Vol. 122, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1984 Pages 394-400

RAT LUNG TISSUE IS A SITE OF α_1-PROTEINASE INHIBITOR SYNTHESIS: EVIDENCE BY CELL-FREE TRANSLATION

Werner Budek, Peter Bünning, and Peter C. Heinrich

Biochemisches Institut, Universität Freiburg,
Hermann-Herder-Str. 7, D-7800 Freiburg, West Germany

Received June 11, 1984

Summary: Poly(A)$^+$RNA isolated from lungs of normal rats and of rats suffering from experimental inflammation was translated in a cell-free translation mixture from rabbit reticulocytes. The translation products were immunoprecipitated with specific antisera against α_1-proteinase inhibitor and α_2-macroglobulin. Comparable levels of mRNA for α_1-proteinase inhibitor were found in rat lung tissue from control and experimentally inflamed animals. α_2-Macroglobulin mRNA could not be detected in rat lung tissue.

Introduction: Proteolytic processes in blood and extravascular tissue fluids are controlled by various proteinase inhibitors. Among these inhibitors α_1-proteinase inhibitor (α_1PI)[1] and α_2-macroglobulin (α_2M)[1] play a predominant role (1). As potent inhibitors of elastase and cathepsin G they prevent tissue destruction during inflammation (2-4). α_1PI is synthesized in the liver and secreted into the plasma (5-8). In addition, α_1PI has been found in mast cells (9), platelets (10), polymorphonuclear leucocytes (11) and macrophages (12) as well as in a large number of tissues and body fluids (13). However, it is not clear whether α_1PI is synthesized at these various locations or is taken up from the circulation. Although it is assumed that the major site of synthesis of α_1PI is the liver (5-8), evi-

[1] Abbreviations: α_1PI, α_1-proteinase inhibitor, also known as α_1-antitrypsin, α_1-antiproteinase, and α_1-trypsin inhibitor; α_2M, α_2-macroglobulin.

0006-291X/84 $1.50

dence for the synthesis of this proteinase inhibitor in macrophages (14) and leucocytes (15) has also been obtained.

An important site of action of α_1PI is the lung where the proteinase inhibitor prevents the development of emphysema during chronic inflammation. Therefore, it is of great interest to investigate whether α_1PI in lung tissue is derived from the liver or whether lung tissue is capable to synthesize this proteinase inhibitor. In the present paper the cell-free synthesis of α_1PI directed by mRNA from rat lung is described. These results demonstrate that lung tissue is a site of α_1PI synthesis.

Materials and Methods: L-[^{35}S]Methionine (>600 Ci/mmol) was purchased from Amersham Buchler (Braunschweig, W.Germany); Protosol was from New England Nuclear (Boston, MA); protein A-Sepharose CL-4B and poly(U)-Sepharose 4B were from Pharmacia (Freiburg, W.Germany); guanidinium HCl, grade I from Sigma (St. Louis, MO). Male Wistar rats (250 g) which had free access to water and a carbohydrate-rich 20% protein diet were obtained from Thomae (Biberach, W.Germany). The purification of α_1PI and α_2M from rat serum and the preparation of rabbit antisera has previously been described (8,16).
Isolation of poly(A)$^+$RNA: Poly(A)$^+$RNA from liver and lung tissue was isolated from control and turpentine-treated rats (4 ml turpentine/kg body weight, 18 hours prior to sacrifice). The tissues were perfused with ice-cold phosphate buffered saline until all blood was removed and poly(A)$^+$RNA was prepared essentially as described by Cox and Smulian (17).
In vitro translation of poly(A)$^+$RNA and immunoprecipitation: 6-8 µg of poly(A)$^+$RNA were incubated for 90 min in 100 µl nuclease-treated rabbit reticulocyte lysate (18) containing 70 µCi [^{35}S]methionine, 100 units RNase inhibitor from human placenta (19), 0.01 µg of pepstatin, chymostatin, leupeptin and antipain, each, and 1 unit of kallikrein trypsin inhibitor (Trasylol). Synthesized α_1PI and α_2M were immunoprecipitated with specific antisera and protein A-Sepharose (20). The immunoprecipitates were analyzed by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (21) and fluorography (22). Radioactive bands were excised from the gel; the gel slices were solubilized with 90% Protosol in water at 40°C overnight and counted for radioacitivity.

