Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1449-1454

ISOLATION AND PROPERTIES OF OXIDIZED ALPHA-1-PROTEINASE INHIBITOR FROM HUMAN RHEUMATOID SYNOVIAL FLUID

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Received September 2,1980

Summary: Human alpha-1-proteinase inhibitor (α-1-PI) from synovial fluid has been isolated to near 90% purity. The preparation has a molecular weight near 52,000, contains 3.5 residues of methionine sulfoxide, and an amino terminal glutamine residue. Sequence studies indicate that the first 17 residues, normally present in plasma α-1-PI, are missing from this protein. The inhibitor did not form a complex with porcine pancreatic elastase but, instead, was converted to a lower molecular weight form. Sequence studies on the latter indicated that two methionyl residues, one at the P_1 reactive site and the other at P_8, had been oxidized. These data confirm the fact that oxidized α-1-PI may be formed in vivo, presumably by the action of myeloperoxidase. This latter effect may alter the proteinase-proteinase inhibitor balance in tissues so that excess proteolysis and abnormal tissue degradation may occur.

Tissue resporption in inflammatory processes is now believed to be caused by a proteinase-proteinase inhibitor imbalance whereby proteinase excess causes the degradation of structural proteins such as elastin, collagen, and proteoglycan. This process has been well described in the development of pulmonary emphysema (1), as well as in rheumatoid arthritis (2). Several of the enzymes believed to be involved in the resorptive process are elicited by neutrophils, including elastase and cathepsin G, and control of their activities is normally due to the circulating plasma proteinase inhibitors (3). In particular, alpha-1-proteinase inhibitor (α-1-PI) has been found to regulate the activity of neutrophil elastase and in its absence rapid degradation of lung tissue found to occur (5).

Recently, we described an important pathway whereby the levels of active, circulating α-1-PI could be reduced in normal, healthy individuals. This process involved the enzymatic oxidation of the reactive site methionine of α-1-PI by neutrophil myeloperoxidase (6) and resulted in the inactivation

0006-291X/80/191449-06$01.00/0

of the inhibitor towards both pancreatic and neutrophil elastase. At that time we speculated that such a process might be occurring in vivo, and a similar suggestion has also been made by Janoff and colleagues (7). We have now isolated α-1-PI from human rheumatoid synovial fluid and found that it cannot inactivate either elastolytic enzyme, yet still retaining some inhibitory activity towards trypsin. Thus, it appears to be related to both the chemically and enzymatically oxidized form of α-1-PI which we have described elsewhere (6,8). This report describes the isolation and properties of the protein as well as structural studies before and after treatment with porcine elastase.

Materials and Methods

Human rheumatoid synovial fluid was obtained from the Marshfield Clinic, Marshfield, Wisconsin, and was a gift of Dr. Ronald C. Roberts. Porcine pancreatic elastase and porcine trypsin were obtained from Sigma Chemical Co. Human α-1-PI and human neutrophil elastase were prepared by methods developed in this laboratory (9,10). Antiserum to human α-1-PI was obtained from Calbiochem-Behring Corp.

Amino acid analyses, amino terminal sequence studies, and methionine sulfoxide analyses were performed as previously described (8).

Results and Discussion

Isolation of α-1-PI from Synovial Fluid

Rheumatoid synovial fluid (250 ml) was diluted 1:1 with 0.05M sodium citrate buffer, pH 5.5. The mixture was stirred for several hours, dialyzed against the same buffer, and centrifuged. The supernatant was fractionated with $(NH_4)_2SO_4$ and the 0.50-0.80 sat. precipitate retained. This fraction was dissolved in the citrate buffer and dialyzed against it with several changes. The sample (300 ml) was applied to a column of Cibacron Blue sepharose (1l) equilibrated with 0.05M sodium citrate buffer, pH 5.5, and the fall through peak which contained all of the α-1-PI, as judged by immunoprecipitation with anti-α-1-PI, was collected. After dialysis against

0.03M sodium phosphate, pH 6.5, the α-1-PI was isolated in a homogenous state by chromatography on DEAE-cellulose at pH 6.5, using a linear gradient from 0-0.15M NaCl to elute the protein (9). The final yield was 30 mg. On occasion, it was necessary to further purify the α-1-PI by passage through a column of Sephadex G-75, in order to remove high molecular weight contaminants. The final product ran as a single band on $NaDodSO_4$-polyacrylamide gels (Figure 1) and could not be differentiated from plasma α-1-PI in this manner. The calculated molecular weight using marker standards was 52,000.

Interaction of Synovial Fluid α-1-PI with Serine Proteinases

The inactivation of porcine elastase by plasma α-1-PI can be readily observed by the formation of an $NaDodSO_4$-stable complex (Figure 2). However,

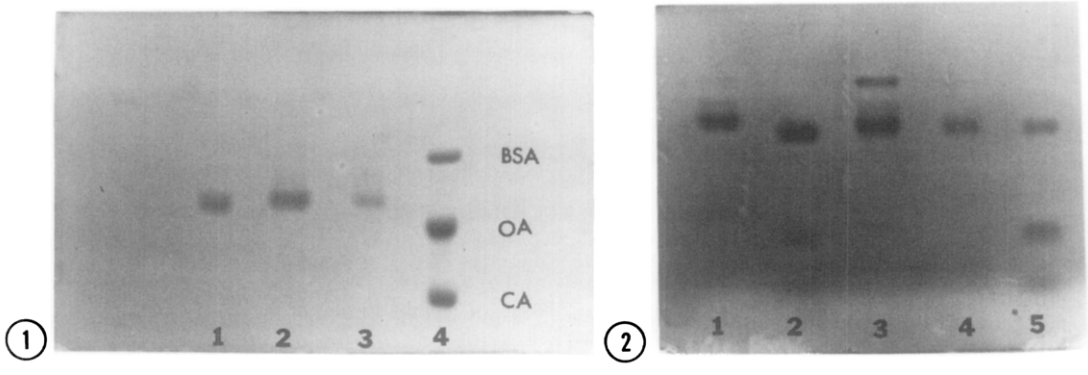

Figure 1. $NaDodSO_4$-gel electrophoresis of human plasma α-1-PI and human synovial fluid α-1-PI. The samples were boiled in 1% $NaDodSO_4$ at pH 8.0 for 5 min. and then submitted to gel electrophoresis. The gel was stained with Coomassie brilliant blue G. Direction of migration is from top to bottom. (1), plasma α-1-PI; (2), synovial fluid α-1-PI; (3) mixture of plasma and synovial fluid α-1-PI; (4) protein markers (BSA = bovine serum albumin, OM = ovomucoid, CA = carbonic anhydrase).

Figure 2. $NaDodSO_4$-gel electrophoresis of incubation mixtures of plasma and synovial fluid α-1-PI with porcine pancreatic elastase. Samples of enzyme and inhibitor were mixed at pH 8.0 for five minutes, made 1% in $NaDodSO_4$, and treated as in Figure 1. (1), synovial fluid α-1-PI; (2), synovial fluid α-1-PI + porcine elastase; (3), plasma α-1-PI + porcine elastase; (4), plasma α-1-PI; (5) synovial fluid α-1-PI + porcine elastase mixed after boiling separately in $NaDodSO_4$.

when synovial fluid α-1-PI is treated in this manner, conversion to a product of slightly lower molecular weight is instead observed, with no evidence of complex formation. These latter results are identical to those found when chemically oxidized α-1-PI is mixed with porcine elastase (8). Apparently, the enzyme simply uses the damaged α-1-PI as a substrate since k_{ass} for the interaction of oxidized α-1-PI with pig elastase has been found to be zero (4). It should be added, however, that the inhibitor does show weak activity towards porcine trypsin after prolonged incubation, although not as much as was reported with oxidized plasma α-1-PI (0.40 moles inhibited per mole of synovial fluid α-1-PI vs 0.90 moles inhibited per mole of oxidized plasma α-1-PI (8).

Structural Studies on Synovial Fluid α-1-PI

The amino acid composition of synovial fluid α-1-PI was found to be quite similar to that of plasma α-1-PI, with the exception that 3.5 residues of a total of seven methionyl residues had been converted to the sulfoxide form. When this protein was subjected to eight cycles in the Beckman sequencer, both a major and a minor sequence were obtained. The major sequence represented 70% of the material analyzed and was identified as follows: Gln-asp-his-pro-thr-phe-asn-lys-. This represents residues 18-25 of the native protein (12) and indicates that cleavage of residues 1-17 must have taken place either before or during the isolation of the protein. This is probably unimportant since an active form of plasma α-1-PI, in which the first nine residues were missing, has been isolated (13). The minor sequence was found to be as follows: Leu-glu-ala-ile-pro-met(O)-ser-ile-. This represents the sequence around the reactive center of α-1-PI (8) and indicates that the active site methionyl residue has been oxidized. Furthermore, because of oxidation cleavage during the isolation of this component could not have occurred at the reactive site, and this has already been reported with plasma α-1-PI (8).

When synovial fluid α-1-PI was incubated with porcine elastase and the modified inhibitor re-isolated (Figure 2) and examined in the sequencer, the amino terminal sequence was as follows: Met(O)-phe-leu-glu-ala-ile-pro-met(O)-ser-ile- . This was the major sequence found and only traces of the sequence: Leu-glu-ala-ile-pro-met(O)-ser-ile- could be detected. This major sequence was also found when myeloperoxidase oxidized plasma α-1-PI was treated with elastase and suggests that the oxidized plasma inhibitor is very similar to that isolated from synovial fluid. In particular, the sequence results show that two methionyl residues at or near the reactive center of α-1-PI have been oxidized. The placement of the other 1-2 residues which were oxidized is, as yet, unknown.

It is obvious from the data shown here that α-1-PI from synovial fluid has undergone oxidation, presumably by myeloperoxidase elicited from synovial leukocytes, and that this has led to a loss of protective activity for the connective tissue proteins in the synovium. We, and others, have suggested such a process as a major contributing factor in the development of a proteinase inhibitor imbalance in the lung, and ultimately to the symptoms associated with emphysema (6,7). In fact, it has been recently reported that a large percentage of α-1-PI in lung washings from cigarette smoke may be oxidized (14). The results given here establish, at a molecular level, the fact that oxidized α-1-PI can be found in human tissue where connective tissue breakdown is occurring. It remains to be proven, through structural analyses, that this process is also occurring in the lung.

Acknowledgements:

This research was supported in part by grants from NIH and The Council for Tobacco Research-U.S.A.

References

1. Snider, G., Hayes, J., Franzblau, C., Kagan, H., Stone, P., and Korthy, A. (1974) Am. Rev. Respir. Dis. 116, 254-270.
2. Steven, F.S., Torre-Blanco, A., and Hunter, J.J.A. (1975) Biochim. Biophys. Acta 405, 188-200.

3. Reilly, C.F. and Travis, J. (1980) Biochim. Biophys. Acta 621, 147-157.
4. Beatty, K., Bieth, J., and Travis, J. (1980) J. Biol. Chem. 255, 3931-3934.
5. Laurell, C.B. and Eriksson, S. (1963) Scand. J. Clin. Invest. 15, 132-140.
6. Matheson, N.R., Wong, P.S., and Travis, J. (1979) Biochem. Biophys. Res. Commun. 88, 402-409.
7. Carp, H. and Janoff, A. (1979) J. Clin. Invest. 63, 793-797.
8. Johnson, D. and Travis, J. (1979) J. Biol. Chem. 254, 4022-4026.
9. Pannell, R., Johnson, D., and Travis, J. (1974) Biochemistry 13, 5439-5445.
10. Baugh, R. and Travis, J. (1976) Biochemistry 15, 836-841.
11. Travis, J., Bowen, J., Tewksbury, D., