for various time intervals at 101°C and cooled in an ice bath prior to activation at pH 8.0. When the samples were thus heated in capped plastic test tubes for 5, 10, and 20 min, the acrosin activity that resulted after activation was 61, 50, and 46%, respectively, of the untreated control values.

Dilute proacrosin solutions could be routinely concentrated with a recovery of 85 to 95% of the total potential acrosin activity when sufficient 1 M benzamidine, pH 3.0, was added to result in a 50 mM solution and this was placed in dialysis tubing and covered with Sephadex G-200 in the cold. After the desired concentration had been achieved, the sample was dialyzed against 1 mM HCl, pH 3.0, to remove the benzamidine.

**DISCUSSION**

Proacrosin is rapidly converted to acrosin when sperm are removed from the seminal plasma (27). However, when benzamidine (a competitive acrosin inhibitor) was present throughout the washing procedure, proacrosin accounted for 100% of the total acrosin extracted from the sperm.

The purification procedures described in this paper resulted in better than a 50% yield of highly purified proacrosin. The largest loss of proacrosin occurred with the sodium chloride precipitation procedure. Although there was only a slight increase in purification, this step was included because almost all of the active acrosin in the preparations remained in the supernatant fluid while the majority of the proacrosin was

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Precipitated and thus, the zymogen was essentially free of active enzyme.

If the incubation with urea was omitted prior to the Sephadex gel filtration procedure, analogous results were found. Several earlier-eluting proacrosin bands appeared and were contaminated with a protein whose molecular weight was approximately 29,000 as judged by sodium dodecyl sulfate-gel electrophoresis. This acidic protein has been purified to homogeneity and has been shown to bind to both the proacrosin and the α-acrosin. However, it is not presently known if this is a modulator protein, a nonspecific binding protein, or possibly a fragment of a higher molecular weight zymogen. This latter possibility is intriguing, for a larger molecular weight zymogen could have been synthesized in the testis and subsequently degraded to the form described in this paper. This process could have occurred either in the epididymis, during the “ripening” process (a 12- to 14-day procedure in which sperm undergo numerous physiological and chemical changes (28)), or by an acrosomal proteinase not inhibited by benzamidine. This second possibility could be the result of acrolysin (29).

The final proacrosin preparations were highly purified as judged by the specific activity (253 units/mg of protein versus the highest previously reported value of 0.108 unit/mg of protein (18)) and the presence of only two closely migrating protein bands in both the sodium dodecyl sulfate-gel electrophoresis and the analytical disc gel electrophoresis. Various lines of evidence indicate that both of the proteins observed in these preparations are in fact zymogens. Although there was no enzymatic activity in the sample prior to the electrophoresis, both bands of protein eventually hydrolyzed BzArgNNab.

The time course of proacrosin activation followed with analytical disc gel electrophoresis and analyzed for protein. Proacrosin (100 μg/ml) was activated in 0.1 M Tris/HCl, pH 8.4, at 0°C containing 0.02% sodium azide. At the time intervals given, 200-μl aliquots were removed and added to 5 μl of 1 M benzamidine. Acrylamide disc gel electrophoresis, pH 4.6, was performed in duplicate using 100 ml of the aliquot per gel at a constant current of 2 mA/gel until the tracking dye had migrated 7 cm into the gel. One set of gels was stained for protein using Coomassie brilliant blue R-250 and de-stained as described in the text.

**Figure 6 (left).** Time course of proacrosin activation followed the analytical disc gel electrophoresis and analyzed for protein. Proacrosin (100 μg/ml) was activated in 0.1 M Tris/HCl, pH 8.4, at 0°C containing 0.02% sodium azide. At the time intervals given, 200-μl aliquots were removed and added to 5 μl of 1 M benzamidine. Acrylamide disc gel electrophoresis, pH 4.6, was performed in duplicate using 100 ml of the aliquot per gel at a constant current of 2 mA/gel until the tracking dye had migrated 7 cm into the gel. One set of gels was stained for protein using Coomassie brilliant blue R-250 and de-stained as described in the text.

**Figure 7 (right).** Time course of proacrosin activation followed with analytical disc gel electrophoresis and analyzed for BzArgNNab hydrolysis. Proacrosin was activated and aliquots were subjected to acrylamide disc gel electrophoresis as described for Fig. 6. One set of gels was analyzed for BzArgNNab hydrolysis as described in the text.

**Table II.** Influence of various proteinases on proacrosin activation

<table>
<thead>
<tr>
<th>Enzyme added</th>
<th>t_{50}^*</th>
<th>min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>Acrosin (1:15)</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Acrosin (1:30)</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Trypsin (1:125)</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Trypsin (1:250)</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Chymotrypsin (1:1)</td>
<td>28.5</td>
<td></td>
</tr>
</tbody>
</table>

* t_{50} is defined as the time required for 50% of the acrosin to be formed.

