THE INFLUENCE OF α_2-MACROGLOBULIN ON THE ELASTOLYTIC AND ESTEROLYTIC ACTIVITY OF ELASTASE

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1. Introduction

It has recently been shown that the elastolytic activity of elastase (pancreatopeptidase E, EC 3.4.4.7.) is inhibited by human α_2-macroglobulin [1, 2]. Elastase thus belongs to a class of hydrolytic enzymes (trypsin, chymotrypsin, thrombin, plasmin) which form complexes with α_2-macroglobulin (α_2 M). These complexes are active against synthetic substrates but inactive against proteins; also, the α_2M-bound enzymes are not inhibited by high molecular weight inhibitors (see [3] and references therein). It was thus of interest to see whether the above properties can be extended to elastase.

The results presented in this letter concern the effect of human and porcine α_2M on porcine elastase. The α_2M-elastase complexes are inactive against Remazol-brilliant-blue elastin but retain approximately 80% of the activity of free elastase on *t*-BOC-alanine-*p*-nitrophenyl ester. One molecule of α_2M binds two or three molecules of enzyme. The complex between human α_2M and elastase is only partially protected against the inhibition by soybean trypsin inhibitor or α_1-antitrypsin.

2. Materials and methods

Elastase, an electrophoretically pure preparation from Worthington (code ESFF, 64 Sachar Units/mg) was dissolved either in 0.05 M *N,N*-bis(2-hydroxyethyl) glycine (bicine) buffer, pH 8.8, for the elastolytic assay or in 0.066 M Soerensen phosphate buffer, pH, 6.5, for the esterolytic assay. The molar concentrations of the enzyme solutions were calculated by taking 25,000 as the molecular weight of elastase.

Human or porcine α_2M, the generous gifts of Dr. Steinbuch [4] and Dr. Jacquot-Armand [5] were dissolved in either one of the above buffers. The molar concentrations of the α_2M solutions were determined by using the appropriate absorbance coefficients ($A^{1\%\,1\,cm}_{280\,nm}$ = 8.1 and 10.2) and molecular weights (820,000 and 960,000). Soybean trypsin inhibitor (3 X cryst., Calbiochem, M. W. = 20,000) or human serum albumin (4 X cryst., NBCo) were dissolved in the phosphate buffer. Human α_1-antitrypsin (M.W. = 60,000) was a crude preparation obtained by filtering human plasma on Sephadex G-200. Its concentration was determined immunochemically.

The elastolytic and esterolytic activities were determined with remazol-brilliant-blue elastin (RBB-elastin) [6] and *N*-terbutyloxycarbonyl-L-alanine-*p*-nitrophenylester (NBA, MANN) [7] respectively. Elastase and α_2M (or soybean inhibitor or α_1-antitryspin) were allowed to react for 7 min before the residual enzymatic activity was assayed. The experiments designed to test the inhibition of the esterolytic activity of the α_2M-elastase complex were conducted as follows: elastase was reacted with α_2M for 7 min at 25°, then soybean inhibitor (or α_1-antitrypsin) was added and the residual activity was determined after an additional reaction time of 7 min. Controls for non-enzymatic hydrolysis of NBA were run similarly.

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3. Results and discussion

In fig. 1, the effects of human and porcine α_2M on the catalytic activity of elastase using either a natural substrate or a synthetic one are compared. With RBB-elastin as the substrate, a linear inhibition is observed with increasing concentrations of α_2M. The association between α_2M and elastase is apparently non-reversible since the inhibition is stochiometric down to 0% activity. This stochiometry may however be due to an exceedingly high association constant between the two proteins. Elastase:α_2M ratios at 100% inhibition are 0.53 and 0.37 for human and porcine α_2M respectively. To the nearest integer, the molar ratios of enzyme to α_2M are thus 2:1 (human α_2M) and 3:1 (porcine α_2M).