Results: The synthesis of the proteinase inhibitors α_1PI and α_2M in rat lung tissue was investigated by cell-free translation of poly(A)$^+$RNA which was isolated from lungs of control animals and of experimentally inflamed rats treated with turpentine. For comparison poly(A)$^+$RNA was obtained from livers of turpen-

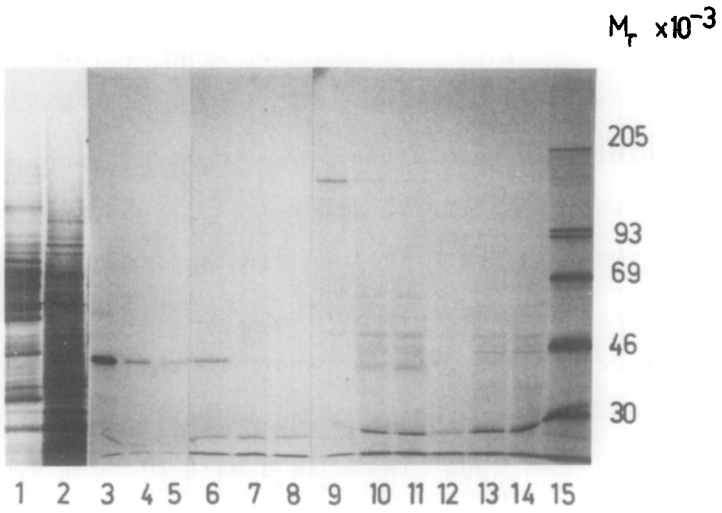

Figure 1: Cell-free synthesis of α_1-proteinase inhibitor and α_2-macroglobulin directed by poly(A)+RNA from lung and liver of turpentine-treated rats.
With liver approximately 8 x 10^6 cpm and with lung about 44 x 10^6 cpm trichloroacetic acid-precipitable [^{35}S]radioactivity were used for immunoprecipitation and sodium dodecyl sulfate polyacrylamide gel (7.5%) electrophoresis:
Total translation products of poly(A)$^+$RNA from liver (lane 1) and lung (lane 2); immunoprecipitation of α_1PI and α_2M synthesized by poly(A)$^+$mRNA from liver (lanes 3 and 9) and lung (lanes 6 and 12); lanes 4,5 and lanes 7,8, same as lanes 3 and 6, except that 15 µg or 45 µg of α_1PI have been added prior to the immunoprecipitation; lanes 10,11 and 13,14, same as lanes 9 and 12, except that 15 µg or 30 µg α_2M have been added prior to the immunoprecipitation. Molecular weight markers (lane 15): myosin (205,000), phosphorylase b (two bands, 92-94,000), bovine serum albumin (69,000), ovalbumin (46,000), and carbonic anhydrase (30,000).

tine-treated and untreated control animals. The mRNAs were translated in a cell-free system from rabbit reticulocytes and the synthesized proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

The products of cell-free translation directed by poly(A)$^+$RNA from liver and lung tissue of turpentine-treated rats are shown in Figure 1. Polypeptides up to high molecular weights are synthesized; their patterns differ characteristically for liver (lane 1) and lung tissue (lane 2). From the total translation products α_1PI was isolated by immunoprecipitation with a specific antiserum and subjected to electrophoretic analysis. Poly(A)$^+$RNA

which directs the synthesis of α_1PI was found in liver (lane 3) as well as in lung tissue (lane 6). An apparent molecular weight of 43,000 was estimated for the cell-free synthesized α_1PI from liver as it has previously been described (8). An identical electrophoretic mobility was observed for α_1PI from lung tissue. The specificity of the immunoprecipitation was further demonstrated by the competition of unlabeled α_1PI with the cell-free synthesized radioactive protein from liver (lanes 3-5) and lung (lanes 6-8).

Translatable mRNA for α_2M was found in liver and the electrophoretic analysis of the cell-free synthesized protein yielded an apparent molecular weight of 160,000 (Figure 1, lanes 9-11) as previously described (16). However, poly(A)$^+$RNA from lung does not direct the synthesis of α_2M (lanes 12-14). Even when the standard translation assay was increased five-fold it was not possible to stimulate cell-free synthesis of α_2M by poly(A)$^+$ RNA from rat lung tissue.

In order to compare the levels of translatable mRNAs for α_1PI and α_2M from liver and lung tissue of turpentine-treated and untreated control animals, the respective mRNAs were translated in vitro and the synthesized proteins were quantified as described in the experimental section (Table I). The first column of Table I shows that the total amounts of poly(A)$^+$RNA isolated from liver and lung tissue differ by a factor of about two and that treatment of the animals with turpentine does not significantly affect the poly(A)$^+$RNA levels. The stimulation of protein synthesis was similar for these poly(A)$^+$RNAs (column 2). The quantification of in vitro synthesized α_1PI shows that poly(A)$^+$ RNA from lung stimulates the cell-free synthesis of this protein to 0.5 - 1% of that of liver (column 3). When translatable α_2M mRNA was determined it was only found in the liver of turpentine-

Table I
Translatability of mRNA for α_1-proteinase inhibitor and α_2-macroglobulin from lung and liver tissues of control- and turpentine-treated rats

Tissue	Poly(A)$^+$RNA (µg/g wet weight)	Total TCA-precipitable radioactivity[a] (cpm x 10^{-6})	Immunoprecipitable radioactivity: α_1-proteinase inhibitor (cpm)	Immunoprecipitable radioactivity: α_2-macroglobulin (cpm)
Control lung	22	17	153	not detected
Lung, 18 h after turpentine injection	20	18	168	not detected
Control liver	46	15	20 000	not detected
Liver, 18 h after turpentine injection	43	18	32 000	7 400

[a] Total trichloroacetic acid-precipitable radioactivity per 100 µl translation mixture containing 6-8 µg of poly(A)$^+$RNA

treated rats (column 4). In contrast, mRNA for α_2M was not detected in lung tissue of either control or inflamed animals.

