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RAPID INACTIVATION OF α_1-PROTEASE INHIBITOR (α_1-ANTITRYPSIN) BY ELASTASE *

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Summary

When purified α_1-protease inhibitor, of P_i MM phenotype, was reacted with a 1.3 fold molar excess of elastase at 37°C, 47% of the inhibitor was immediately inactivated while the remaining 53% formed an electrophoretically separable complex with the enzyme. Zone, followed by antigen-antibody crossed electrophoresis of time-course mixtures (0 to 30 min at 37°C) showed that the α_1-protease inhibitor-elastase complex is further degraded to inactivated α_1-protease inhibitor and that the degradation follows first order kinetics ($k = 2.1 \cdot 10^{-3}$ s^{-1}). α_1-Protease inhibitor in the presence of 1.3 fold molar excess of elastase is 99% inactivated in 30 min at 37°C. The inactivation reaction was not followed by the release of equivalent amounts of active enzyme i.e., only a 4.0% increase in active enzyme was apparent after 30 min at 37°C. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the above α_1-protease inhibitor-elastase mixtures gave 49 000 and 78 000, respectively, for the molecular weights of inactivated α_1-protease inhibitor and the α_1-protease inhibitor-elastase complex. Two other proteins intermediate between the α_1-protease inhibitor-elastase complex and inactivated α_1-protease inhibitor possessed molecular weights of 74 000 and 65 000. The reaction also produced a 2200 dalton peptide fragment.

Introduction

Several years ago this laboratory reported that α_1-antitrypsin (currently α_1-protease inhibitor) was the principal elastase inhibitor of human serum [1]. These studies also revealed that free α_1-protease inhibitor was demonstrable in

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electrophoretically separated elastase-serum reaction mixtures even in the presence of an excess of elastase [1]. Assuming a mol/mol interaction and assuming the α_2-macroglobulin inhibitor to be fully saturated with elastase it was calculated that approximately 23% of the available α_1-protease inhibitor was chemically incapable of reacting with the enzyme [1].

In view of the implication of α_1-protease inhibitor deficiency in obstructive lung disease (emphysema) [2] it was considered important to determine the physicochemical reason why a portion of the α_1-protease inhibitor in human serum is not effective as an elastase inhibitor.

The present communication presents data which show that the fraction of α_1-protease inhibitor in serum which apparently did not react with elastase [1] had indeed reacted and that the resulting product is chemically incapable of forming an electrophoretically separable complex with, and therefore does not inhibit, either elastase or trypsin; presented also are data which demonstrate that at 37°C, in the presence of excess elastase, the α_1-protease inhibitor-elastase complex is very short-lived.

Preliminary accounts of portions of this work have been published [3,4].

Materials and Methods

Porcine pancreatic elastase (EC 3.4.21.11) was prepared by column chromatography on DEAE-cellulose as previously described [5]. The homogeneity of the product has been demonstrated [1,5,6] and 100% activity is assumed. Trypsin (2 × crystallized, EC 3.4.21.4) was a product of the Sigma Chemical Company, and contained 45% active trypsin as determined by the Chase and Shaw titration [7].

Separation of α_1-protease inhibitor from human serum

Human plasma (500 ml), of phenotype P_i MM [8]*, was dialyzed against 10 volumes of 0.01 M $CaCl_2$ + 0.14 M NaCl [10]. After removal of fibrin by centrifugation the supernatant (serum) was brought to 50% of saturation with ammonium sulfate. After centrifugation the supernatant was brought to 70% of saturation with ammonium sulfate, centrifuged and the precipitate dissolved in 0.01 M phosphate buffer, pH 8.3, (molar ratio $K_2HPO_4/KH_2PO_4 = 24$) and dialyzed against 17-liter aliquots of the same buffer until the conductivity of the dialysate reached that of the 0.01 M buffer ($1.5 \cdot 10^{-3}\,\Omega^{-1}$, Radiometer CDM2e with PP1042 cell). The dialyzed 70% fraction was added to a 5 × 110 cm column of DEAE cellulose (Whatman DE 23, 0.9 mEquiv. N/g), equilibrated with the pH 8.3 buffer, which was poured and operated as previously described [11,12]. Following collection of 700 ml of effluent (0.01 M phosphate buffer, pH 8.3) a 3 × 2 liter gradient was added. The limit buffer [13] was 0.5 M KH_2PO_4 and the percentage of limit buffer in each chamber was 0 (chamber 1), 20, 100. α_1-Protease inhibitor in the effluent fractions, was detected in agar gel using the elastase impregnation method previously described [1]. The α_1-protease inhibitor-containing fractions (20 ml/fraction) were pooled, dialyzed

