Volume 323, number 3, 236–238 FEBS 12518 June 1993

Conformational changes of the α_1-proteinase inhibitor affecting its cholesterol binding ability

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Received 22 March 1993

The effect of conformational changes of the α_1-proteinase inhibitor (α_1PI) on α_1PI–cholesterol complex (1:2 mol/mol) formation in vitro was studied with electrophoretic and gel chromatographic methods. Native α_1PI was modified by adding free thiol agents such as glutathione, cysteine HCl, or DL-homocysteine, by heating, or by cleavage with pancreatic elastase or trypsin. Conformational changes of the α_1PI molecule induced by these procedures were all accompanied by a loss of its ability to bind cholesterol in vitro under standard experimental conditions. The data suggest α_1PI–cholesterol binding to be affected by both direct and indirect modifications of the α_1PI-reactive center, that is situated on a mobile peptide loop.

α_1-Proteinase inhibitor; Cholesterol; Complex formation

1. INTRODUCTION

Complex formation in vitro between human α_1-proteinase inhibitor (α_1PI) and cholesterol was demonstrated in a recent study [1]. It was shown that this interaction results in an abnormal α_1PI immunoprecipitation pattern, diminished antiproteinase activity, and a gel filtration peak at 67 kDa (as compared to 52 kDa for native α_1PI). Like other serine antiproteinases (serpins), α_1PI has a highly ordered structure and its properties are critically dependent on the conformationally constrained active site loop [5]. To function as inhibitors the serpins have a native, stressed (*S*) conformation that renders them vulnerable to proteolytic cleavage, the cleavage being accompanied by an irreversible transition to a stable relaxed (*R*) form [3]. Modifications in the loop sequence due to species differences, genetic variation or physical and chemical alterations result in changes of the predominant function of α_1PI as an inhibitory serpin [4]. The single cysteine at position 232 in the α_1PI molecule has been shown to participate in mixed disulfide formation with cysteine, glutathione and plasma proteins with free thiols [5]. Conformational changes induced by modification of Cys-232 directly affect the conformationally constrained active site loop and changes the affinity of α_1PI for neutrophil elastase [5]. Our goal in this study was to investigate the effects on the conserved α_1PI tertiary structure induced by temperature, disulfide bond formation, cleavage with target enzymes on the α_1PI–cholesterol complex formation in vitro.

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2. MATERIALS AND METHODS

Electrophoretically pure α_1PI was isolated from human plasma in our laboratory as previously described [1]. A standard aqueous solution of cholesterol was obtained from Boehringer Mannheim, Germany, glutathione (GSH), DL-homocysteine and cysteine-HCl were from Sigma, St. Louis, MO, porcine pancreas elastase type III from Sigma, and lyophilized bovine trypsin (266 U/mg) from Worthington Biochemical Corporation, Freehold, NJ. Monospecific rabbit antiserum against human α_1PI was purchased from DAKO, Denmark and agarose, Sephadex G-75 superfine and the gel filtration calibration kit, from Pharmacia, Uppsala, Sweden. All other chemicals were of reagent grade.

In the experiments we used stock solutions of α_1PI (1.78 mg/ml, 0.34 μM/ml) and cholesterol (2 mg/ml) in 0.015 M Tris buffer, pH 7.4, containing 0.15 M NaCl. Solutions of glutathione, cysteine-HCl and DL-homocysteine (300 μmol/ml) were prepared in 1 M glycine, 0.01 M Na_2-EDTA buffer, pH 7.4.

Disulfide forms of α_1PI were prepared by mixing α_1PI (0.34 μM/ml) with glutathione, cysteine or DL-homocysteine (300 μmol/ml) at volume ratios 5 or 10 of α_1PI to 1 free thiol reagent in Tris buffer, pH 7.4. Samples were kept overnight at +4°C. Temperature modified α_1PI was prepared by keeping α_1PI at 56°C for 5 min resulting in a 50% reduction of antielastase activity. Elastase inhibitory activity was analyzed as described by Gaillard and coworkers [7].

For elastase–α_1PI or trypsin–α_1PI complex formation, reaction mixtures containing proteinase and α_1PI (1:1 1 mol/mol) were incubated at 37°C in the Tris buffer (15 min for elastase and 1 h for trypsin).

