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THE DUAL NATURE OF THE REACTION BETWEEN PORCINE ELASTASE AND HUMAN PLASMA α_1 PROTEINASE INHIBITOR*

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SUMMARY: Only a portion of human plasma α_1 proteinase inhibitor (α_1PI) forms a 1:1 complex with porcine elastase; the other portion is inactivated via proteolysis. High temperature (37°) and high salt (2 M) enhance complex formation. The complex is unstable, but no significant liberation of active elastase could be demonstrated. Probably the same two major products of ∿50,000 and ∿4,000 daltons are formed from α_1PI via proteolysis and via disintegration of the complex. Iodination of α_1PI or oxidation with chloramine-T prevents complex formation with elastase but not with trypsin. Iodinated elastase, however, forms a complex with α_1PI.

Recently, it was reported that about one-half of α_1PI formed a 1:1 complex with porcine elastase. The other half was hydrolyzed to form one large and one small fragment (1). Independently, several laboratories showed that α_1PI was susceptible to limited proteolysis by a variety of proteinases (2-5), and that the inactivating cleavage occurred in the same region as that observed during inactivation by trypsin (6,7). It was decided to extend our studies of α_1PI (6) to the pair, α_1PI-elastase, hoping to find conditions that suppress or enhance the pathway leading to complex formation.

EXPERIMENTAL PROCEDURES

α_1PI was prepared as described (6). Porcine trypsin was from Novo. Elastase was prepared according to Shotton (8) and further purified from trypsin and chymotrypsin by passing it through an affinity column containing immobilized soybean trypsin inhibitor and a second column containing turkey ovomucoid to remove elastase II (9,10). The final elastase preparation had no detectable tryp-

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sin, chymotrypsin, or elastase II activity and was devoid of any contaminant capable of direct proteolysis of α_1PI. Molarity was calculated using $E_1^{1\%}{}_{cm,\ 280\ nm}$ = 20.2 (elastase), 5.0 (α_1PI), and 14.9 (trypsin); the latter was corrected for active trypsin (11). For titration curves elastase solution containing a specified amount in 0.2 ml of phosphate buffer, pH 6.8, was mixed with 0.8 ml containing variable amounts of α_1PI in the same buffer. The mixture was preincubated 60 min at 1°, 10 min at 25° or 37°. An aliquot (0.5 ml) was withdrawn to the cuvette containing substrate and the remaining elastase activity determined by the method of Visser and Blout (12) using a Cary Model 16 Spectrophotometer at 25°. Trypsin was determined according to Schwert and Takenaka (13). In the experiments using gels α_1PI and elastase were mixed in predetermined ratio and temperature in a total volume of 10 µl. To stop the reaction quickly 2 µl of a mixture containing 10% sodium dodecyl sulfate (SDS) and 10% 2-mercaptoethanol (kept in a boiling water bath) were added, and the tube was immediately transferred to a boiling water bath for 5 min. After cooling 2 µl of fluorescamine solution (5 mg/ml acetone) were added, followed by 1 µl of 50% sucrose solution. One µl of this mixture was applied to an appropriate channel of the gradient gel slab (3).

Elastase-α_1PI complex was prepared by gel filtration of the reaction mixture (α_1PI/elastase = 2) through a column of Biogel P-150 (1.5 x 90 cm). The first peak contained a polymer of the ~50,000 dalton fragment. The second peak contained crude complex. Similar distribution has been observed with α_1PI-chymotrypsin complex (14). Crude complex was further purified on DEAE Biogel A10 with a gradient of 0 to 0.15 M NaCl in 0.01 M sodium phosphate, pH 7.5. The peak containing the complex was concentrated with Ficoll 400. The complex was identified by gel electrophoresis with and without SDS (not shown).

Iodination was performed as described (15).

RESULTS AND DISCUSSION

Fig. 1 shows the difference in shape between the titration curves of trypsin and elastase with α_1PI. Titration of trypsin is a typical straight line representing uni-unimolecular reaction. From the curving near the equivalence point the dissociation constant can be calculated (16). With elastase the intercept and the convex shape of the titration curves depend on temperature and salt concentration. If the inactivation curve results from two independent reactions, one leading to complex formation, the other to direct proteolysis, the convex shape and variability of the intercept would be expected. Lowering of temperature increases both the convexity and the value of the intercept, suggesting that the rate of complex formation is affected more than the rate of

