CONVERSION OF RABBIT TESTIS PROACROSIN TO ACROSIN

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1. Introduction

The mammalian sperm trypsin-like enzyme acrosin (E.C. 3.4.21.10) appears to play an important role in fertilization [1]. Work from this laboratory on rabbit testis has suggested that rabbit testis acrosin is present as a zymogen, proacrosin [2,3], and our preliminary studies have also indicated this proacrosin is present in rabbit epididymal sperm [4].

The aim of the present communication is to present new evidence which supports the zymogen nature of rabbit testis proacrosin, and evidence which strongly suggests that proacrosin can be activated by an autocatalytic process regulatable by Zn^{2+} and Ca^{2+}.

2. Materials and methods

Sources of chemicals and rabbit testes have been previously described [2,3]. Proacrosin was purified 53-fold by acid extraction from rabbit testes, and chromatography on Sephadex G-75 and on SE-Sephadex columns [3]. Before 'autoactivation' protein was estimated by measuring the optical density at 280 nm and 260 nm. Acrosin activity was determined spectrofluorometrically [2,3] by measuring the β-naphthylamide (βNA) produced by enzymatic hydrolysis of α-*N*-benzoyl-D, L-arginine-β-naphthylamide (BANA). Proacrosin was 'autoactivated' to acrosin (47 nmol βNA/min/mg protein) by incubation at 25°C with an equal vol of 0.05 M Tris buffer of various pH's and cation concentrations (see figure legends).

3. Results and discussion

Fig.1A shows that increasing the concentration of the proacrosin preparation decreased the lag phase (time required for the first appearance of detectable arcrosin activity) of the sigmoidal time course of 'autoactivation'. This result suggests that the rate of 'autoactivation' is dependent upon the proacrosin concentration. As previously mentioned [2] the sigmoidal 'autoactivation' time course of proacrosin is typical of the autocatalytic activation of zymogens [5]. The autocatalytic conversion of a zymogen to its active enzyme can be described by a second order equation which states that the rate of reaction at any time is a function of both zymogen and active enzyme concentrations, and a plot of log [zymogen (time)/ active enzyme (time)] versus time will give a straight line [6]. In the present report (fig.1B) such plots gave straight lines at all the proacrosin concentrations tested. These results are further evidence for the zymogen nature of proacrosin and for the autocatalytic conversior of the proacrosin to acrosin (i.e., the product formed, acrosin, accelerated the conversion).

Fig.2 shows that the 'autoactivation' lag phase was 50, 30, 20, and 15 min at pH's 7.0, 8.0 and 8.5–9.0 respectively. Thus the pH optimum for proacrosin 'autoactivation' was pH 8.5–9.0 which is close to the pH optima (pH 8.0–8.5) reported for rabbit acrosin assayed with small synthetic substrates [1,4]. This lends further credence to the idea that the proacrosin 'autoactivation' involves acrosin.

Fig.3 demonstrates that the rate of proacrosin 'autoactivation' was increased by increasing the con-