The complex between native or modified α_1PI and cholesterol was prepared by mixing α_1PI with cholesterol (1:2 mol/mol) in Tris buffer and incubated for 24 h at 37°C. Under these standard experimental conditions the complex formed had a typical immune precipitate pattern and a gel filtration peak at 67 kDa [1].

Gel filtration, electrophoresis in 1% agarose, and crossed immunoelectrophoresis were performed as previously described [1]. Rocket immunoelectrophoresis was performed as described by Laurell [8].

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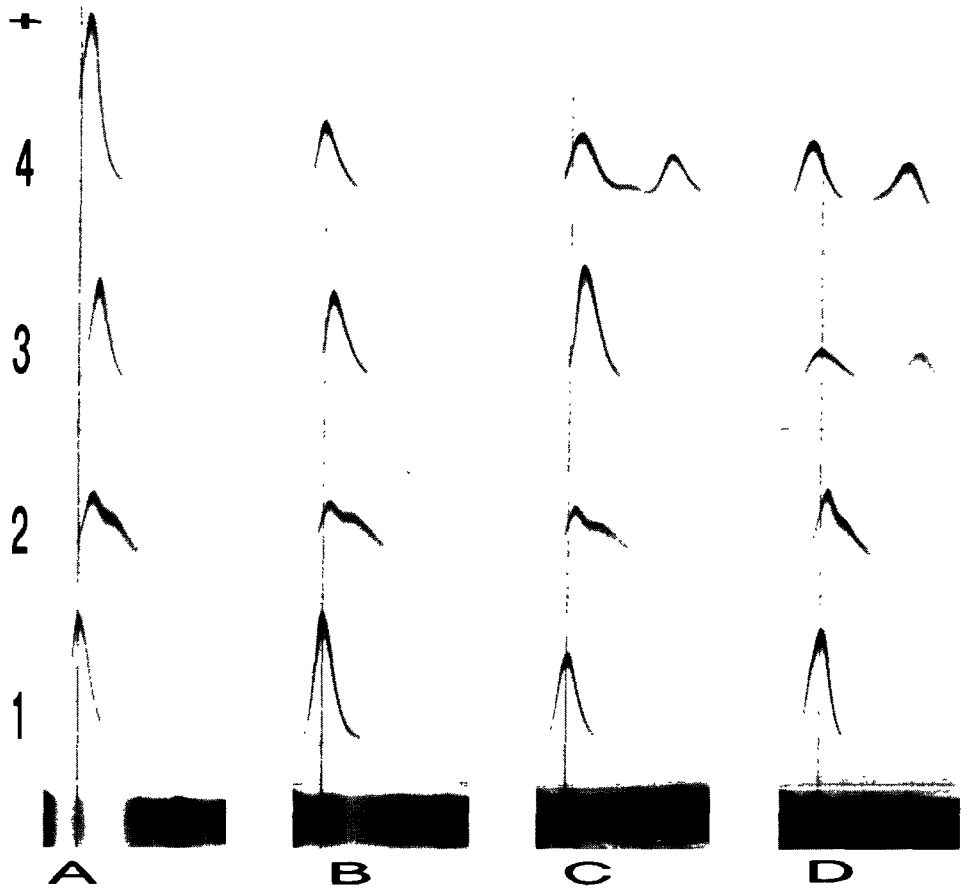

Fig. 1. Crossed immunoelectrophoresis of modified forms of α_1PI (see text). A, B, C, D: 1, native α_1PI; A, B, C, D: 2, α_1PI–cholesterol complex showing the retarded double peak; A3, disulfide form of α_1PI mixed with cholesterol; A4, disulfide form of α_1PI prepared by adding glutathione; B3, heated α_1PI–cholesterol mixture; B4, α_1PI heated at 56°C for 5 min; C3, elastase cleaved α_1PI–cholesterol mixture; C4, elastase–α_1PI–cholesterol mixture; D4, trypsin–α_1PI complex Anode (+) to the left. Vertical lines indicate identical mobilities.

3. RESULTS AND DISCUSSION

3.1. *Effects of disulfide formation*

Complex formation between native or modified α_1PI and cholesterol (1:2 mol/mol) was visualized with crossed immunoelectrophoresis. As shown previously KP[1], and as seen in Fig. 1A-2, the complex formation results in typically double peaked immune precipitate with retarded mobility towards the anode. When the disulfide form of α_1PI prepared by adding glutathione was mixed with cholesterol, no effect on the immunoprecipitate pattern (as compared to native PI) could be seen (Fig. 1A-3). Identical results (not shown) were obtained when cysteine-HCl or homocysteine were used for disulfide formation.

