Volume 316, number 3, 269–272 FEBS 12064 February 1993

In vitro complex formation between cholesterol and α_1-proteinase inhibitor

Sabina Janciauskiene and Sten Eriksson

Gastroenterology and Hepatology Division, Department of Medicine, Malmö General Hospital, University of Lund, S-214 01 Malmö, Sweden

Received 9 December 1992

The in vitro interaction between human α_1-proteinase inhibitor (α_1-PI) and cholesterol was studied with electrophoretic and gel chromatographic methods. The addition of cholesterol (from 1 to 20 mol/mol α_1-PI) at 37°C resulted in retarded electrophoretic mobility of α_1-PI towards the anode, diminished immunoreactivity and antiproteinase activity. At a molar ratio of 2:1 (cholesterol/α_1-PI), antitryptic activity was reduced by 15% but antielastase activity by 50%. At this ratio the gel filtration α_1-PI peak appeared at 67 kDa, as compared to 52 kDa for native α_1-PI. No size difference was noted on SDS-PAGE. These results suggest the occurrence of noncovalent complex formation between cholesterol and α_1-PI in vitro.

α_1-Proteinase inhibitor; Cholesterol; Complex formation

1. INTRODUCTION

Human plasma α_1-proteinase inhibitor (α_1-PI), a member of the serpin superfamily, is an acute-phase protein synthesized in increased amounts by hepatocytes during inflammation [1]. Functionally α_1-PI acts as a barrier to prevent the breakdown of vulnerable elastic tissue, principally in the lung, by the enzymes released from inflammatory cells [2]. α_1-PI diffuses into most tissues and is found in most body fluids. Increased fecal concentrations of α_1-PI are found in association with inflammatory lesions of the bowel [3].

α_1-PI-like albumin and α_2-macroglobulin have been found in human aortic intima [4]. Pinocytic uptake of the α_1-PI-elastase complex was observed on the surface of endothelial cells and in smooth-muscle cells bordering the elastic lamina of arterioles [5]. Local sites of synthesis such as human blood monocytes and bronchoalveolar macrophages also make important contributions to the α_1-PI pool in these tissues and body fluids [6].

Findings in other studies have suggested α_1-PI to have functional additional properties apart from the inhibition of serine proteases. The carboxy-terminal fragment of α_1-PI, which can be generated during formation of complexes with serine proteases or during proteolytic inactivation, may also be involved in the regulation of α_1-PI synthesis [7]. Corticosteroid binding globulin (CBG) is structurally related to the serpins and is able to bind a hydrophobic compound, cortisol [17]. Hydrophobic peptides from α_1-PI have recently been isolated from human bile [8]. Taken together, these findings indicate that α_1-PI may possibly interact with cholesterol. Such an in vitro interaction is described in this report.

Correspondence address: S. Eriksson, Gastroenterology and Hepatology Division, Department of Medicine, Malmö General Hospital, University of Lund, S-214 01 Malmö, Sweden. Fax: (46) (40) 33 62 08.

2. MATERIALS AND METHODS

Electrophoretically pure α_1-PI was isolated from human plasma in our laboratory, using the method Pannell and coworkers [9]. We also used two commercially available α_1-PI preparations, Prolastin from Bayer and α_1-AT from Sigma. Human transferrin was obtained from Sigma, neuraminidase (Sialidase) and a standard aqueous solution of cholesterol (2 mg/ml) from Boehringer Mannheim, GmbH, Germany. Net - 030 [7-^{3}H(N)]cholesterol spec.act. 21.8 Ci/mmol, was obtained from New Research Products, Boston, MA, lyophilized bovine trypsin (266 U/mg) from Worthington Biochemical corporation, Freehold, NJ, and porcine pancreatic elastase Type III from Sigma , St Louis, MO. The chromogenic substrate for trypsin [*N*-α-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA)] was obtained from BDH Chemicals, England, and that for elastase [*N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (SAPNA)] from Sigma. Monospecific rabbit antiserum against human α_1-PI 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_1-PI and cholesterol in 0.015 M Tris buffer, pH 7.4, containing 0.15 M NaCl. Pure α_1-PI 1.78 mg/ml (3.42×10^{-5} M) was mixed with an aqueous solution of cholesterol (2 mg/ml (5.17×10^{-3} M)) at increasing molar ratios (1:1, 1:2, 1:5, 1:10, 1:20, 1:40, 1:60) and incubated for 24 h at 37°C. In the isotopic experiments with [^{3}H(N)]- cholesterol, labelled and unlabelled cholesterol were mixed at a ratio 1:10 by weight, immediately added to α_1-PI samples and incubated for 24 h at 37°C. Gel filtration of the mixtures of α_1-PI and cholesterol at a molar ratio of 1:2 was performed on a sephadex G-75 superfine column (0.9×60 cm) at room temperature. The column was equilibrated with the Tris buffer and run at a flow rate of 4 ml/h. 1 ml fractions were collected and analyzed spectrophotometrically (A_{280}) and for radioactivity using the

Packard TRI-Carb 300C liquid screen infiltration system. Electrophoresis in 1% agarose at pH 8.6 and crossed immunoelectrophoresis were performed using standard methods [11,12]. SDS-PAGE was carried out as previously described with 20 × 30 cm slab gels containing a 10–15% polyacrylamide gradient with a 2 cm spacing gel [14]. Determination of trypsin inhibitory activity was performed with the method of Dietz and coworkers [13], and elastase inhibitory activity as described by Gailbard and coworkers [15]. Desialylation of α_1-PI was performed as previously described [16].

3. RESULTS AND DISCUSSION

3.1. *Electrophoresis*

Samples of α_1-PI/cholesterol mixtures at increasing molar ratios (1:1, 1:2, 1:5, 1:10 and 1:20) were studied by 1% agarose electrophoresis at pH 8.6. As shown in Fig. 1, the α_1-PI/cholesterol mixture manifested somewhat retarded mobility towards the anode as compared to free α_1-PI. In addition, the bands were broader and less well demarcated than native α_1-PI. When identical samples were analyzed with SDS-PAGE using a 10–15% gradient (results not shown), no unequivocal differences between the a_1-PI/cholesterol mixture and native α_1-PI could be seen.

The patterns of α_1-PI/cholesterol mixtures were also analyzed with crossed immunoelectrophoresis using monospecific antisera against human α_1-PI (Fig. 2). The addition of increasing amounts of cholesterol to α_1-PI produced three different effects: there was some loss of immunereactivity already at a molar ratio of 1:1, the mobility of the immune precipate towards the anode was decreased, and the configuration of the precipitate changed and tended to become double peaked. Identical results were obtained both in experiments using different antisera to human α_1-PI and in experiments with other α_1-PI preparations (results not shown). When transferrin was used instead of α_1-PI, no mobility or configurational changes were noted. The interaction was unaffected by desialylation

3.2. *Proteinase inhibitory activity of α_1-PI/cholesterol mixtures*

The elastase and trypsin inhibitory capacities of α_1-PI/cholesterol mixtures are shown in Fig. 3. Cholesterol inhibits α_1-PI activity. With increasing amounts of cholesterol, α_1-PI completely lost antielastase activity, but as can be seen in Fig. 3 even at a molar ratio of 1:60 (cholesterol/α_1-PI), 30% trypsin inhibitory activity remained.

3.3. *Gel filtration*

Native α_1-PI at a concentration of 1.78 mg/ml (3.8×10^{-5} M) and mixed with cholesterol (2 mg/ml (5.1×10^{-3} M)) at molar ratio of 1:1 or 1:2, was analyzed with chromatography on a Sephadex G-75 column. As can be seen in Fig. 4, pure α_1-PI and α_1-PI in mixture with cholesterol elute from the column in different fractions. The molecular weight of α_1-PI after mixing with cholesterol was calculated to be about 67 kDa. The experiments were repeated under identical conditions, but with tritiated cholesterol. Radioactivity could only

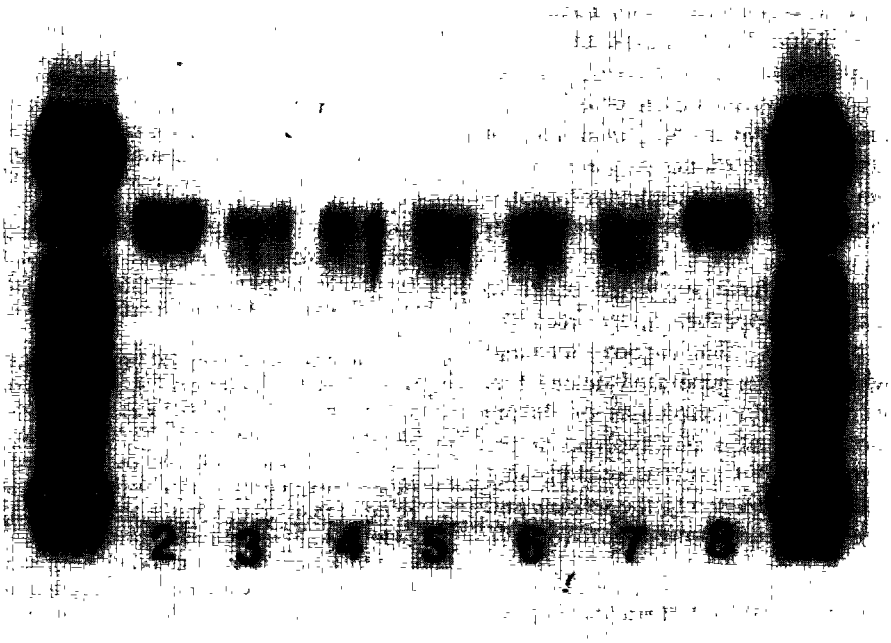

Fig. 1. 1% agarose gel electrophoresis of native α_1-PI and α_1-PI/cholesterol mixtures at pH 8.6. Lanes 1 and 9, normal human plasma; lanes 2 and 8, native α_1-PI; lanes 3, 4, 5, 6 and 7, α_1-PI/cholesterol mixtures: 1 M to 1, 2, 5, 10 and 20 M, respectively. Anode at the top.

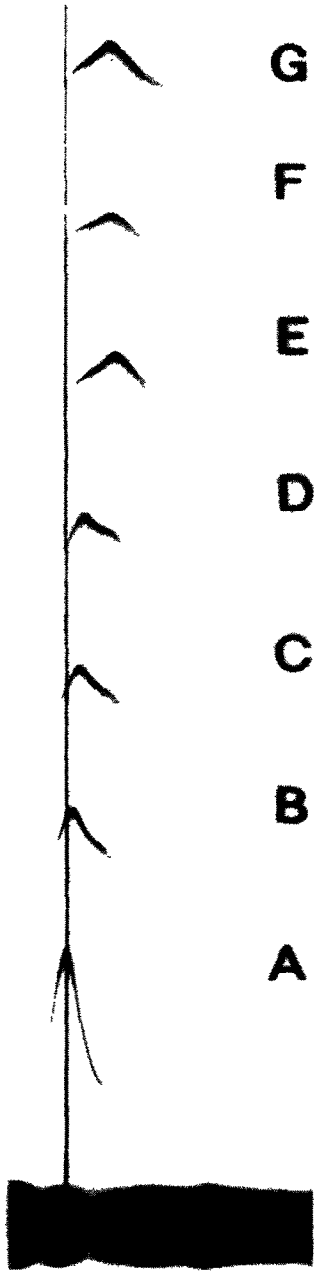

Fig. 2. Crossed immunoelectrophoresis of native α_1-PI and α_1-PI/cholesterol mixtures. Normal plasma: A, native α_1-PI; and B, C, D, E, F and G, mixtures of pure α_1-PI with increasing amounts of cholesterol (1 M α_1-PI to 1, 2, 5, 10 and 20 M cholesterol, respectively. Anode to the left.

be found in the fractions containing α_1-PI/cholesterol (Fig. 4). It should be noted that identical results were obtained with two other α_1-PI preparations in mixtures with cholesterol. In control experiments where α_1-PI was replaced with transferrin, no differences in elution pattern could be seen between native transferrin and that mixed with cholesterol.

It is evident from the electrophoretic data presented in Figs. 1 and 2 that an interaction between cholesterol and α_1-PI takes place at 37°C, which results in altered physicochemical properties of α_1-PI; a loss of net charge and an abnormal precipitation pattern. This interaction also results in diminished antiproteinase activity, most pronounced for elastase and less pronounced for trypsin (Fig. 3). The inhibitory specificity of serpins is to a predominant extent determined by the reactive center. Whether cholesterol affects the conformation of the molecule so that the reactive center becomes less accessible to proteases or binds directly to the reactive center is presently unknown. The different effects on elastase and trypsin also remain unexplained. However, the results suggest the occurrence of complex formation between cholesterol and α_1-PI, as clearly shown by the findings in gel filtration experiments (Fig. 4). The appearance of a relatively high molecular weight (67 kDa) complex may be compatible with dimerization, and does not simply reflect an addition of 2 mol cholesterol to one mol α_1-PI.

Moreover, the complex formation is specific. No complex formation takes place with transferrin, another (but non-serpin) transport glycoprotein. A covalent binding to sialic acid residues of α_1-PI is excluded by the results of experiments with the desialylated protein and with SDS-PAGE. The interaction is probably explained by hydrophobic bonds. The binding site(s) is (are) presently unknown, but a hydrophobic peptide of α_1-PI has recently been isolated both from human bile, plasma and spleen [8]. This peptide may well be responsible for cholesterol binding. Its proximity to the reactive center of α_1-PI is of particular interest with respect to the loss of biological activity of α_1-PI in complex. The complex between cholesterol and α_1-PI may play an important in vivo role in cholesterol transportation, for instance in bile.

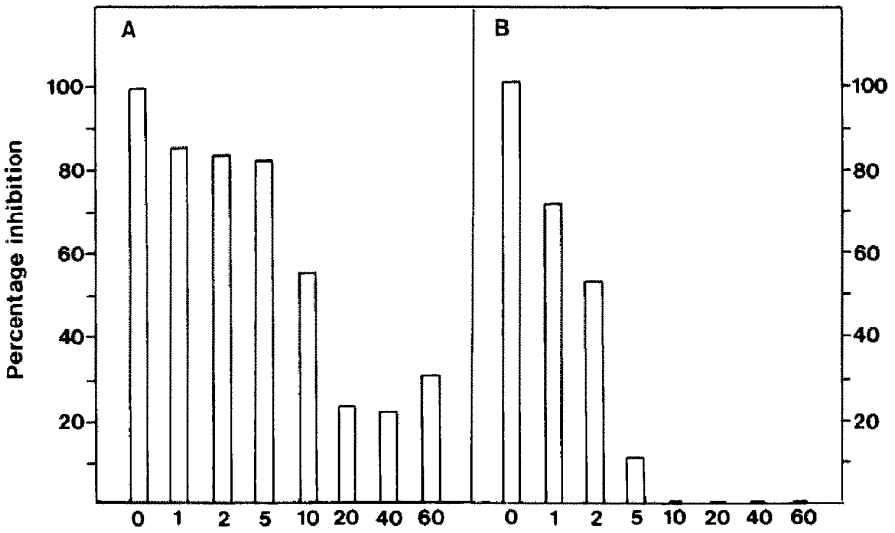

Fig. 3. Percentage inhibition of (A) trypsin and (B) elastase by α_1-PI/cholesterol mixtures with increasing amounts of cholesterol. Figures on the x-axis indicate the molar ratios between cholesterol and α_1-PI.

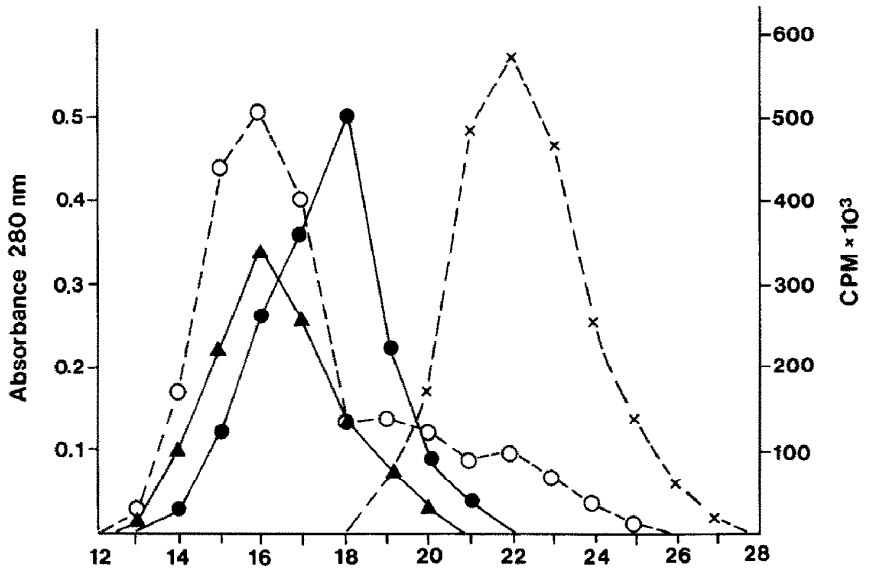

Fig. 4. Gel filtration of native α_1-PI and α_1-PI/cholesterol mixture (1 M:2 M) on Sephadex G-75 superfine. Native α_1-PI (●–●); α_1-PI/cholesterol (1 M:2 M) mixture (▲–▲); α_1-PI-labelled [^{3}H(N)]cholesterol (1 M:2 M) mixture (○–○); labelled [^{3}H(N)]cholesterol (×–×).

Acknowledgement: This work has been supported by grants from the Ernold Lundströms Foundation.

REFERENCES

[1] Carrell, R.W. (1986) J. Clin. Invest. 78, 1427–1431.
[2] Eriksson, S. (1990) Lung, Suppl. 523–529.
[3] Hill, R.E., Hercz, A., Corey, M.L., Gilday, D.L., Eng, B. and Hamilton, J.R. (1981) J. Pediat. 90, 416–418.
[4] Smith, E.B. (1990) Eur. Heart J. 11. Suppl. E, 72–81.
[5] Tsujii, T., Katayama, K., Naito, I. and Seno, S. (1988) Histochemistry 88, 443–451.
[6] Perlmutter, D.H., Cole, F.S., Kilbridge, P., Rossing, T.H. and Colten, H.R. (1985) Proc. Natl. Acad. Sci. USA 82, 795–799.
[7] Perlmutter, D.H., Travis, J. and Punsal, R.I. (1988) J. Clin. Invest. 81, 1744–1780.
[8] Johansson, J., Gröndal, S., Sjövall, J., Jörnvall, H. and Curstedt, T. (1992) FEBS Lett. 146–148.
[9] Smith, L. (1991) Free Radical Biol. Med. 11, 46–61.
[10] Pannell, R., Johnsson, D. and Travis, J. (1974) Biochemistry 13, 5439–5445.
[11] Johansson, B. (1972) Scand. J. Clin. Lab. Invest. 29, Suppl. 124, 7–19.
[12] Ganrot, P.O. (1972) Scand. J. Clin. Lab. Invest. 29, Suppl. 124, 39–41.
[13] Dietz, A.A., Rubinstein, H.M. and Hodges, L.K. (1976) Clin. Chem. 22, 1754–1755.
[14] Carlsson, J., Eriksson, S., Alm, R. and Kjellström, T. (1984) Hepatology 4, 235–241.
[15] Gaillard, M.C., Kilroe-Smith, T.A., Nagueira, C., Dunn, D., Jenkins, T., Fine, B. and Kallenbach, J. (1992) Am. Rev. Respir. Dis. 145, 1311–1315.
[16] Eriksson, S. and Larsson, Ch. (1976) Biochim. Biophys. Acta 445, 67–73.
[17] Hammond, G.L., Smith, S.L. and Underhill, D.A. (1991) J. Steroid Biochem. Mol. Biol. 40, 755–762.