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Matrix degrading properties of sperm serine proteinase, acrosin

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The serine proteinase acrosin plays an important role in sperm penetration of the *zona pellucida*. In the present study we investigated the effect of the enzyme on various matrix proteins. Acrosin degraded proteolytically fibronectin, type IV collagen and heat denatured type I collagen, whereas neither native type I collagen nor laminin were cleaved by the enzyme. The specific activity of acrosin with type IV collagen as substrate (66.6 g/h/g) was 125-fold higher than that of known type IV collagenase or stromelysin. These results suggest that acrosin may act as a matrix-degrading proteinase.

Acrosin; Matrix-degrading activity; Collagen IV; Fibronectin

1. INTRODUCTION

Acrosin plays a central role in mammalian fertilization. The enzyme was found in spermatozoa of vertebrates and non-vertebrates (reviewed in [1]). It is a disulfide-boned two-chain glycosylated serine proteinase, exhibiting trypsin-like cleavage specificity [2,3]. In intact spermatozoa, the enzyme is associated with the acrosomal peripheral membrane in form of proacrosin which is activated by limited proteolysis before fertilization [4,5].

The high amount of acrosin in the acrosome of spermatozoa is believed to be essential for gamete fusion, particularly for binding to and penetration of the *zona pellucida* ([6], reviewed in [7]). The molecule of acrosin combines a specific proteolytic activity with *zona*- and carbohydrate-affinity, a property unusual for a serine proteinase [8,9]. It was suggested that in vivo this special affinity of acrosin directs the proteolytic activity to its structural target. Sperm acrosin may thus function in penetration of *zona pellucida* and of a highly structured extracellular matrix of the previtellin space [6].

Since little is known about the chemical structure of the *zona pellucida*, the action of acrosin on its presumed physiological substrate has not been characterized on the molecular level and the degradation of matrix proteins was attributed rather to the action of matrix-degrading metalloproteinases.

We have recently described that matrix protein, fibronectin, contains a latent proteolytic system of matrix degradation, that can be activated by external proteinases [10,11]. FN-type IV collagenase, one enzyme of this system, is a metalloproteinase that degrades collagen type IV, heat-denatured collagen type I and laminin. Fibronectinase is a serine proteinase that degrades fibronectin and its fragments. As both the spermatozoid and the previtellin space contain fibronectin, a process of a potential activation of its latent proteolytic system by acrosin had to be taken into consideration.

The intent of this study was to examine the direct effect of the serine proteinase acrosin on various matrix proteins and the possibility of an indirect effect of activation of a matrix-degrading activity from fibronectin [10,11].

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2. MATERIALS AND METHODS

Gelatin-Ultrogel was purchased from IBF Biotechnics (Villeneuve-la-Garenne, France). Phenylmethane sulfonylfluoride (PMSF) and pepstatin A were from Fluka Chemie AG (Buchs, Switzerland); 1,10-phenanthroline and *n*-ethylmaleimide were from Sigma Chemical Co. (St. Louis, USA).

Acrosin (α-form, 14 U/mg) was purified and activated as previously described [12]. The lyophilized sample was solubilized in 1 mM HCl to a final conc. of 1 mg/ml. Lyophilized human plasma fibronectin (Centre National de Transfusion Sanguine, France), was purified as described [13,14], with a final step of purification by gel filtration [15]. Laminin and type IV collagen were purified from the Engelbreth-Holm-Swarm (EHS) tumor [16] and type I collagen from rat tendons [17]. Heat-denatured collagen (gelatin) was prepared by incubation of native type I collagen at 70°C for 15 min.

Enzyme assays with native type I collagen, denatured type I collagen (gelatin), type IV collagen and laminin as substrates (enzyme/substrate ratio of 1:1000) were carried out in 50 mM Tris-HCl, pH 7.4, buffer, containing 0.2 M NaCl and 5 mM $CaCl_2$ with 20 g of substrate in a final volume of 50 litres. In the case of laminin, the assays were performed without NaCl. The samples were incubated for 16 h at 37°C, lyophilized, reduced and analyzed by electrophoresis using 10% acrylamide gel according to Doucet and Trifaro [18]. Substrate digestion was evaluated in Laser Densitometer Ultroscan XL (LKB, Sweden) by comparison of control and enzyme-containing samples.