Similarly, when samples of the disulfide form of α_1PI and cholesterol mixtures were subjected to gel filtration both native α_1PI and the disulfide form mixed with cholesterol eluted at 52 kDa, in contrast to native α_1PI in complex with cholesterol which appeared at a molecular size of 67 kDa (Fig. 2).C Despite the loss of cholesterol binding activity disulfide formation did not cause any reduction in antiproteinase activity.

3.2. *Effects of temperature*

When native α_1PI was subjected to heating at 56°C for 5 min it lost about 50% of its antielastase activity but its ability to bind cholesterol under standard experimental conditions was completely abolished (Fig. 1B-3). The heated protein was also found to elute with a normal size, 52 kDa, from the Sephadex column (not shown).

3.3. *Effects of proteolytic cleavage*

The cleavage of native α_1PI by its target enzyme, elastase, or by trypsin, results in the transition from a stressed (*S*) form to a relaxed (*R*) form of the protein [3]. The cleavage products can be visualized by crossed immunoelectrophoresis. As can be seen in Fig. 1C-4, D-4 cleavage resulted in the appearance of an electrophoretically retarded (α_1PI–proteinase complex) form of α_1PI. It is also seen that the transition from an *S* to

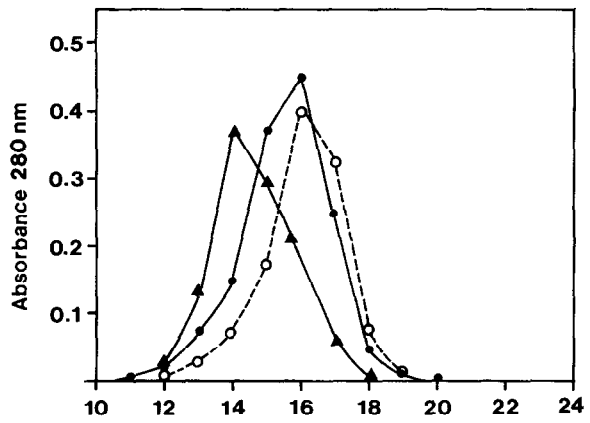

Fig. 2. Gel filtration of native α_1PI (●–●), native α_1PI–cholesterol mixture 1 M:2 M (△–△) and disulfide form α_1PI–cholesterol (○–○) mixture 1 M:2 M on a Sephadex G-75 superfine column.

an *R* form abolishes the ability of α_1PI to bind cholesterol (Fig. 1C-3; D-3). For unknown reasons, α_1PI–elastase and -trypsin complexes differ from each other in their reaction to the addition of cholesterol (C-3, C-4).

These results suggest that the cholesterol binding site of α_1PI is located on or close to the active site loop of the molecule. Modest heating results in some loss of biological activity, but has a marked effect on the tertiary structure sufficient to result in a complete loss of cholesterol binding capacity under standard experimental conditions. Similarly the direct effect of target enzymes on the reactive center abolishes the cholesterol binding ability of α_1PI.

On the other hand the induction of conformational changes in the α_1PI molecule by disulfide bond formation between free thiols and Cys-232, which is protected in a crevice [10], also abolishes the ability of α_1PI to bind cholesterol. Tyagi and coworkers [6] have shown disulfide bond formation involving the Cys-232 of α_1PI to induce conformational changes in the protein's reactive site without causing any decline in antiproteinase activity. Our results are in agreement with their findings; we detected no loss of α_1PI antielastase activity after disulfide bond formation. The fact that, after disulfide bridge formation between Cys-232 and free thiol agents, α_1PI lost its ability to interact with added cholesterol suggests the presence of an additional cholesterol binding site which is located in the hydrophobic [11] Cys-232 environment of the α_1PI molecule.

Our results suggest α_1PI–cholesterol complex formation in vitro to be dependent on a conformationally constrained reactive site loop. Complex formation is blocked by both direct (heating, proteolytic cleavage) and indirect (disulfide formation) processes affecting this region.

Acknowledgements: This work was supported by grants from the Ernhold Lundström's and Anna and Edwin Berger's Foundations.

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