Discussion: In the present study it has been demonstrated by means of cell-free protein synthesis that rat lung tissue contains mRNA for α_1PI. Therefore, the lung is a site of synthesis for this important proteinase inhibitor.

Lung tissue is composed of various cell types: alveolar cells, endothelial cells, smooth muscle cells, fibroblasts, macrophages, and immigrated leucocytes. Although the present investigation does not identify the type(s) of lung cell(s) which synthesize α_1PI, one site of de novo synthesis may be the pulmonary macrophage. Recently, it has been demonstrated that pulmonary macrophages contain α_1PI (12) and also possess the capability to synthesize this proteinase inhibitor (14). The local production of α_1PI by pulmonary macrophages may by far exceed the level which is provided to the lung from the circulation. Through the synthesis of this proteinase inhibitor pulmonary macrophages may

play a predominant role for the control of proteinase activity in lung tissue.

The observation that lung tissue of rats as well as of humans (W. Budek, unpublished experiments) have the potential to synthesize α_1PI may demonstrate the importance of this proteinase inhibitor for the normal function of this organ. In human individuals low plasma levels of α_1PI are associated with the development of severe pulmonary emphysema, and it is assumed that the emphysema is due to the unopposed action of proteinases in the lung (23). In order to support this concept for the development of emphysema it will now be of great interest to determine the levels of translatable mRNA of α_1PI in lung tissue of individuals with α_1PI deficiency.

Acknowledgements: The authors like to thank Dr. J. Bauer for the generous gift of anti-α_2-macroglobulin and Mrs. H. Gottschalk for her help with the preparation of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft.

References

1. Travis, J., and Salvesen, G.S. (1983) Ann. Rev. Biochem. 52, 655-709.
2. Jamieson, J.C., Friesen, A.D., Ashton, F.E., and Chou, B. (1972) Can. J. Biochem. 50, 856-870.
3. Koj, A. (1974) Structure and Function of Plasma Proteins (Allison, A.C., ed.) Vol. 1, pp. 73-125, Plenum Press, London.
4. Kushner, I. (1982) Ann. N.Y. Acad. Sci. 389, 39-48.
5. Rawley, P.T., and Miller, L.L. (1975) Proc. Soc. Exp. Biol. Med. 148, 145-150.
6. Gautier, M., Martin, J.P., and Polini, G. (1977) Biomedicine 27, 116-119.
7. Carlson, J., and Stenflo, J. (1981) FEBS Letters 130, 297-300.
8. Geiger, T., Northemann, W., Schmelzer, E., Gross, V., Gauthier, F., and Heinrich, P.C. (1982) Eur. J. Biochem. 126, 189-195.
9. Benitez-Bribiesca, L., Freyre, R., and De La Vega, G. (1973) Life Sci. 13, 631-638.
10. Bagdasarian, A., and Colman, R.W. (1978) Blood 51, 139-156.
11. Benitez-Bribiesca, L., and Freyre-Horta, R. (1978) Life Sci. 21, 99-104.
12. Gupta, P.K., Frost, J.K., Geddes, S., Aracil, B., and Davidovski, F. (1979) Hum. Pathol. 10, 345-347.
13. Callea, F. (1983) Thesis, University of Leuven.
14. Wilson, G.B., Walker, J.H., Watkins, J.H., and Wolgroch, D. (1980) Proc. Soc. Exp. Biol. Med. 164, 105-114.

15. Rogers, J., Kalsheker, N., Wallis, S., Speer, A., Coutelle, C.H., Woods, D., and Humphries, S.E. (1983) Biochem. Biophys. Res. Commun. 116, 375-382.
16. Andus, T., Gross, V., Tran-Thi, T.-A., Schreiber, G., Nagashima, M., and Heinrich, P.C. (1983) Eur. J. Biochem. 133, 561-571.
17. Cox, R.A., and Smulian, N.J. (1983) FEBS Letters 155, 73-80.
18. Pelham, H.R.B., and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247-256.
19. Blackburn, P. (1979) J. Biol. Chem. 254, 12484-12487.
20. Maccecchini, M., Rudin, Y., Blobel, G., and Schatz, G. (1979) Proc. Natl. Acad. Sci. USA 76, 343-347.
21. Weber, K., Pringle, J.R., and Osborn, M. (1972) Methods Enzymol. 26, 3-27.
22. Bonner, W.M., and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83-88.
23. Carrell, R.W., Jeppsson, Y.-O., Laurell, C.-B., Brennan, S.O., Owen, M.C., Vaughan, L., and Boswell, D.R. (1982) Nature 298, 329-334.