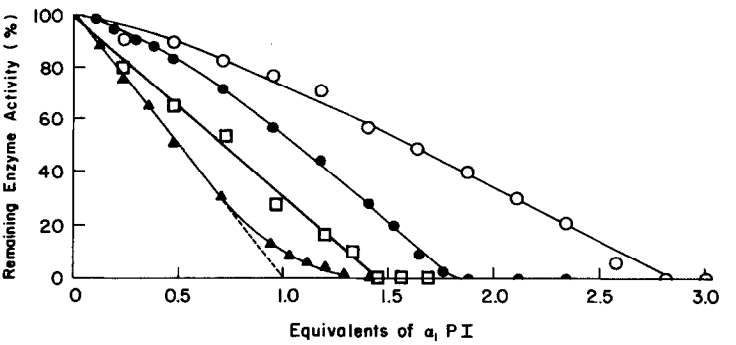

Fig. 1. Titration curves of trypsin (▲—▲) at 25°, and of elastase (o—o) at ∿1° (●—●) at 25° and (□—□) at 37°. Trypsin 6.66 x 10^{-10} moles was titrated in 0.05 M Tris-HCl, pH 8.0 containing 1 mM $CaCl_2$. Elastase (6.72 x 10^{-10} moles) titrations were in 0.05 M phosphate buffer, pH 6.8, except that the 37° (□—□) titration was made in the presence of 2 M NaCl, and corrected for the decrease in elastase activity in NaCl.

proteolysis. Under the most favorable conditions (37° and 2 M NaCl) only 1/3 of α_1PI is directly digested, whereas 2/3 forms the complex. This suggests that hydrophobic forces enhance complex formation with elastase. A similar conclusion was reached from experiments with dioxane (17).

The dual nature of elastase and α_1PI reaction is also shown by gradient gel electrophoresis (3). The experiments were performed using an α_1PI/elastase ratio of 1.6, at two different temperatures, 37° (Fig. 2A) and ∿1° (Fig. 2B). At 37° (without NaCl) only a trace of free elastase should be present, and indeed no band of free elastase is visible (Fig. 2A). The α_1PI band (54,000 daltons) is visible for about 30 sec. Only one type of complex (80,000 daltons) corresponding to 1:1 combination is immediately formed and is already visible at 10 seconds but disappears after 30 min. Two fragments of α_1PI, ∿50,000 and ∿4,000 daltons are formed simultaneously, become visible at 30 sec, and persist throughout the experiment, suggesting that they are relatively stable to further digestion by elastase. At 1° (Fig. 2B) with the

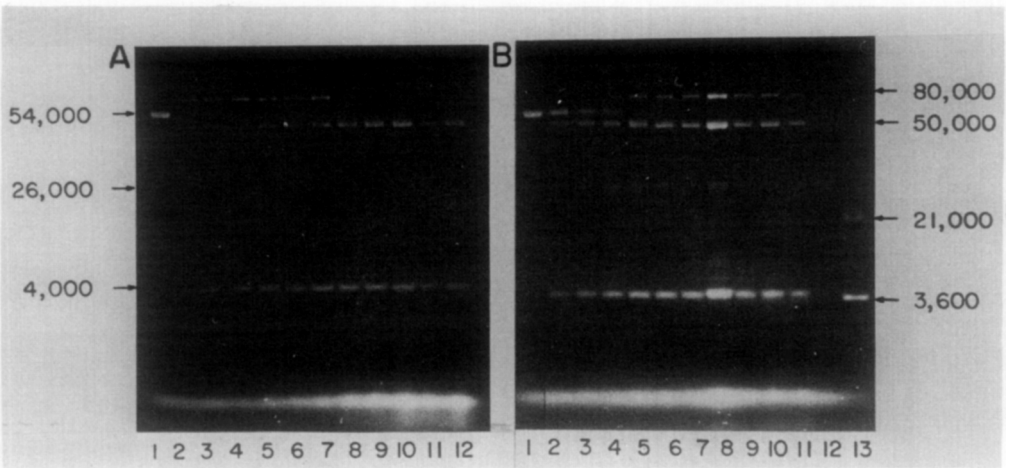

Fig. 2A. Progress of reaction between elastase and α_1PI at 37°. Electrophoresis was run overnight at 100 volts (constant voltage); channel 1) markers: α_1PI and elastase 2) 10 sec incubation 3) 30 sec, 4) 1 min, 5) 5 min, 6) 10 min, 7) 30 min, 8) 1 h, 9) 2 h, 10) 5 h, 11) 15 h, 12) 24 h. *2B.* Similar experiment at ~1°. Channel 1) markers: α_1PI and elastase 2) 30 sec incubation 3) 1 min, 4) 5 min, 5) 10 min, 6) 30 min, 7) 1 h, 8) 2 h, 9) 5 h, 10) 15 h, 11) 24 h, 12) elastase only, 13) markers: soybean trypsin inhibitor (Kunitz) and B chain of insulin.