* The ratio of enzyme to zymogen was based on an activity basis.

* Amount of chymotrypsin on a weight basis.

**Table III.** Influence of zymogen concentration of autoactivation of proacrosin

<table>
<thead>
<tr>
<th>Zymogen</th>
<th>t_{50}^*</th>
<th>min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proacrosin</td>
<td>11.5</td>
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</tr>
<tr>
<td>Acrosin (1:15)</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>Acrosin (1:30)</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>Trypsin (1:125)</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Trypsin (1:250)</td>
<td>13.7</td>
<td></td>
</tr>
</tbody>
</table>

* t_{50} is defined as the time required for 50% of the acrosin to be formed.

proteolytic degradation. In view of these results, the following working hypothesis is suggested:

\[
\text{Proacrosin I} \rightarrow \text{Proacrosin II} \rightarrow \\
\begin{array}{ccc}
M_i &=& 65,000 \\
M_a &=& 63,000 \\
M_r &=& 25,000 \\
M_y &=& 22,000 \\
M_p &=& 19,000 \\
M_s &=& 16,000 \\
M_t &=& 14,000 \\
M_{st} &=& 12,000 \\
M_{st} &=& 10,000
\end{array}
\]

These molecular weight values are not to be taken as absolute numbers, for only one technique has been used and we have seen a variation of 5 to 10% upon repeated analysis. However, the \(\alpha\)-acrosin is similar in molecular weight to the first form of acrosin found in the acrosomal extracts from epididymal (30) and ejaculated rabbit sperm (7). The \(\beta\)-acrosin corresponds to the most extensively studied form from ejaculated boar sperm (8). The \(\gamma\) is similar to the more recently observed rabbit \(\beta\) (31) and human acrosins (32). This is not to imply that other proacrosin or acrosin forms may not be possible, but it is a summary of the data presented in the text.

Examination of Fig 4 demonstrates the apparent antiactivation of proacrosin occurs via the classical S-shaped curve, indicating that activation products catalyze the activation process. This seems reasonable, for the addition of isolated \(\alpha\)-acrosin occurs via the classical S-shaped curve, a summary of the data presented in the text.

Treatment of the purified zymogen with TosLysCh,Cl and analyzed in the pH 4.3 analytical gel electrophoresis system for BzArgNNab hydrolysis. Only two bands of enzymatic activity were eventually obtained at the \(R_c\) values corresponding to the proacrosins. These data demonstrated that trace amounts of acrosin were not responsible for the activation processes. Similar results were obtained with \(iPr\), PF.

Treatment of the zymogen with 8 M urea definitely disrupts at least one complex formed between the zymogen and another protein, and it is probably safe to assume that it disrupts all noncovalent interactions. Gel filtration chromatography, therefore, should separate the zymogen from any other protease present, unless it has a molecular weight very similar to the zymogen. Treatment of the purified zymogen with TosLysCh,Cl or \(iPr\), PF should inactivate any serine proteinase, although there is no reason to presuppose that an activating proteinase would be a serine proteinase. The known substrate specificities of acrosin and trypsin suggest that activation of the zymogen occurs by cleavage of either an arginyl or lysyl peptide linkage. It is therefore reasonable to assume that a contaminating protease that could activate the zymogen would have a similar specificity. In the presence of TosLysCh,Cl, there was no BzArgNNab-hydrolyzing activity detected in the gels, even after extended incubations. This suggests that either TosLysCh,Cl inhibits a BzArgNNab-hydrolyzing protease or that there is no BzArgNNab-hydrolyzing protease present that is not inhibited by TosLysCh,Cl. Either one of these explanations suggests that no contaminating protease was present that could activate the zymogen after TosLysCh,Cl treatment.

The third possibility, namely that proacrosin possesses some catalytic ability, appears, at this time, to be the least easily dismissed possibility for initiation of acrosin formation. Thezymogens of pepsin and trypsin have been shown to be catalytically active (33) and there is, therefore, no compelling reason to suspect that proacrosin is devoid of this property. On the contrary, the data presented in this communication suggest that the zymogen does, in fact, possess some catalytic ability. Unfortunately, assay systems to demonstrate this activity independent of zymogen activation have yet to be developed. This area obviously needs further investigation.

The acrosin involvement in fertilization appears to be a more complex process than originally believed. It has been well documented that the inhibition of active acrosin prevents fertilization and attempts to prepare compounds as inhibitors are well advanced. However, the development of specific reagents that block the proacrosin activation may prove to be superior to those that inhibit acrosin itself, since this is one step further removed from the actual zona pellucida penetration by sperm.

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REFERENCES

Boar Proacrosin

Boar proacrosin. Purification and preliminary activation studies of proacrosin isolated from ejaculated boar sperm.
K L Polakoski and R F Parrish


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