The inhibition studies were done as previously described [10,11]

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with phenylmethane sulfonylfluoride, 1,10-phenanthroline, pepstatin A and *n*-ethyl maleimide in final concentrations of 1.3 mM, 10 mM, 1 mM and 3.3 mM, respectively.

To evaluate the partial digestion of fibronectin with acrosin, the protein (5 mg) was digested at enzyme/substrate ratio of 1:100 in 5 ml of 50 mM Tris-HCl buffer, pH 8, containing 5 mM $CaCl_2$ at 22°C. After 2 h, the digest was applied on a gelatin-Ultrogel column (2.2 × 10 cm), equilibrated with the same buffer. The retained fragments were eluted with the equilibration buffer containing 4 M urea.

3. RESULTS AND DISCUSSION

The effect of acrosin on collagens was studied with native and heat-denatured collagens type I and with native collagen type IV as substrates.

The results in Fig. 1A show that native type I collagen was not cleaved by the enzyme. On the contrary, the heat-denatured collagen was degraded.

The degradation of basal membrane matrix proteins was studied with collagen type IV and with laminin, the substrates of a specific group of matrix-degrading metalloproteinases, comprising type IV collagenases and stromelysin.

Our results (Fig. 1B) have shown that acrosin efficiently degrades type IV collagen at E/S ratio of 1:1000. This degradation, as well as the cleavage of the heat-denatured type I collagen, were inhibited by the serine proteinase inhibitor phenylmethane sulfonylfluoride, while the inhibitor of metalloproteinases, *o*-phenanthroline, was without effect. Therefore the cleavage of type IV and denatured type I collagens can be attributed to acrosin itself and not to an eventual contamination by a metalloproteinase or by its zymogen. Contrary to collagen type IV, laminin was not degraded by acrosin (Fig. 1C).

The effect of acrosin on fibronectin is demonstrated in Fig. 2. The proteolysis of fibronectin at an E/S ratio of 1:100 was accomplished within 2 h (Fig. 2A). Further incubation did not change the electrophoretic pattern of the digest (not shown).

An affinity chromatography of the digest on gelatin-Ultrogel (Fig. 2B) and a subsequent gel electrophoresis of the separated fractions (Fig. 2A) have demonstrated three main fragments of 110, 27 and 25 kDa in gelatin-unretained fraction. All other fragments were retained by gelatin-Ultrogel. When either the gelatin-unretained or gelatin-retained fractions were assayed with various matrix proteins as substrates, no traces of any newly generated proteolytic activity were detected. A 24 h-preincubation of the fractions alone or together (ratio 1:1) that enhanced the activation of FN-proteinases did not change the results.

Therefore the present study has demonstrated that acrosin does not generate any proteolytic activity from fibronectin, but that it can degrade directly matrix proteins. A comparison of the specific activities of acrosin towards the studied matrix proteins with those of known matrix-degrading proteinases (Table I) demonstrates that its specific activity towards type IV collagen is 125-fold higher than that of type IV collagenase (gelatinase) and of stromelysin [19].