When the activity is measured with the synthetic substrate, α_2M acts as a partial inhibitor since the α_2M-elastase complexes are 75% or 80% active (fig. 1). It should be noticed that in both cases the esterolytic

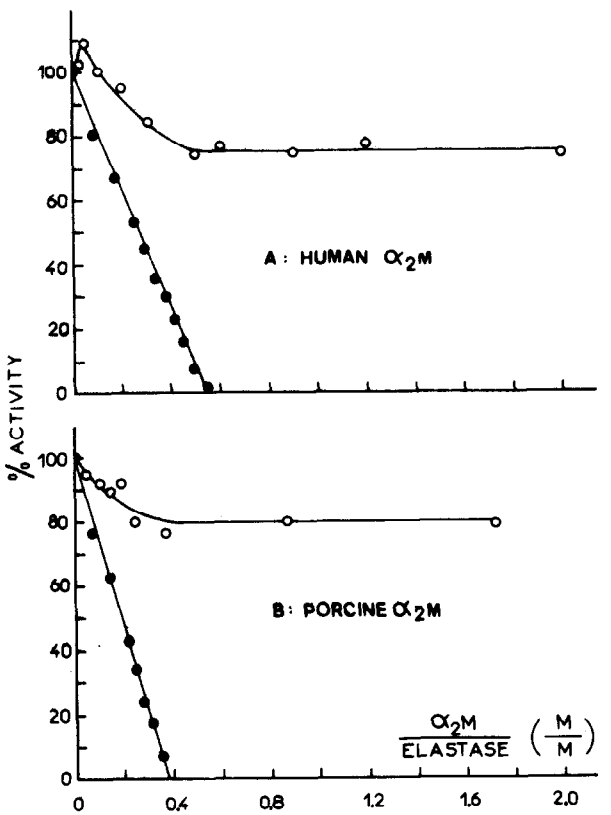

Fig. 1. Influence of porcine and human α_2M on the elastolytic (•) and esterolytic (○) activity of elastase. Elastolytic assay: reaction volume 5 ml; RBB-elastin 4 mg/ml; elastase 5.9×10^{-7}M; α_2M 0 to 3.2×10^{-7} M; 0.05 M bicine buffer, pH 8.8; 37°. Esterolytic assay: reaction volume 3 ml; NBA 4×10^{-4} M; elastase 1.33×10^{-7} M; α_2M 0 to 2.66×10^{-7}M; 0.066 M phosphate buffer pH 6.5; 25°.

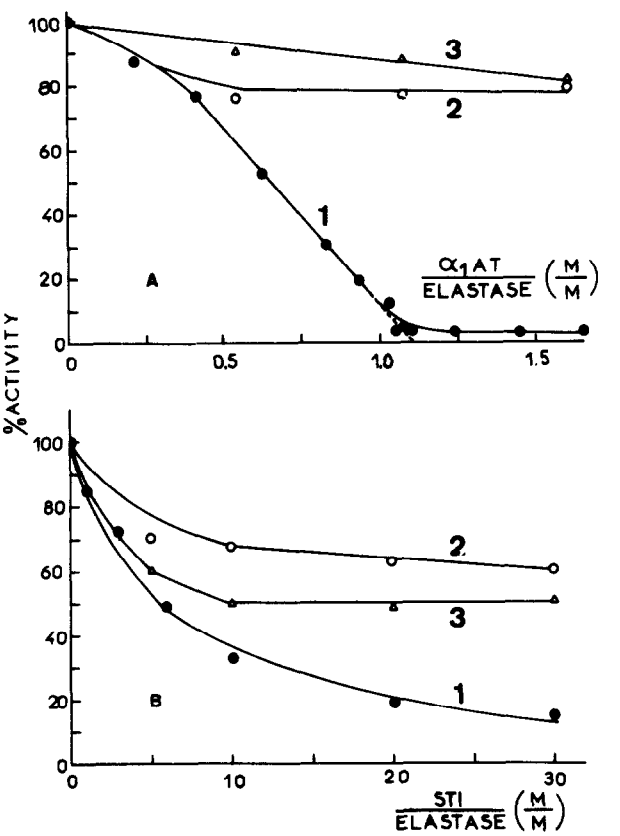

Fig. 2. Inhibition of the esterolytic activity of free and α_2M-bound elastase by soybean trypsin inhibitor (STI) and α_1-antitrypsin (α_1AT). Esterolytic assay as indicated in fig. 1.
A: *α_1AT* elastase 1.33×10^{-7} M; α_1AT 0 to 2.16×10^{-7}M.
curve 1: free elastase;
curves 2 and 3: α_2M-elastase same molar ratios as for STI.
B: *STI* elastase 1.33×10^{-7} M; STI 0 to 3.99×10^{-6} M;
curve 1: free elastase: (——) calculated curve; (•) experimental points;
curve 2: α_2M-elastase: α_2M/elastase molar ratio = 0.5;
curve 3: α_2M-elastase: α_2M/elastase molar ratio = 1.0.

