Observation of Two Proacrosins in Extracts of Human Spermatozoa

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SUMMARY: When human spermatozoa are extracted in the presence of 0.05 M benzamidine, the resulting solutions show a time dependent, sigmoidal increase of trypsin-like activity upon incubation at pH 8. Gel permeation chromatography of these extracts separates two species, P_1 and P_2, with apparent molecular weights of 75,000 and 42,000 respectively. P_1 and P_2 are both autoactivatable at pH 7-8 and the kinetic parameters of activated P_1 and P_2 are indistinguishable from those of human acrosin. That P_1 and P_2 are inactive precursors of human acrosin is shown by the fact that, in the presence of benzamidine, they are obtained instead of and in greater yield than acrosin. That P_1 and P_2 are zymogens is shown by the features of the activation process.

Mammalian sperm acrosomes contain a protease, acrosin (EC 3.4.21.10), whose biological role may be to facilitate the passage of spermatozoa through the zona pellucida of the ovum (1). Several investigations have suggested that in the spermatozoa of the boar (2,3) and the rabbit (4) acrosin is stored in the form of a single zymogen, proacrosin. The observations reported below indicate that extracts of human spermatozoa contain two acrosin zymogens, which are obtainable under conditions inhibiting autoactivation.

Materials and methods

Sperm extracts were prepared by detergent extraction, alcohol precipitation, dialysis and lyophilization as described previously for "D-2" (5), except that 0.3ml of 0.5 M BA* was added to each semen specimen (2-5mg) within 1-2 hours after ejaculation. All solutions for sperm washing and extraction contained 0.05 M BA in addition to the components previously described. Esterolytic activity was measured spectrophotometrically at 253 nm by the hydrolysis of BAEE*, 5.8×10^{-4} M, in 0.1 M Tris buffer, pH 8.0, containing 0.05 M $CaCl_2$ (6). One unit of activity (U) is that amount of esterase which causes an increase of 1.0 absorbancy unit per minute when added to 3.0ml of the substrate solution. Polyacrylamide gel electrophoresis and staining of the gels for amidase activity was as described before (5). Gel filtration estimates of molecular weight were performed by the method of Andrews (7). Autoactivation experiments were performed in 0.1 M Tris buffer, pH 8.0, containing 0.05 M $CaCl_2$ at 25°. Zymogens were assayed by allowing autoactivation to proceed for 90 mins.

* BA : benzamidine hydrochloride (Aldrich)
BAEE: N-benzoyl arginine ethyl ester (Sigma).

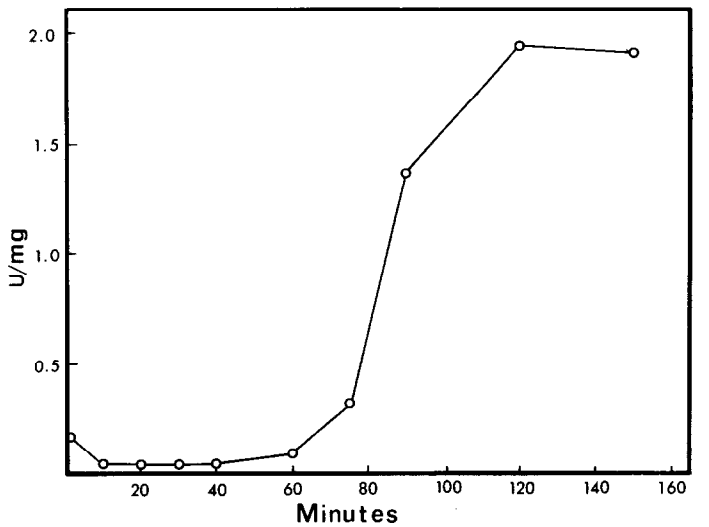

Figure 1: Time course of autoactivation of the crude sperm extract.

Results and Discussion

When the dialyzed and lypohilized sperm extracts are redissolved and incubated at pH 8, they at first display less than the expected BAEE hydrolase activity. However, after a lag period the activity rises sigmoidally (figure 1). In the experiment shown in figure 1, the final specific activity is some four fold higher and the absolute activity one and one-half fold higher, than what would have been obtained if the BA had been omitted from the extraction procedure (5). Gel filtration of the sperm extract obtained in the presence of BA separates the autoactivatable material into two species (see figure 2), P_1 and P_2,eluting at apparent molecular weights of 75,000 and 42,000, respectively. These apparent molecular weights are comparable to those observed for acrosins obtained from human sperm. Recovery of the precursors from the gel filtration column is better than 90 percent. When assayed before autoactivation, column fractions containing P_1 and P_2 exhibit less than 10 percent of the activity observed after autoactivation. With both P_1 and P_2 the same sigmoidal activation kinetics are observed as with the crude extract.

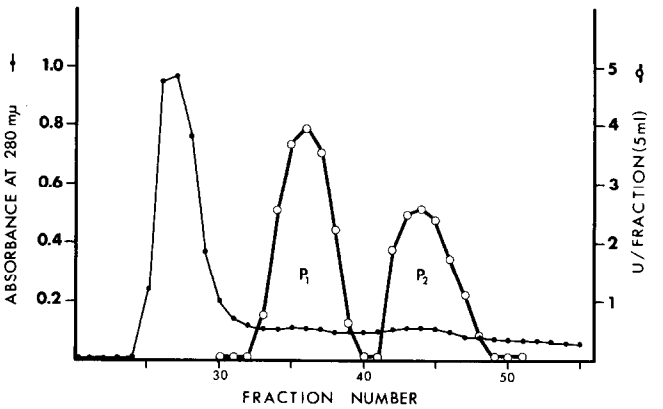

Figure 2: Gel permeation chromatography (Sephadex G-100, 0.1 M formate buffer) of the crude sperm extract.

P_1 and P_2 may also be visualized on polyacrylamide gels (see figure 3). The quantity of acrosin activity present in the aliquots of P_1 and P_2 applied to the gels was too small to be observed by this staining procedure. Thus P_1 and P_2 must have autoactivated while the gels were soaking in the pH 7.5 staining solution. Figure 3 also shows that the electrophoretic mobilities of P_1 and P_2 are very similar to the mobilities of acrosins A_1 and A_{2S}, respectively. When P_1 (or P_2) is assayed before activation in increasingly smaller amounts, the observed activity is found to be directly proportional to the quantity of P_1 taken for assay (figure 4).

These observations raise a variety of questions, two of which can be answered: Are P_1 and P_2 precursors of acrosin? Are P_1 and P_2 zymogens or only enzyme-inhibitor complexes?

That P_1 and P_2 are acrosin precursors follows from the facts that P_1 and P_2 were obtained instead of and in greater yield than acrosin and that acrosin is the major BAEE hydrolase of sperm (1). Furthermore, the apparent molecular weights and electrophoretic mobilities of P_1 and P_2 are very similar to acrosins A_1 and A_{2S}, respectively, and the K_m measured for activated P_1 and P_2 with BAEE as substrate is the same as that for the acrosins, i.e., $1.3 \pm 0.5 \times 10^{-5}$M. Thus P_1 and P_2 are precursors of acrosin.

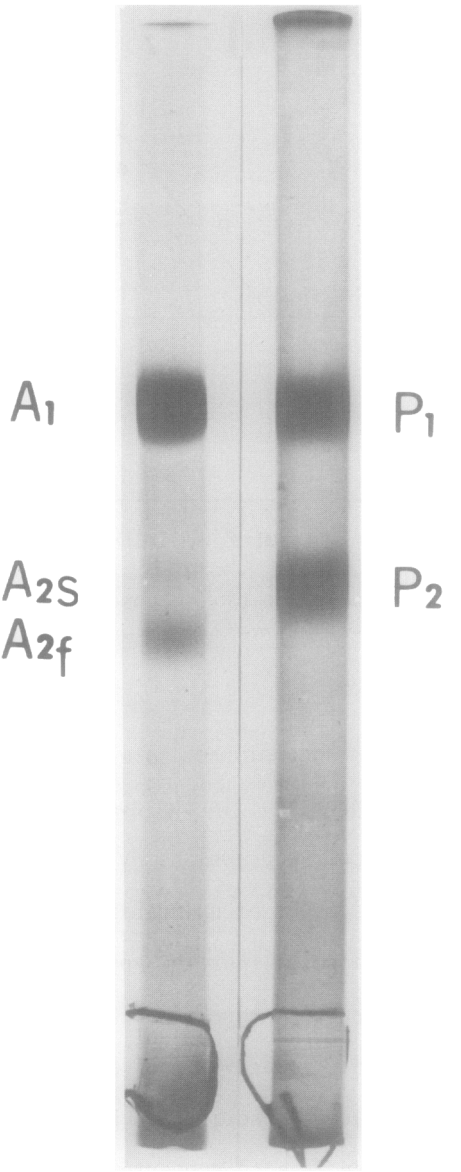

Figure 3: Polyacrylamide gels of acrosin (left) and proacrosin (right) stained for amidase activity.

With regard to the question of whether P_1 and P_2 are zymogens or only enzyme-inhibitor complexes, the sigmoidal time course of activation is a property of all protease zymogens capable of autocatalytic activation. It in-

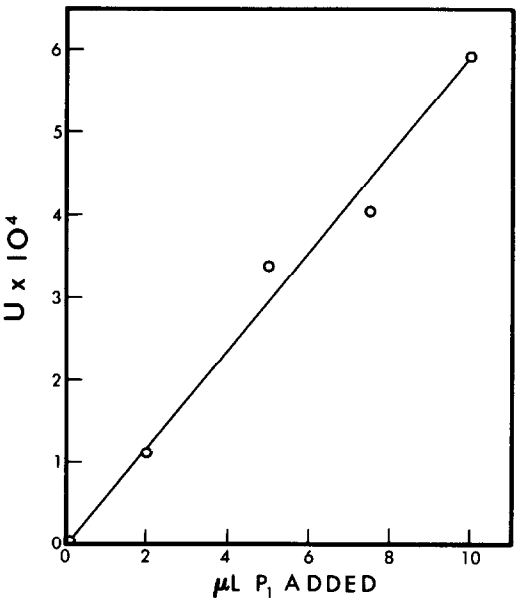

Figure 4: Concentration dependence of the observed activity of a P_1 solution before activation.

dicates that activation is not accomplished solely by inhibitor dissociation, which would be a first order process. In the event that solutions of P_1 and P_2 contain enzyme-inhibitor complexes which may be activated via digestion of the inhibitor by the small amount of uninhibited enzyme, the time course of activation could also be sigmoidal but the apparent initial activity of the solution should not be directly proportional to the size of the aliquot taken for assay. Since the initial activity of a P_1 solution was directly proportional to dilution (figure 4), activation by inhibitor digestion is very unlikely. This conclusion is also supported by the observation that the enzymatic activity in the assay mixtures which yielded the data of figure 4 was constant for the 30 minute duration of the assay. Thus P_1 and P_2 are certainly not readily dissociable enzyme-inhibitor complexes. If these comments are deemed insufficiently convincing, it is informative to construct a profile of what the properties of the inhibitor would have to be: The maximum value of the enzyme-inhibitor dissociation constant at pH 8 would have to be

10^{-12}-10^{-13}M, if one takes the turnover number reported for boar acrosin (8) as a guide to the turnover number for active P_1 or P_2. The inhibitor must be capable of remaining bound to P_1 and P_2 during gel filtration at pH 3. The inhibitor must be a substrate for P_1 and P_2 as well as an inhibitor. It must have a molecular weight less than 5000 [cf. apparent molecular weights of P_1 and A_1, P_2 and A_{2S}(9)]. Finally, its adhesion to spermatozoa must be dramatically affected by 0.05 M BA. By comparison with known protease inhibitors such a combination of properties is extremely unlikely. We conclude, therefore that P_1 and P_2 are indeed zymogens of human acrosin.

Further questions such as the relationship between P_1 and P_2 and the pathways of their activation in vitro and in vivo will be the subjects of future reports.

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