ratio 1.6 about 50% of the elastase should be free (see Fig. 1), and indeed free elastase is seen throughout the experiment (Fig. 2B). Intact α_1PI persists longer than in Fig. 2A. The complex (80,000 daltons) forms more slowly but remains visible for 24 hours. Neither at 37° nor at 1° are bands observed in the regions between 80,000 and 54,000 daltons. This contrasts with the α_1PI-trypsin reaction (6,7) and earlier reports on α_1PI-elastase reaction (1,18). An increase in intensity of the bands in the range of a few hundred daltons is apparent in Fig. 2. This is probably due to side reactions, one of which is instability during SDS 2-mercaptoethanol treatment, because a weak band is seen even with markers. Additional experiments of this type in which the α_1PI/elastase ratio was 1 or 2, and temperature was either 1° or 37° were performed (data not shown). The conclusion was the same;

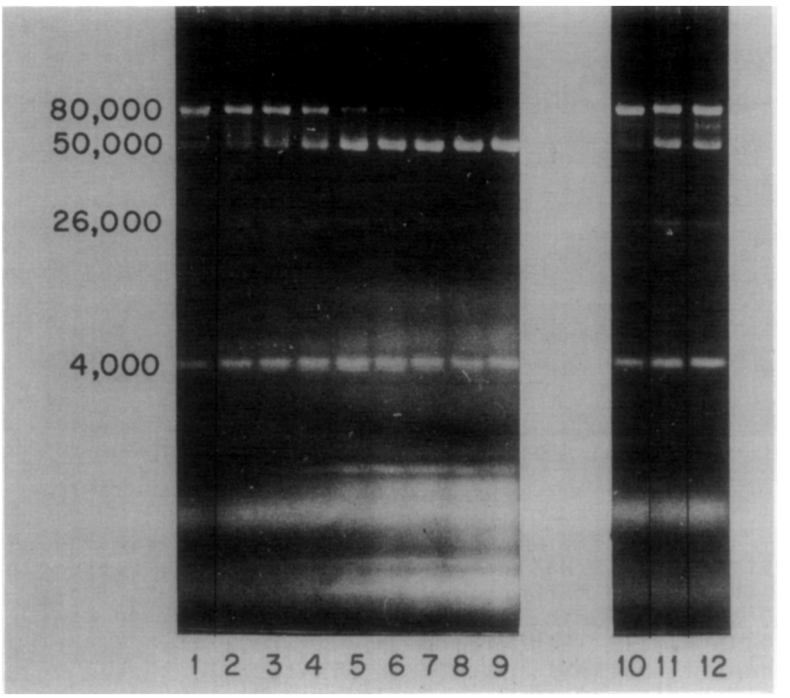

Fig. 3. Stability of the isolated α_1PI-elastase complex in the presence and absence of free elastase at 37°. Complex (2.67 A_{280} units) and 5 µg elastase were incubated in 50 µl of 0.05 M sodium phosphate, pH 7.0. Electrophoresis as in *Fig. 2*. Channel 1) 10 sec incubation, 2) 30 sec, 3) 1 min, 4) 5 min, 5) 10 min, 6) 30 min, 7) 1 h, 8) 2 h, 9) 5 h; 10-12) controls incubated at 37° without added elastase: 10) 30 min, 11) 1 h, 12) 5 h.

namely, that 2 independent reactions occur. Fig. 3 shows an experiment in which isolated complex was treated with elastase and incubated at 37°. The results confirm that ∿50,000 and ∿4,000 dalton major bands are formed from the complex, and that the degradation reactions are accelerated by added elastase.