* Between 86 and 99% of the world population possess the P_i MM phenotype. For an explanation of the P_i system see ref. 9.

against 0.01 M phosphate buffer, pH 8.3, and added to a 2.1 × 80 cm column of DEAE cellulose equilibrated with the same buffer. Following the collection of 1 liter of effluent (10 ml/fraction), a 9 × 300 ml gradient (Varigrad, Buchler Instrument Co.) was begun. The limit buffer was 0.8 M KH_2PO_4 and the percentage of limit buffer in each chamber was 0 (chamber 1), 2, 2, 10, 10, 2, 20, 40, and 100. The α_1-protease inhibitor-containing fractions, detected as above, were pooled and rechromatographed on a 2.1 × 80 cm column of DEAE-cellulose using the same gradient. The α_1-protease inhibitor-containing fractions were pooled, concentrated (Diaflo UM-10) and added to a 4 × 100 cm column of Sephadex G-200 equilibrated with 0.01 M phosphate buffer, pH 8.3 [12]. The α_1-protease inhibitor-containing fractions (5 ml/fraction) were pooled and added to a 2.1 × 20 cm column of Con A-Sepaharose (Pharmacia) for removal of albumin as described by Liener et al. [14]. Purity was determined by disc gel [15], sodium dodecyl sulfate gel [16], and by immunoelectrophoresis [1] (antihuman whole serum antibody, Behring Diagnostics). Further, the concentrated product (15—30 mg/ml) was subjected to double diffusion in agar gel against antibody (Behring Diagnostics) to the following possible contaminents: albumin, α_1-acid glycoprotein, transferrin, hemopexin, prealbumin, haptoglobin and ceruloplasmin. The product produced single bands or arcs using the above electrophoretic methods, and was negative by double diffusion analysis, for the possible contaminents listed above. The yields of α_1-protease inhibitor varied between 20—25%.

All concentrations of α_1-protease inhibitor were determined by radial immunodiffusion in anti α_1-protease inhibitor-containing agarose gel (Quantiplate, Kallestad Laboratories, Chaska MN).

Isolation of crude "non-reactive" α_1-protease inhibitor

Elastase and serum were mixed at 0°C (0.1 ml volumes) and immediately subjected to zone electrophoresis (9.3 V · cm^{-1} for 1 h) in agar gel at pH 8.6 on 5 × 25 cm photographic plates (three, 0.2 × 4 cm sample slits) as previously described [5]. The molar ratio of elastase/α_1-protease inhibitor was 1.5. The "non-reactive" α_1-protease inhibitor-containing zones were removed and frozen. After collection of a sufficient quantity of zones the gel segments were thawed, thoroughly macerated, centrifuged and extracted twice with 100-ml aliquots of 0.027 M diethylbarbiturate buffer, pH 8.6. The supernatants were pooled, dialyzed against distilled water and lyophilized.

Time-course studies on the inactivation of α_1-protease inhibitor by excess elastase and trypsin at 37°C

Aliquots of α_1-protease inhibitor (in 0.027 M diethylbarbiturate buffer, pH 8.6) at a concentration of 1.0 mg/ml were prepared, mixed with an equal volume of freshly prepared elastase solution (0.6 mg/ml, in the above buffer) and the reaction mixture(s) incubated at 37°C for up to 30 min. The molar ratio of elastase/α_1-protease inhibitor was 1.3 *. Following each incubation period the reaction mixture(s) was plunged into an ice bath and 5 μl ($4.6 \cdot 10^{-5}$ μmol α_1-protease inhibitor) immediately subjected to zone electrophoresis in

* (0.6/25 000)/(1.0/54 000) = [Elastase]/[α_1-protease inhibitor] = 1.3.

agarose gel (15°C) followed by crossed electrophoresis into α_1-protease inhibitor antibody (Behring Diagnostics) containing agarose gel (15°C) overnight (18 h) according to the microtechnique of Weeke [17]. After processing in the usual way [1] the plates were dried under filter paper and stained with Amido Black 10B. The areas of the peaks, representative of inactivated α_1-protease inhibitor and the α_1-protease inhibitor-elastase complex, were determined by planimetry of a 6.4 × enlargement (S.D. of three separate tracings was negligible), equated with the areas of the controls and the resulting concentrations plotted as function of time at 37°C. $2.6 \cdot 10^{-5}$ μmol of inactivated α_1-protease inhibitor was used for the control. Details of the preparation of and physicochemical properties of inactivated α_1-protease inhibitor will be presented in a forthcoming publication.

The concentration of α_1-protease inhibitor in the α_1-protease inhibitor-elastase complex was calculated as follows. First, the concentration of inactivated α_1-protease inhibitor was determined for the zero h sample as described above. The amount of inactivated α_1-protease inhibitor thus determined was subtracted from the amount of α_1-protease inhibitor initially present and this amount was equal to the α_1-protease inhibitor present in the complex at zero time. In order to compensate for slight differences in areas determined for succeeding incubation times the ratio of $cm^2 \cdot \mu mol^{-1}$ inactivated α_1-protease inhibitor of the control to that of the complex ($cm^2 \cdot \mu mol^{-1}$ α_1-protease inhibitor in the complex) was determined for the zero time and this was used in the following equation to calculate the α_1-protease inhibitor concentration in the complex for subsequent incubation times:

$$(C)\,(A_{\text{control}})\,(B)/(A_{\text{control}}) = \mu\text{mol } \alpha_1\text{-protease inhibitor (complex)} \qquad (1)$$

where C is the ratio calculated above, A is the area of the peak in cm^2 and B is the concentration of inactivated α_1-protease inhibitor ($2.6 \cdot 10^{-5}$ μmol) in the control sample.

The elastase activity of 0.1 ml samples representative of zero and 30 min incubation times was determined using the method of Visser and Blout [18].

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of elastase-α_1-protease inhibitor reaction mixtures

These experiments were carried out with elastase-α_1-protease inhibitor reaction mixtures according to Weber and Osborn [16]. The molecular weights of inactivated α_1-protease inhibitor and the α_1-protease inhibitor-elastase complex were determined using human serum albumin (separated from human serum in the last step of the α_1-protease inhibitor purification procedure presented above), ovalbumin (Worthington), lysozyme (Worthington) and elastase as standards. The molecular weight of a peptide produced in the reaction was determined using the method of Swank and Munkres [19]. Elastase, lysozyme and bacitracin (Upjohn) were used as standards.

Interaction of elastase and the protein zones of α_1-protease inhibitor separated by zone electrophoresis in acid starch gel

Since we had previously shown that the protease-binding property of α_1-protease inhibitor was not destroyed by acid starch gel electrophoresis [8] and

since the latter method provides for an excellent separation of this polymorphic protein [9] it was of interest to determine if each of the separated zones react equally with elastase. This was accomplished by subjecting serum (P_i MM phenotype) to acid starch gel electrophoresis [8] followed by removal of a 1.5 × 3 × 82 mm strip of starch gel (origin to anode). The gel strip was incubated at 25° C for 20 min in a solution of 0.1 M diethylbarbiturate buffer, pH 8.6, containing 0.4 mg/ml of elastase. A control starch gel strip was incubated in buffer only. The gel strips were subjected to crossed electrophoresis into anti α_1-protease inhibitor antibody-containing agarose gel as above and the areas of the resulting peaks determined (as above).

Since the α_1-protease inhibitor-elastase complex possesses a slower electrophoretic mobility than the inhibitor [1] one would expect the areas of the peaks produced by the elastase-treated sample to be lower than the control peaks. Therefore, if the separated α_1-protease inhibitor reacted similarly then the ratios of the areas of the elastase-treated peaks to that of the control peaks (without elastase) should be the same.

Results

Fig. 1 presents the results obtained when elastase-serum and trypsin-serum mixtures are subjected to immunoelectrophoresis and when the enzymes and α_1-protease inhibitor are allowed to interact during the electrophoretic separation. Both enzymes are present (Fig. 1) in excess of equivalence i.e., the molar ratio of enzymes/α_1-protease inhibitor > 1.0. The results in Fig. 1A illustrate the phenomenon observed previously [1] i.e., in the presence of excess elastase not all of the α_1-protease inhibitor in the sample forms an electrophoretically separable complex with elastase. However, the results for trypsin (Fig. 1B) show that 100% of the α_1-protease inhibitor does react with trypsin as evidenced by the firm electrophoretically separable complex and the absence of free α_1-protease inhibitor. When solutions of electrophoretically isolated "non-reactive" α_1-protease inhibitor (= 0.6 mg/ml α_1-protease inhibitor) were reacted with elastase and trypsin and subjected to immunoelectrophoresis, according to Fig. 1A, a single arc of precipitate in the α_1-protease inhibitor position was produced. These results conclusively demonstrated that the "free" α_1-protease inhibitor produced when elastase was in excess (Fig. 1A1) had indeed reacted with the enzyme and that the product of the reaction (inactivated α_1-protease inhibitor) was chemically incapable of forming a complex with either elastase or trypsin.

Time-course studies on the inactivation of α_1-protease inhibitor by elastase

Since the production of inactivated α_1-protease inhibitor occured almost instantaneously (Fig. 1A) it was of interest to determine the extent of the reaction at 37° C in slight molar excess of elastase (molar ratio elastase/α_1-protease inhibitor = 1.3) and to determine the fate of the α_1-protease inhibitor-elastase complex under these conditions. Briefly, the results show that in the presence of excess elastase approximately half of the α_1-protease inhibitor was immediately inactivated, the remainder formed an electrophoretically separable complex with the enzyme, and that the complex upon further incubation was

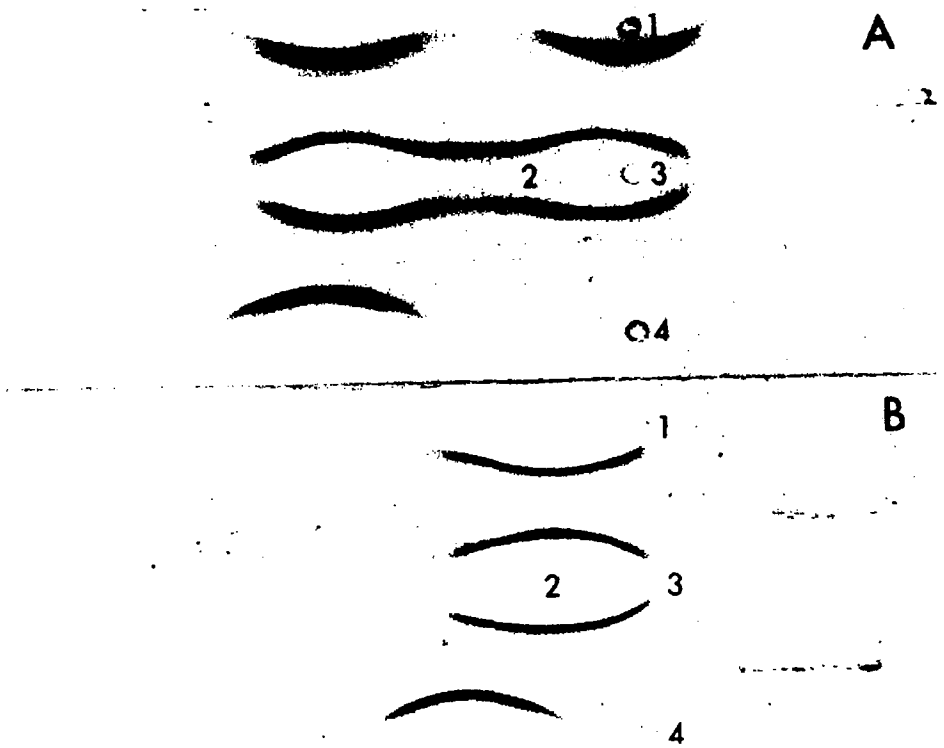

Fig. 1. Interaction of elastase and trypsin with human serum α_1-protease inhibitor during zone electrophoresis in agar gel. (A) Elastase: 1, elastase-serum reaction mixture (molar ratio elastase/α_1-protease inhibitor = 1.1); 2, elastase, 2 mg/ml; 3, serum (α_1-protease inhibitor, 4 mg/ml); 4, serum. Note in wells 2 and 3 that the cathodically migrating elastase has reacted with only a portion of the anodically migrating α_1-protease inhibitor. (B) Trypsin: 1, trypsin-serum reaction mixture (molar ratio trypsin/α_1-protease inhibitor = 1.9); 2, trypsin 2.7 mg/ml; 3, serum (α_1-protease inhibitor, 3.1 mg/ml); 4, serum. Note in wells 2 and 3 that the cathodically migrating trypsin has reacted with 100% of the anodically migrating α_1-protease inhibitor. Distance between sample wells 2 and 3, 0.5 cm. Enzyme-serum mixtures, designated as sample 1 in A and B were mixed and immediately subjected to electrophoresis. All troughs contained anti α_1-protease inhibitor antibody (Behring Diagnostics). One percent agar in 0.027 M diethylbarbiturate buffer, pH 8.6. Field intensity 7.5 V · cm^{-1}; A, 2 h; B, 1.5 h. Sample volume, 1.5 µl. The anode is at the left.

gradually degraded to the inactivated inhibitor and inactivated elastase. The results of this experiment are presented in Figs. 2 and 3. Fig. 2 illustrates the crossed electrophoretic patterns obtained for the zero, 10, 20, and 30 min samples. As can be seen, when the reactants are mixed and immediately subjected to zone electrophoresis there is an instantaneous conversion of 47% of the native α_1-protease inhibitor to inactivated α_1-protease inhibitor with 53% of the available inhibitor being accounted for in the α_1-protease inhibitor-elastase complex (zero h, Fig. 3). Fig. 3 shows that for the first 10 min of incubation the production of inactivated α_1-protease inhibitor, resulting from the degradation of the complex, and the decrease in α_1-protease inhibitor concentration in the complex are approximately equivalent ($3.5 \cdot 10^{-8}$ µmol · s^{-1} vs. $-2.6 \cdot 10^{-8}$ µmol · s^{-1}). As the incubation proceeds (Fig. 3) the decrease in the concentration of α_1-protease inhibitor in the complex should result in an equal increase in the concentration of inactivated α_1-protease inhibitor. This, however, as

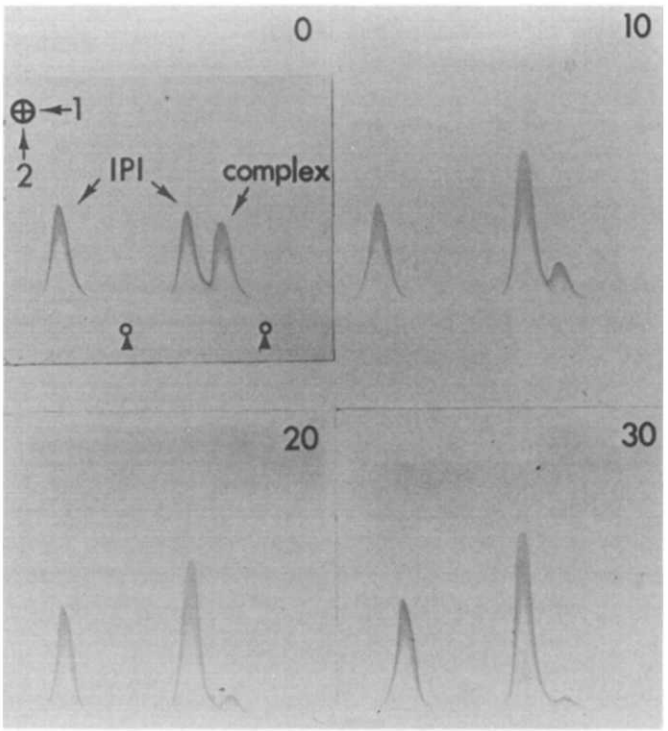

Fig. 2. Zone electrophoretic-crossed electrophoretic patterns of elastase-α_1-protease inhibitor reaction mixtures. Numbers in the upper right hand corners refers to incubation times (min) at 37°C. Sample well locations (black circles designated by shaftless arrows), direction of migration during the first and second dimensions, location of inactivated α_1-protease inhibitor (IPI) and the α_1-protease inhibitor-elastase complex (complex) are shown in the upper left of the figure (zero h sample). The peak to the left (control) in all cases is representative of $2.6 \cdot 10^{-5}$ μmoles of inactivated α_1-protease inhibitor. Sample volume, 5 μl. One percent agarose in 0.027 M diethylbarbiturate buffer, pH 8.6. Field intensity; first dimension: 1 h at 3.5 V · cm^{-1} (agarose); second dimension: 18 h at 2.0 V · cm^{-1} (anti α_1-protease inhibitor antibody-containing agarose). See text for further details.

shown in Fig. 3, was not the case. The latter is in all probability the result of further degradation of inactivated α_1-protease inhibitor, possibly that portion of the molecule which contains the antigenic determinant(s). After 30 min at 37°C, 97% of the α_1-protease inhibitor in the complex has been converted to inactivated α_1-protease inhibitor and at this time 98.7% of the total α_1-protease inhibitor has undergone conversion to the inactivated inhibitor. When the logarithm of the decreasing α_1-protease inhibitor concentration in the complex was plotted as a function of time (Fig. 3) a straight line resulted indicating that the degradation followed first order kinetics. The first order rate constant calculated from the slope (Olivetti Programma 101, Microcomputer Code 219) of this plot was $k = 2.1 \cdot 10^{-3}$ s^{-1}. A second experiment gave $k = 2.4 \cdot 10^{-3}$ s^{-1}. A single run with trypsin under the same conditions yielded $k = 9.4 \cdot 10^{-3}$ s^{-1}. Thus, the trypsin-α_1-protease inhibitor complex is degraded, in the presence of excess trypsin, in a manner analogous to that observed with elastase. The reaction is more rapid and the initial fast reaction exhibited in the elastase-α_1-protease inhibitor interaction (47% degradation at zero h) does not occur. In addi-

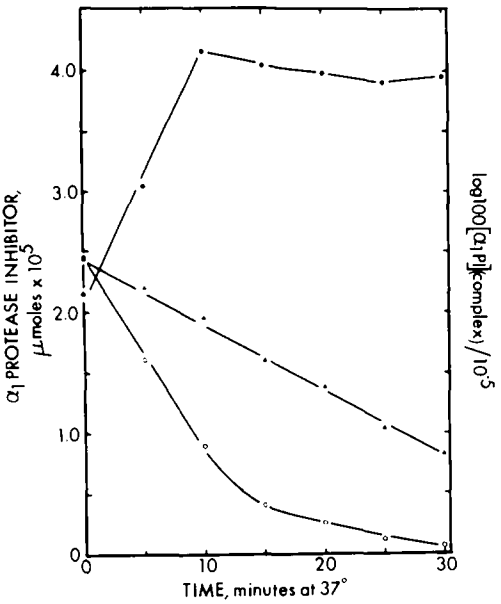

Fig. 3. Time-course plots of the production of inactivated α_1-protease inhibitor and degradation of the α_1-protease inhibitor-elastase complex at pH 8.6 and 37°C. Values were calculated from areas of peaks presented in Fig. 2. ●, inactivated α_1-protease inhibitor; ○, α_1-protease inhibitor in the α_1-protease inhibitor-elastase complex; △, log plot of decreasing α_1-protease inhibitor concentration in complex (○). See text for further details.

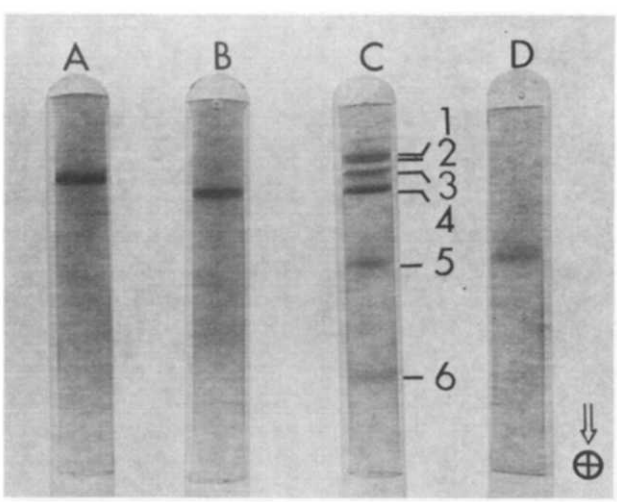

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns of A, α_1-protease inhibitor (54 000 daltons), 6.25 μg; B, purified inactivated α_1-protease inhibitor (49 000 daltons), 6.25 μg; C, zero hour mixture (Figs. 2 and 3) of α_1-protease inhibitor, 6.25 μg and elastase, 3.75 μg (molar ratio, elastase/α_1-protease inhibitor = 1.3), 1, α_1-protease inhibitor-elastase complex No. 1, 78 000 daltons, 2, α_1-protease inhibitor-elastase complex No. 2, 74 000 daltons, 3, intermediate complex, 65 000 daltons, 4, inactivated α_1-protease inhibitor, 49 000 daltons, 5, elastase, 25 000 daltons, 6, peptide fragment, 2200 daltons. D, elastase, 3.75 μg. All samples were heated at 100°C for 5 min prior to electrophoresis. Stained with Coomassie Brillant Blue R.

tion, all of the available α_1-protease inhibitor, as shown in Fig. 1B, could be accounted for (at zero h) in the trypsin-α_1-protease inhibitor complex.

When mixtures equivalent to the above (Fig. 3) were analyzed for residual elastase activity only a 4.0% increase was demonstrable at the end of 30 min at 37° C. A second experiment gave 5.0%. The results in Figs. 2 and 3 clearly show that disappearance of the α_1-protease inhibitor-elastase complex with a concomitant increase in inactivated α_1-protease inhibitor occurs. However, no increase in elastase activity equivalent, on a molar basis, to the formation of inactivated α_1-protease inhibitor is apparent.

A control elastase sample (0.8 mg/ml) was also incubated at 37°C and $1.7 \cdot 10^{-4}$ μmol analyzed for activity at 0, 5, 10, 15, 20, 25, and 30 min intervals. The results gave a mean and standard error of 0.088 ± 0.008 (absorbance at 357.5 nm) indicating that little or no self digestion occurred under the experimental conditions.

Molecular weights of reactants and products of the inactivation reaction

It was also of interest to determine the molecular weights of the α_1-protease inhibitor-elastase complex and inactivated α_1-protease inhibitor and the molecular weights of any intermediates or fragments produced in the inactivation reaction. The results of this experiment are shown in the SDS gel electrophoretic patterns presented in Fig. 4 *. Fig. 4C shows that in addition to the α_1-protease inhibitor-elastase complex (78 000 daltons) at least two additional proteins of high molecular weight (74 000 and 65 000) are present in the zero hour mixture. These proteins no doubt represent intermediates in the inactivation reaction. A single low molecular weight peptide fragment of 2200 daltons was demonstrable in the zero hour reaction mixture (Fig. 4C). The principal product of the reaction was inactivated α_1-protease inhibitor which possessed a molecular weight of 49 000.

Interaction of elastase and electrophoretically separated zones of α_1-protease inhibitor

Since it was shown above that 47% of the inhibitor (Fig. 3) was converted immediately by elastase to the inactivated inhibitor it was desirable to determine if all of the zones produced by α_1-protease inhibitor upon acid starch gel electrophoresis would react equally with the enzyme. When acid starch gel strips, containing the separated zones of α_1-protease inhibitor were reacted with elastase and cross electrophoresed into anti α_1-protease inhibitor antibody-containing agarose gel the results in Fig. 5 were obtained. Fig. 5 shows a decrease in area of all of the peaks in the elastase treated sample (Fig. 5B) as compared to the control (Fig. 5A). When the areas of the individual peaks were determined, the mean and standard error (Fig. 5) of the ratio of the elastase treated sample (Fig. 5B) to the control (Fig. 5A) was 0.48 ± 0.02 indicating that the α_1-protease inhibitor zones (P_i MM phenotype) appear to react equally with elastase. An additional P_i MM sample gave 0.71 ± 0.03.

* These samples were heated for 5 min at 100°C in the presence of SDS and 2-mercaptoethanol to prevent proteolysis. Note the absence of additional bands in Fig. 4D, the elastase control. Unheated samples resulted in random proteolysis leading to the production of many bands with lower molecular weights.

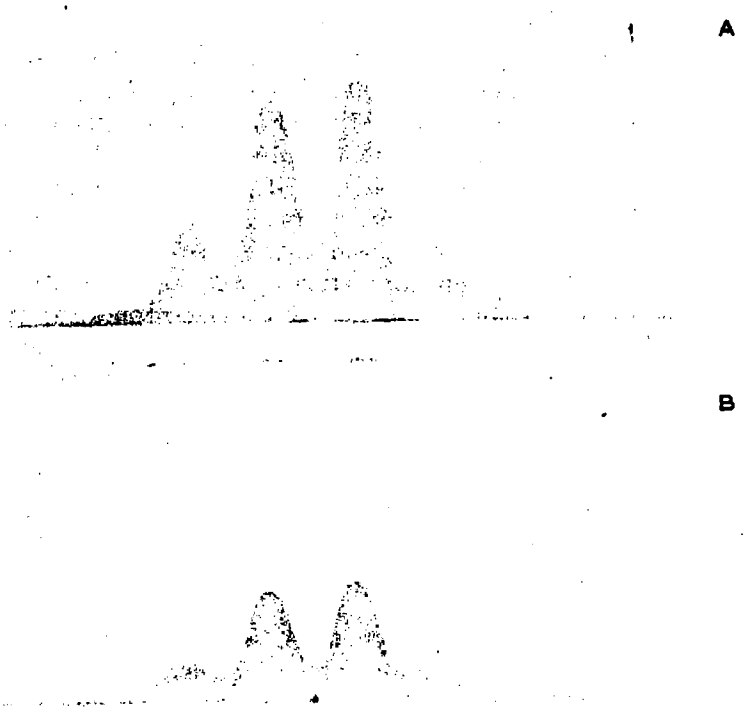

Fig. 5. Interaction of elastase and the electrophoretically separated protein zones of α_1-protease inhibitor. A, control. B, separated α_1-protease inhibitor (acid starch gel electrophoresis) reacted with elastase prior to crossed electrophoresis into anti α_1-protease inhibitor antibodycontaining agarose. The anode is at the left (starch gel) and top (crossed electrophoresis) of the figure. Please see text for details.

Discussion

The previous study [1] had shown that a portion of the α_1-protease inhibitor in human serum did not form an electrophoretically separable complex with elastase when the enzyme was present in excess of equivalence. This inability was not reflected in any appreciable change in electrophoretic mobility of the non-reactive α_1-protease inhibitor, in the reaction of the protein with anti α_1-protease inhibitor antibody or in any change in electrophoretic mobility when the elastase-serum mixture was incubated at 37°C for 30 min [1]. These results were taken to mean that a portion of the α_1-protease inhibitor in human serum was chemically incapable of reacting with the enzyme.

The present work has clearly shown that, in the presence of excess elastase α_1-protease inhibitor is rapidly converted to the inactivated inhibitor (Figs. 2 and 3). Although inactivated α_1-protease inhibitor is the principal and most obvious product of the reaction the most important and salient feature is the fact that active elastase equivalent, on a molar basis, to the inactivated α_1-protease inhibitor produced is not demonstrable. These results suggest that cleavage of the α_1-protease inhibitor molecule occurs at the binding site and that the fragment produced is still associated with and inhibits the activity of the enzyme.

It is also possible that the elastase moiety of the α_1-protease inhibitor-elastase complex is cleaved by excess elastase prior to or at the same time as the release of inactivated α_1-protease inhibitor. The results in Fig. 4 offer evidence that cleavage does occur and that a fragment of 2200 daltons was produced. The molecular weight of this fragment is approximately half that of the difference (5000) between the molecular weights of α_1-protease inhibitor and inactivated α_1-protease inhibitor. Whether it is identical with, or a degradation product of the fragment which is still associated with elastase, at this juncture, is not clear.

Travis et al. [20] have incubated human trypsin-α_1-protease inhibitor mixtures at various trypsin/α_1-protease inhibitor ratios, presumably at room temperature, and subjected them to polyacrylamide slab gel electrophoresis. At enzyme/α_1-protease inhibitor ratios greater than 1.0 a decrease in Coomassie blue staining of the complex was noted [20]. These authors also were unable to demonstrate any "re-gain" of tryptic activity [20]. Our results with bovine trypsin are similar to the above in that the trypsin-α_1-protease inhibitor complex, at a molar ratio of enzyme/α_1-protease inhibitor of 1.3, is degraded in less than 30 min at 37°C. It is also of interest to note that the first order rate constants show that the trypsin-α_1-protease inhibitor complex is degraded about 4.5 times more rapidly than the elastase-α_1-protease inhibitor complex ($9.4 \cdot 10^{-3}\ s^{-1}$ vs. $2.1 \cdot 10^{-3}\ s^{-1}$) *.

It is difficult, at best, to explain the physicochemical reason why a portion (47%, Fig. 3, zero h) of the α_1-protease inhibitor molecules should, when exposed to excess elastase, be immediately converted to inactivated α_1-protease inhibitor whereas the remaining 53% is complexed by the enzyme (Fig. 3). We have examined many sera [3] and several other purified α_1-protease inhibitor preparations and all, to varying degrees, possess this property. It is possible that α_1-protease inhibitor while in vivo could have been subjected to mild proteolytic attack or otherwise conformationally altered (age of the molecules?). Either of the above could conceivably result in a molecule more susceptible to attack by elastase. Interestingly, all of the α_1-protease inhibitor molecules form an electrophoretically separable complex with trypsin. Therefore, the initial fast reaction (47% inactivation at zero h) appears to be, at present, a property specific for elastase and is apparently unrelated to the α_1-protease inhibitor in any one or more individual protein zones characteristic of the P_i MM phenotype (Fig. 5). The inactivation reaction also could be of importance in the normal turnover of α_1-protease inhibitor in vivo and it is perhaps significant that, like the degradation of the α_1-protease inhibitor-elastase complex (Fig. 3) the catabolic reactions involved in the turnover of all plasma proteins appear to be first order [21].

In conclusion, the mechanism of the interaction between excess elastase and α_1-protease inhibitor appears not to be inhibition via simple complex formation but a reaction resulting in the inactivation of both moieties. Although the interaction does indeed result in the inactivation of α_1-protease inhibitor this fact should not be taken as evidence that the entire population of the protein is an ineffective inhibitor of elastase.

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* These constants are dissociation rate constants (k_{dissoc}) of the α_1-protease inhibitor-enzyme complexes. How they relate to the association rate constants (k_{assoc}) and the enzyme-inhibitor association constants (K_{assoc}) will be detailed in a forthcoming publication.

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