activity becomes constant with concentrations of α_2M which give 100% inhibition of the elastolytic activity. The initial decrease in activity is thus a measure of the binding of α_2M to elastase. Unlike porcine α_2M, the human protein activates the esterolytic activity of elastase when the α_2M/elastase molar ratio is lower than 0.1 (fig. 1A). This activation has been observed repeatedly in separate experiments; besides α_2M does not hydrolyze the substrate by itself. It was thought that the initial activation was non-specific; to check this hypothesis, human serum albumin (HSA) was added to the reaction mixture instead of α_2M: a 33% activation was observed with a concentration of HSA of 1.1×10^{-2} mg/ml. HSA (and probably other proteins) may thus induce a conformational change of the elastase molecule with a subsequent increase of the catalytic activity of the enzyme. For an α_2M/elastase molar ratio lower than 0.1, the reaction medium contains free elastase and a small proportion of α_2M-elastase complexes: these complexes may exert a potentiating effect on the free elastase molecules in the same way as does HSA. The non-observable initial activation in the case of porcine α_2M (fig. 1B) may be due to the higher binding capacity of this protein which leaves less free enzyme molecules in the reaction medium.

Fig. 2 shows the inhibition of the esterolytic activity of free elastase and α_2M-bound elastase by soybean trypsin inhibitor (STI, fig. 2B) and α_1-antitrypsin (α_1AT (fig. 2A). Both inhibitors form inactive complexes with free elastase (100% inhibition was obtained with an STI/elastase molar ratio of 50). The data with STI and free elastase (fig. 2B) were analyzed by assuming the reversible formation of a one to one enzyme-inhibitor complex whose K_i is not affected by the presence of substrate. An Easson-Stedman plot [8] gave a value of $K_i = 6.6 \times 10^{-7}$ M; the theoretical inhibition curve calculated with this value fits well the experimental points (fig. 2B). Human serum α_1AT which inhibits the elastolytic activity of elastase [1, 2] also inhibits the esterolytic activity of this enzyme (fig. 2A). Extrapolation of the linear part of the inhibition curve gives an inhibitor:enzyme ratio of 1.11 at 100% inhibition. It is thus reasonable to conclude that one mole of α_1AT inhibits one mole of elastase. The K_i value, determined by activity measurements near the equivalence point was found to be 3.5×10^{-10}M.

Because α_2M-elastase does not hydrolyze the macromolecular substrate RBB-elastin, it was thought that its esterolytic activity is not inhibited by the macromolecular inhibitors STI and α_1AT. The results of fig. 2 only partially confirm this prediction: with an α_2M/elastase molar ratio of 0.5, STI inhibits the α_2M-elastase activity to 40% and α_1AT to 20%. It must thus be assumed that these proteins can be bound to the α_2M-elastase complex. The differences in inhibitory potencies may be related to the differences in molecular weights of STI (20,000) and α_1AT (60,000) whose binding to elastase may be more or less sterically hindered by α_2M. The results obtained with a higher concentration of α_2M (α_2M/elastase = 1) are somewhat contradictory and difficult to interpret: α_1AT inhibits less but STI inhibits more than with the former concentration of α_2M. No reasonable interpretation was found to account for these discrepancies.

In conclusion, our results confirm with more quantitative details the inhibition of the elastolytic activity of elastase by human α_2M demonstrated by others [1, 2], extend this inhibitory property to porcine α_2M and indicate that α_2M does not bind to the catalytic center of elastase (or does not "bury" it) since α_2M-elastase is able to hydrolyze a small substrate. In addition, it is shown that soybean inhibitor and α_1-antitrypsin which inhibit the elastolytic activity [1, 2, 9] also abolish the esterolytic activity of free elastase and that these proteins can probably form ternary complexes with α_2M-elastase indicating that elastase possesses several protein binding sites. An extensive study of these binding properties is now in progress.

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