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EFFECT OF α_1-PROTEINASE INHIBITOR AND SULPHATED POLYSACCHARIDES ON THE ACTIVITY OF m_β-ACROSIN

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Purified m_β-acrosin catalysed amidolysis *in vitro* of several p-nitroanilides with C-terminal arginine residues. α_1-Proteinase inhibitor inhibited amidolysis catalysed by the enzyme. This effect of α_1-proteinase inhibitor was not prevented by pre-incubation of the enzyme with heparin or any other glycosaminoglycan. Pre-incubation of the enzyme with sulphated dextran or sulphated cellulose alleviated the effect of α_1-proteinase inhibitor. These results are discussed in terms of possible *in vivo* modulation by α_1-proteinase inhibitor of acrosin activity.

The proteinase acrosin (EC 3.4.21.10), largely in zymogen form, is present within the acrosome, an intracellular spermatazoon organelle. Prevention of fertilization *in vitro* and *in vivo* by acrosin inhibitors suggests the importance of acrosin during fertilization, possibly in enabling spermatazoon penetration of the zona pellucida of the ovum and/or in participation in the acrosomal reaction, a sequence of events leading to release or exposure of acrosomal contents. These considerations have stimulated interest in the effectiveness of proteinase inhibitors on acrosin activity, and in their possible function as physiological modulators of acrosin activity and possible use as pharmacological contraceptives (1,2). In this communication we report the ability of α_1-proteinase inhibitor to affect the action *in vitro* of m_β-acrosin. Because the action of this inhibitor on some proteinases may be affected by sulphated polysaccharides (3-6), the effects of these compounds on the m_β- acrosin-α_1-proteinase inhibitor system is also examined.

MATERIALS AND METHODS

Materials: Human plasma α_1- proteinase inhibitor (preparation A: lot no. 70F-9340; preparation B: lot no. 111F-9355; preparation C: lot no. 42F-9420; preparation D: lot no. 51F-9345; preparation E: lot no. 92F-9325), heparin from porcine intestinal mucosa (lot no. 46C-0035) and Polybrene (1,5-dimethyl 1,5-diazaundecamethylene polymethobromide) were from Sigma Chemical Co., Poole, U.K. Dermatan sulphate

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from porcine skin (lot no. S9401) was from Seikagaku Fine Biochemicals, Tokyo, Japan. River sturgeon notochord chondroitin 4-sulphate and 6-sulphate, bovine lung heparan sulphate and human umbilical cord hyaluronic acid, all produced under contract no. NO1-AM-5-2205 for the U.S.A. National Institues of Health, were kindly given by Professors M.B. Mathews and J.A. Cifonelli, Dept. of Pediatrics, University of Chicago, IL, U.S.A. Sulphated cellulose (lot no. 185) was obtained from Kelco, San Diego, CA, U.S.A. Sulphated xylan (preparation SP54) was obtained from Benechemie GmbH, Munich, F.D.R. Sulphated dextran from dextran of molecular weight 5×10^5 was obtained from Pharmacia, Uppsala, Sweden. All polysaccharides were in the form of sodium salts. Chromogenic substrates S-2222 (N-benzoyl-L-isoleucyl-L-glutamyl-glycyl L-arginine-p-nitroanilide hydrochloride and its methyl ester), S-2238 (D-phenylalanyl-L-pipecolyl-L-arginine-p-nitroanilide dihydrochloride), S-2266 (D-valyl-L-leucyl-L-arginine-p-nitroanilide dihydrochloride), S-2288 (D-isoleucyl-D-prolyl-L-arginine-p-nitroanilide dihydrochloride), S-2302 (D-prolyl-L-phenylalanyl-L-arginine-p-nitroanilide dihydrochloride) and S-2444 (L-pyroglutamyl-glycyl-L-arginine-p-nitroanilide hydrochloride) were from KabiVitrum, Ealing, U.K. m_β-Acrosin (approximate activity 130 units/mg protein) purified from ovine spermatazoa (7) was kindly given by Dr. R.A.P. Harrison, Agricultural Research Council Institute of Animal Physiology, Cambridge, U.K. This is the major and most stable form of active ovine acrosin; for a definition of nomenclature and of unit of activity of ovine acrosins see (7). Reagents were dissolved and diluted in buffer (0.11 M-NaCl/0.05M-Tris/HCl, pH 7.7 at 37°C).