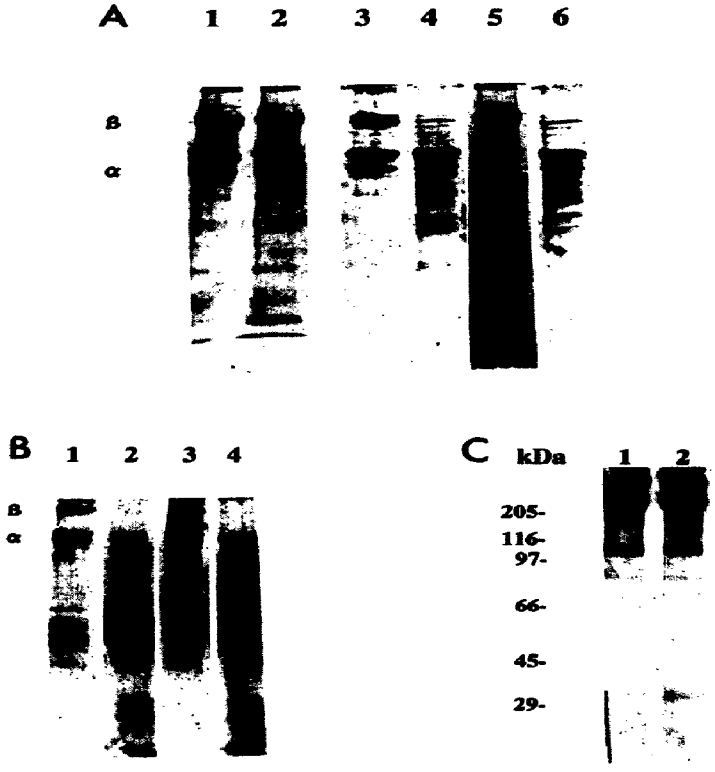

Fig. 1. Analysis of proteolytic activity of acrosin. Substrates were incubated with acrosin (E/S ratio of 1:100) for 16 h at 37°C. (A) Native type I collagen (20 g) alone (lane 1) or with acrosin (lane 2); (lanes 3–6) heat denatured collagen (20 g) as substrate: incubated alone (lane 3), with acrosin (lane 4), with acrosin and phenylmethane sulfonylfluoride (lane 5), with acrosin and 1,10-phenanthroline (lane 6). (B) Collagen type IV (15 g) alone (lane 1); with the enzyme (lane 2); with the enzyme and PMSF (lane 3); with the enzyme and 1,10-phenanthroline (lane 4). (C) Laminin (15 g) alone (lane 1); laminin with the enzyme (lane 2).

Up to now, the degradation of type IV collagen was considered as a specific property of metalloproteinases, in particular of type IV collagenases. This group of enzymes was exhaustively studied by several laboratories, since a correlation was established between their proteolytic activity and the metastatic potential of tumor cells [20,21].

Our present results brought evidence that serine proteinase acrosin can also display a high specific proteolytic activity towards collagen type IV. Therefore a degradation of this matrix protein characteristic for the basal membrane is not an exclusive property of metalloproteinases. Further study is required to understand the biological significance of this particular property of acrosin.

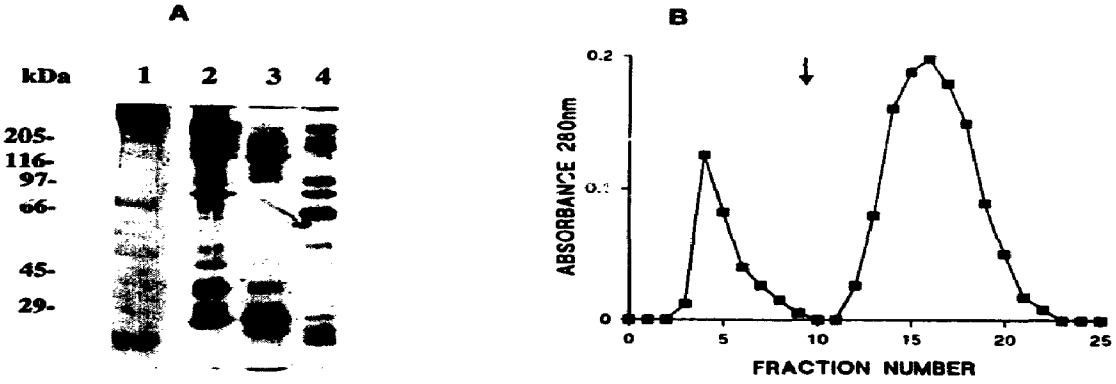

Fig. 2. Digestion of fibronectin with acrosin and affinity chromatography of the digest on gelatin-Ultrogel. (A) Gel electrophoresis. Fibronectin (lane 1); acrosin digest of fibronectin (lane 2); gelatin-unretained fraction (lane 3) and retained fraction (lane 4). (B) Affinity chromatography of the digest. Arrow indicates elution with urea-containing buffer.