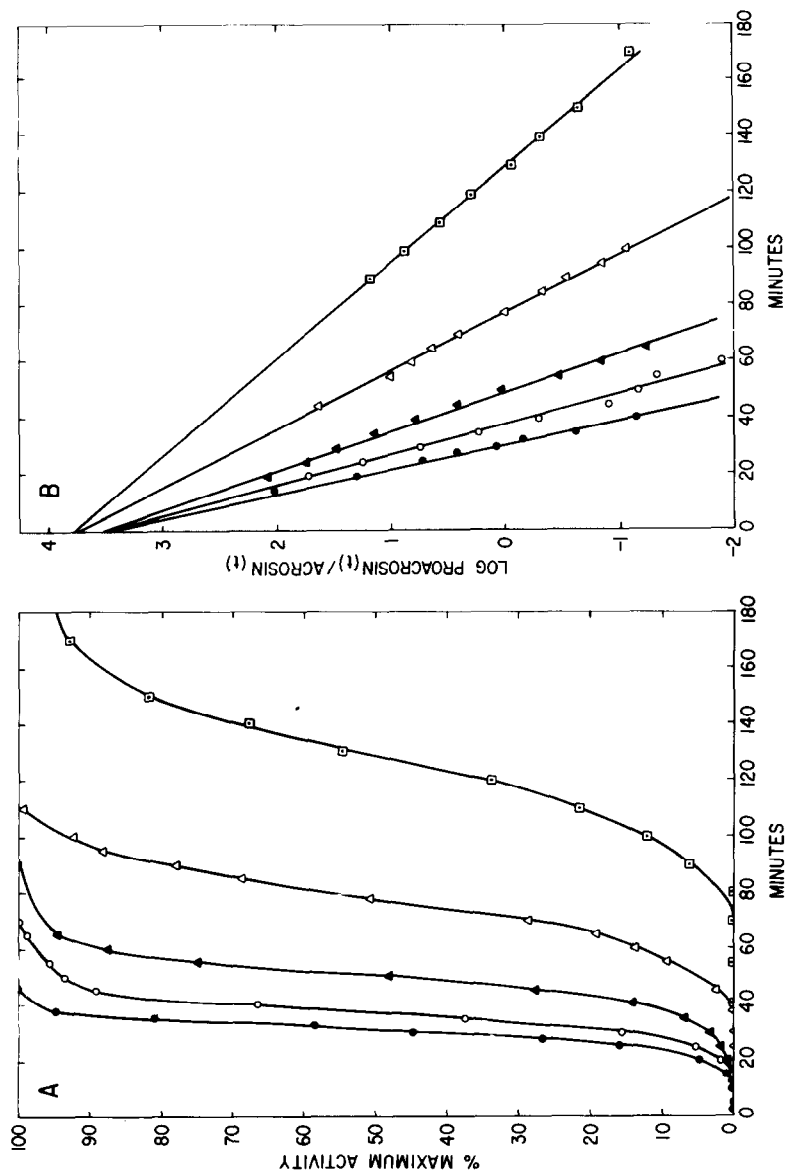

Fig. 1. Effect of zymogen concentrations on the time course of 'autoactivation' for proacrosin. All activations were carried out in 0.05 M Tris–HCl, pH 8.0 containing 0.1 M $CaCl_2$. Final protein concentrations of proacrosin were 0.0156 (■), 0.0312 (△), 0.0625 (▲), 0.1250 (○) or 0.250 (●) mg per ml. (A) Direct plot (B) Semilog plot. The initial concentrations of proacrosin were calculated from the points at full activation.

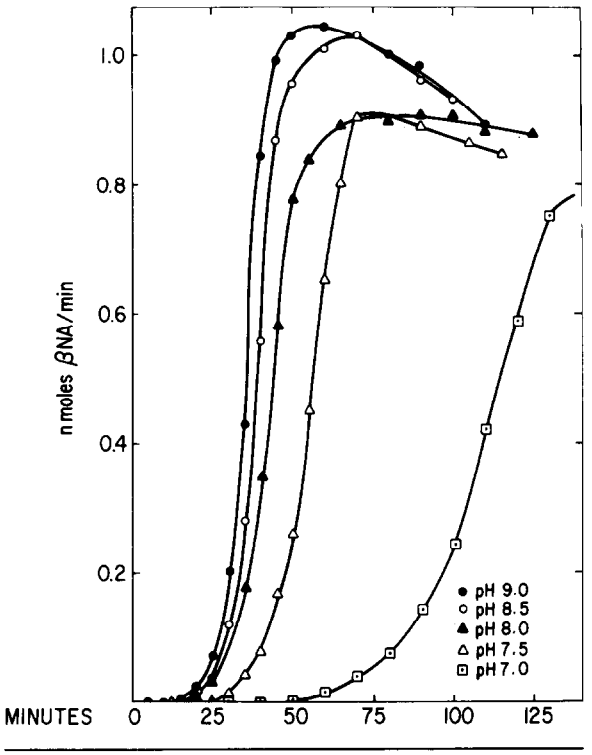

Fig.2. Effect of pH on the time course of proacrosin 'autoactivation'. The zymogen was incubated with 0.05 M Tris–HCl (pH's 7.0–9.0) containing 0.05 M $CaCl_2$.

centrations of Ca^{2+}, with a maximum reached at a final Ca^{2+} concentration of 0.1 M to 0.15 M. Proacrosin could be 'autoactivated' in the presence of Tris buffer, pH 8.0 alone, but the total acrosin activity was only about 60% of that with 0.1 M Ca^{2+} (fig.3). Ca^{2+} may stabilize the structure of acrosin similar to its effect on trypsin during trypsinogen autoactivation [5]. The relative rates of proacrosin 'autoactivation' with several buffers were Tris > veronal > borate. Fig.4 shows that at increasing concentrations of Zn^{2+} the time course lag phase was prolonged and the final acrosin activity was reduced. The final acrosin activity obtained from 'autoactivation' in the presence of 39 μM Zn^{2+} was only 65% of that obtained in the presence of 0.1 M Ca^{2+} (fig.5), but complete activation was achieved when Ca^{2+} was added along with Zn^{2+}. The Ca^{2+} and Zn^{2+} results were not due to changes in