Methods: A 30µl portion of polysaccharide solution was incubated at 37°C for 5 min with 20µl of m_β-acrosin solution (4.4 g/ml). Then 20µl of a solution of α_1-proteinase inhibitor was added and the mixture incubated for 5 min. Next 20µl of a mixture of chromogenic substrate (0.12 mg/ml) and Polybrene (0.12 mg/ml) was added. Initial reaction rates at 37°C were determined by measuring released p-nitroaniline spectrophotometrically. In control experiments, polysaccharides, enzyme and α_1-proteinase inhibitor were, in various combinations, replaced by appropriate volumes of buffer. The polycation Polybrene was included in order to prevent possible electrostatic interaction between polysaccharides and substrates (8). Concentrations quoted are final concentrations in the reaction mixture after addition of substrate and Polybrene. Methodology of experiments involving thrombin (EC 3.4.21.5) has been described previously.

RESULTS

In the assay system described, m_β- acrosin in the absence of α_1-proteinase inhibitor or polysaccharide catalysed the release of p-nitroaniline from p-nitroanilides containing C-terminal arginine residues at the following initial reaction rates (ΔA_{405}^{1cm}/µl/min x 10^3): substrates S-2288 5.8; S-2222 4.0 ; S-2266 3.9; S-2238 3.5; S-2302 2.7; S-2444 2.3. In subsequent experiments, substrate S-2288 was used. Incubation of α_1-proteinase with enzyme before addition of substrate inhibited enzyme activity (Table 1). No amidolysis was observed when α_1-proteinase inhibitor preparations were incubated with substrate in the absence of enzyme.

Incubation of heparin with m_β-acrosin before addition of α_1- proteinase inhibitor did not affect the action of the inhibitor; in contrast, incubation of heparin with thrombin before addition of α_1- proteinase inhibitor prevented

Table 1

Concentration of inhibitor (mg/ml x 10^2)	Initial amidolytic rate catalysed by m_β- acrosin in presence of α_1-proteinase inhibitor (ΔA^{1cm}_{405}/µl/min x 10^3)					
	Human preparations					Bovine
	A	B	C	D	E	
200	1.3	0.8	1.0	1.2	1.1	<0.2
100	3.4	1.4	3.0	3.5	1.6	<0.2
50	5.4	3.0	5.5	5.8	3.3	<0.2
10	5.8	5.6	5.7	5.9	5.7	1.4
5	N.D.	N.D.	N.D.	N.D.	N.D.	4.8
1	N.D.	N.D.	N.D.	N.D.	N.D.	5.4

Amidolytic rate catalysed by m_β-acrosin in absence of inhibitor was 5.8 (ΔA^{1cm}_{405}/µl/min x 10^3) N.D. = not done

the action of the inhibitor on thrombin-catalysed amidolysis (Fig. 1a). Incubation of m_β- acrosin with other glycosaminoglycans or with a sulphated xylan before addition of inhibitor did not affect the action of α_1-proteinase inhibitor (Fig. 1b,c). Incubation of m_β-acrosin with a sulphated dextran or with a sulphated cellulose before addition of α_1-proteinase inhibitor prevented the action of the inhibitor (Fig. 1c).

No amidolysis was observed when any of the polysaccharides, with or without α_1-proteinase inhibitor, were incubated with substrate in the absence of enzyme. None of the polysaccharides, when incubated with enzyme in the absence of α_1-proteinase inhibitor, altered the amidolytic rate.

DISCUSSION

Inhibitors immunologically indistinguishable from plasma $\alpha_1$proteinase inhibitor have been detected in seminal plasma, cervical mucous and Fallopian tube fluids (9). Although the pharmacological contraceptive potential of such naturally-occurring high molecular-weight proteinase inhibitors may be limited by their wide specificity and by their probable inability to penetrate effectively to sites of acrosin activity (9), it is possible that endogenous proteinase inhibitors modulate acrosin activity in vivo.