Table I

Comparison of substrate specificity of acrosin with extracellular matrix metalloproteinases

Substrate	Enzyme			
	Acrosin	Type IV [19] collagenase (gelatinase)	Stromelysin [19]	Collagenase interstitial [19]
Collagen IV	66.6	0.5	0 5	0.1
Collagen I	–	–	–	54.1
Collagen I (D)	79.1	96	7.5	3.0
Laminin	–	–	+	–
Fibronectin	83.3	+	++	++

Activity values were expressed in g of substrate degraded per hour per g of enzyme; data from [19] were recalculated accordingly. (D), denatured.

REFERENCES

[1] Müller-Esterl, W. and Fritz, H. (1981) Methods Enzymol. 80, 621–632.
[2] Fock-Nuzel, R., Lottspeich, F., Henschen, A. and Müller-Esterl, W. (1984) Eur. J. Biochem. 141, 441–446.
[3] Schleuning, W.D. and Fritz, H. (1976) Methods Enzymol. 45, 330–342.
[4] Čechová, D., Henschen, A. and Töpfer-Petersen, E. (1988) FEBS Lett. 241, 136–140.
[5] Meizel, M. and Mukerji, S.K. (1975) Biol. Reprod. 13, 83–93.
[6] Talbot, P. and diCarlantonio, G. (1984) Dev. Biol. 103, 159–167.
[7] Hedrick, J.R., Urch, U.A. and Hardy, D.M. (1988) in: Enzymes in Agricultural Research (Shoemaker, S., Sonnet, P. and Whitaker, J.) pp. 1–11, Symp. Series, ACS Books, Washington.
[8] Töpfer-Petersen, E. and Henschen, A. (1987) FEBS Lett. 226, 38–42.
[9] Jones, R. (1987) Cell. Biol. Int. Reports 11, 833.
[10] Lambert-Vidmar, S., Lottspeich, F., Emod, I., Planchenault, T. and Keil-Dlouha, V. (1991) Eur. J. Biochem. (in press).
[11] Lambert Vidmar, S., Lottspeich, F., Emod, I., Imhoff, J.-M. and Keil-Dlouha, V. (1991) Eur. J. Biochem. (in press).
[12] Železná, B. and Čechová, D. (1982) Hoppe-Seyler's Z. Physiol. Chem. 363, 757–766.
[13] Keil-Dlouha, V. and Planchenault, T. (1986) Proc. Natl. Acad. Sci. USA 83, 5377–5381.
[14] Keil-Dlouha, V. (1990) Biol. Chem. Hoppe-Seyler 371, Suppl. 283–287.
[15] Johansson, S. and Smedsrod, B. (1986) J. Biol. Chem. 261, 4363–4366.
[16] Kleinman, H.K., McGarvey, M.L., Liotta, L.A., Cheron, R.P., Trygvason, K. and Martin, G.R. (1982) Biochemistry 21, 6188–6193.
[17] Berman, M.B., Manabe, R. and Davison, P. (1973) Anal. Biochem. 54, 522–534.
[18] Doucet, J.-P. and Trifaro, J.-M. (1988) Anal. Biochem. 168, 265–271.
[19] Collier, I.E., Wilhelm, S.M., Eisen, A.Z., Marmer, B.L., Grant, G.A., Seltzer, J.L., Kronberger, A., He, C., Bauer, E.A. and Goldberg, G.I. (1988) J. Biol. Chem. 263, 6579–6587.
[20] Liotta, L.A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C.M. and Shafie, S. (1980) Nature 284, 67–68.
[21] Liotta, L.A. and Rao, C.N. (1985) Ann. NY Acad. Sci. 460, 333–344.