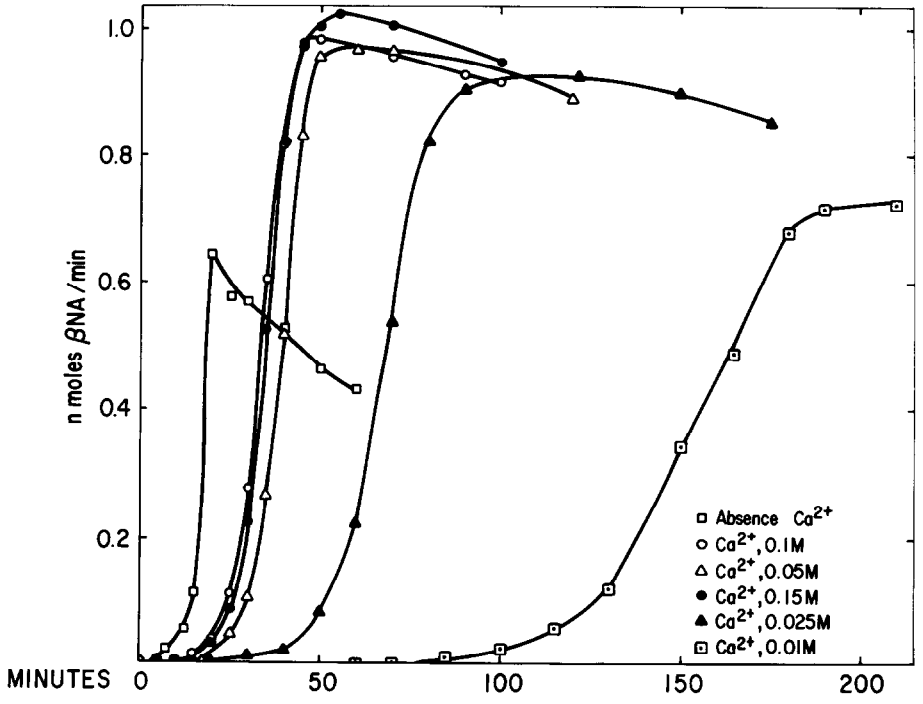

Fig.3. Effect of the presence and absence of Ca^{2+} on the time course of proacrosin 'autoactivation' at pH 8.0.

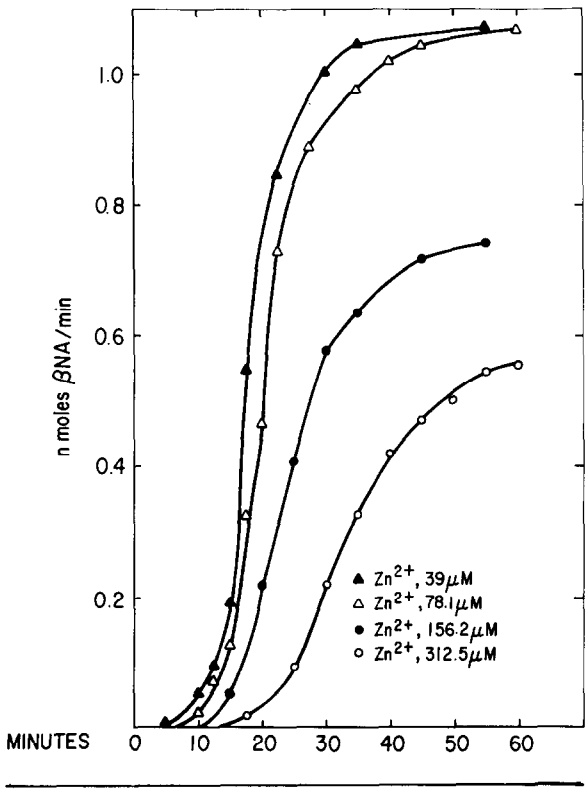

Fig.4. Effect of Zn^{2+} on the time course of proacrosin 'autoactivation' at pH 8.0.