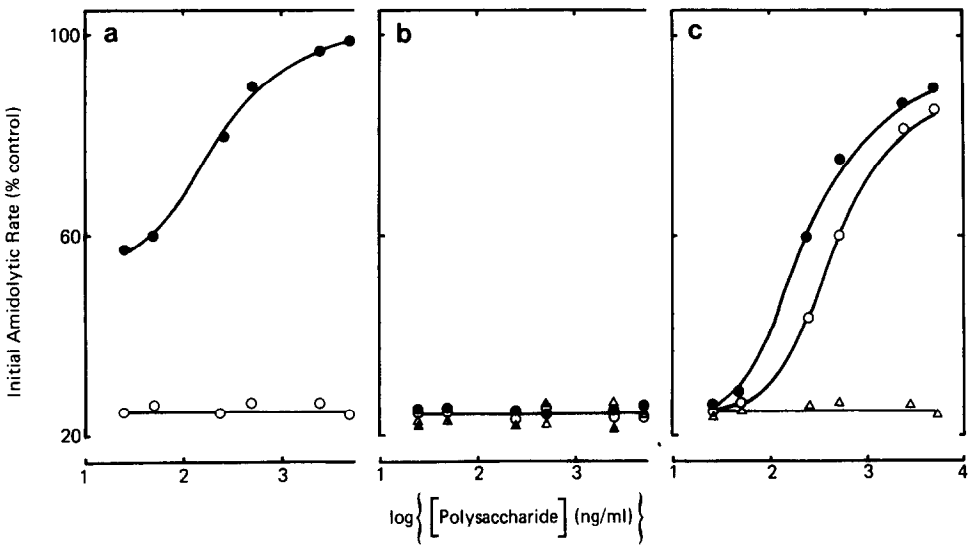

Fig. 1 Effect of polysaccharides on inhibition of $\underline{m}_\beta$- acrosin and thrombin by α_1-proteinase inhibitor.
(a) Effect of heparin on inhibition of (○) $\underline{m}_\beta$- acrosin; (●) thrombin;
(b) Effect of (○) heparan sulphate, (●) dermatan sulphate, (△) chondroitin 4-sulphate, (▲) chondroitin 6-sulphate on inhibition of $\underline{m}_\beta$- acrosin.
(c) Effect of (●) cellulose sulphate, (○) dextran sulphate (△) xylan sulphate on inhibition of $\underline{m}_\beta$- acrosin.
Initial amidolytic rate is expressed as a percentage of that initial rate occurring when enzyme was incubated with substrate and Polybrene and in the absence of inhibitor and polysaccharide; these control rates (ΔA_{405}^{1cm}/µl/min x 10^3) were 5.8 for $\underline{m}_\beta$-acrosin and 0.26 for thrombin. Concentrations of α_1-proteinase inhibitor were 0.1 mg/ml for $\underline{m}_\beta$-acrosin experiments, and 0.05 mg/ml for thrombin experiments. Reaction rates (as percentage control) in the presence of enzyme and these concentrations of inhibitor, and in the absence of polysaccharide were: $\underline{m}_\beta$-acrosin 24%; thrombin 58%.

Such modulation may include effects on acrosin activity at fertilization and during anovulatory periods. A partial inhibition of ovine acrosin by very high concentrations of human α_1-proteinase inhibitor preparations has been reported (2). In the experimental system described in this communication, an apparently rapid and complete inhibition of enzyme activity occurred when purified bovine α_1-proteinase inhibitor, present in 5-10 fold molar excess over the enzyme, was incubated with ovine $\underline{m}_\beta$- acrosin of high specific activity. Inhibition of thrombin by α_1-proteinase inhibitor may be prevented by pre-incubation of enzyme with heparin (Fig. 1a; 3-6); this effect depends upon polysaccharide-proteinase interaction, and may be clinically significant (3). In contrast, inhibitor of factor Xa (EC 3.4.21.6) and plasmin (EC 3.4.21.7) by α_1-proteinase inhibitor is not prevented by heparin (4,5)

presumably because of the less avid interaction between these enzymes and the glycosaminoglycan (10). Results reported here suggests that m_β- acrosin in this respect resembles factor Xa and plasmin rather than thrombin.

In summary, the results of this study using purified α_1-proteinase inhibitor and m_β- acrosin *in vitro* suggest possible physiological modulation of acrosin activity *in vivo* by endogenous inhibitors resembling α_1 -proteinase inhibitor. It seems likely that endogenous glycosaminoglycans would not affect such modulation.

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