Experiments involving radioiodination of either α_1PI or elastase were then performed. The method of iodination requires simultaneous oxidation (15). Labeling of α_1PI decreased its activity against trypsin by only 20%, but abolished its ability to form a complex with elastase. Formation of α_1PI fragments of ∿50,000 and ∿4,000 daltons by elastase was not affected (data not

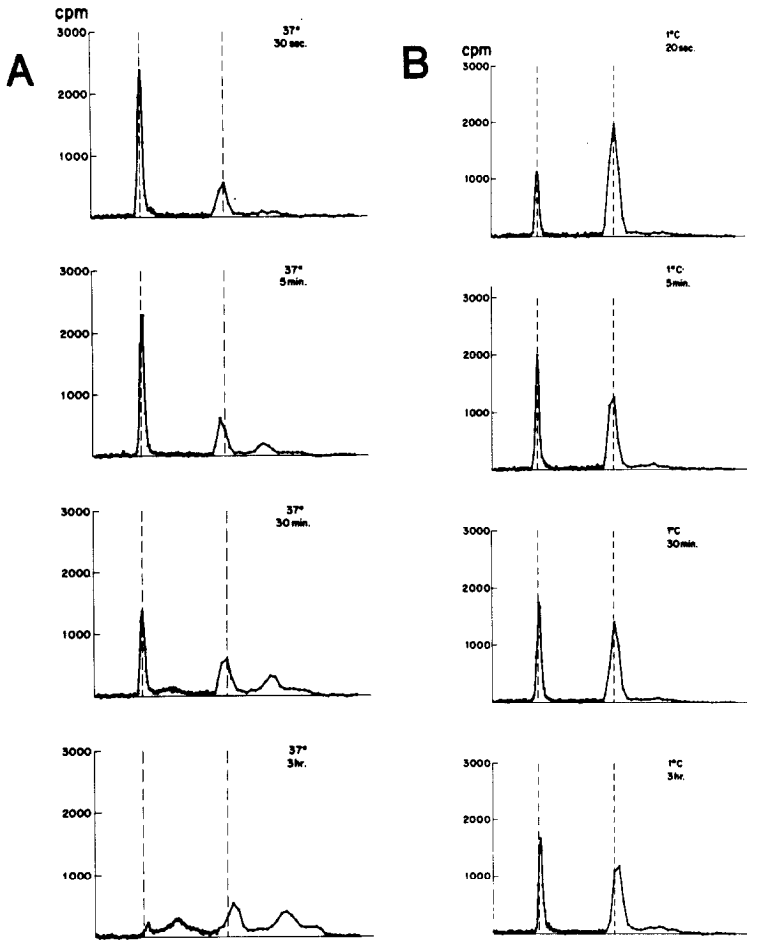

Fig. 4A. Reaction between ^{125}I-elastase and native α_1PI at 37° at α_1PI/elastase ratio = 2. At indicated time intervals the aliquots were withdrawn, terminated and electrophoresed for 5 h at 10 mA per tube (constant current). The gels were sliced into 0.5 mm fragments with a Gilson automatic gel slicer. The fragments were placed into scintillation vials and radioactivity was counted using a Packard Auto-Gamma Spectrometer. Two dotted lines were drawn at 80,000 and 26,000 daltons (the peak of complex at 30 sec and the peak of elastase). *4B.* Similar experiment to that in *4A* except that the temperature of the reaction was ∿1°.

shown). Oxidation of α_1PI with chloramine-T without iodination was sufficient to produce the same effects. Since only methionine is oxidized under the conditions used (19), this suggests that methionine is at the reactive center of α_1PI for elastase, and agrees with a recent independent conclusion (20). The reason for the different behavior of oxidized or iodinated α_1PI *vs* trypsin

remains temporarily unresolved.

Iodination of elastase, however, did not prevent complex formation. At 37° complex formed rapidly and disappeared within 3 hours (Fig. 4A). The elastase peak remained almost constant, but the counts increased in the regions between 80,000 and 26,000 daltons. This was further evidence that only a single α_1PI-elastase complex is formed under the conditions used. The formation of products in both regions is due to direct digestion of the complex, because a control experiment with elastase alone showed only ∿5% loss of activity during the 3-hour incubation. At 1° (Fig. 4B) complex forms slowly, never reaches the same height as at 37°, but lasts much longer. The amount of free elastase first decreases, and then stays constant. There is little radioactive material in the region from 80,000 to 26,000, but some accumulation of radioactive material in the region below 26,000 daltons is detectable. Neither at 37° nor at 1° is there any accumulation of counts in the regions corresponding to 50,000 and 4,000 daltons. The disintegration of complex (Fig. 3) proceeds by either cleaving the α_1PI moiety into 50,000 and 4,000 dalton fragments, or by cleaving the elastase moiety at several sites (Figs. 3 and 4). Both processes apparently occur simultaneously.

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