ionic strength because unlike the cation effects, 'autoactivation' in the presence of increasing amounts of NaCl (not shown) resulted in both an increased lag phase and increased final acrosin activity. Rabbit testis acrosin produced by proacrosin 'autoactivation' was stimulated 40% by 0.1 M Ca^{2+} and inhibited 44%, 56%, 64%, and 79% by 39, 78, 312 and 625 μM Zn^{2+} respectively. These results indicate that Ca^{2+} stimulation and Zn^{2+} inhibition of proacrosin 'autoactivation' were due at least in part to the effects of the cations on acrosin's activity. The inhibitory effects of very low concentrations of Zn^{2+} (0.1 to 1% of Ca^{2+} concentrations used) on proacrosin 'autoactivation' was of particular interest since mammalian sperm have a high zinc content [7].

Results presented in table 1 show that the addition of increasing concentrations of highly purified rabbit sperm acrosin in the activation mixture decreased the lag time of proacrosin 'autoactivation'. These results

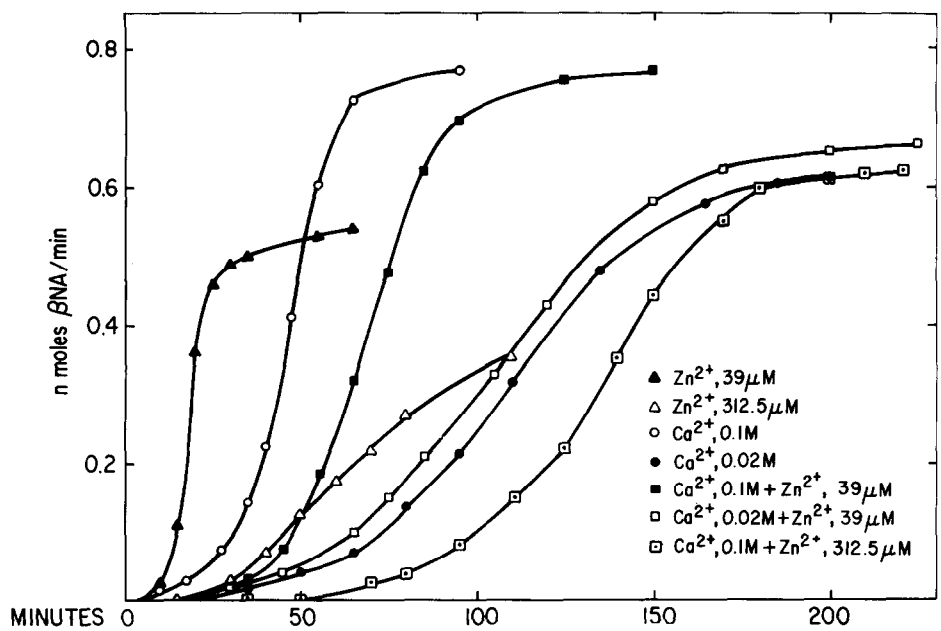

Fig.5. A comparison of the effect of Ca^{2+} and Zn^{2+} added alone or together on the time course of 'autoactivation' at pH 8.0.

Table 1
'Autoactivation' of proacrosin in the presence of added acrosin[a]

Acrosin (μg)	Lag Phase[b] (min)
0	40
0.23	30
0.46	20
0.92	15
1.84	10

a Highly purified rabbit sperm acrosin (203 nmol βNA/min/mg) was prepared by Sephadex G-75 and SE-Sephadex column chromatography (unpublished experiments). The sperm acrosin was added to the testis proacrosin preparation (final proacrosin protein concentration of 0.034 mg/ml in 1 mM HCl, pH 3.0) and the entire mixture was 'autoactivated' as described in the text.

b The time required for the first appearance of acrosin activity produced from testis proacrosin (determined by subtraction of the activity of added sperm acrosin from total acrosin activity present at various times of 'autoactivation').

provide further support for the autocatalytic nature of the proacrosin 'autoactivation'.

Further experiments will be required to determine whether 'autoactivation' in the absence of exogenous acrosin was due to the presence of a low amount of acrosin undetectable by the present assay methods or to the innate catalytic activity of proacrosin as described for certain zymogens [5]. The possibility that proacrosin is activated in vivo by an autocatalytic process regulatable by cations and/or pH also warrants investigation.

Acknowledgement

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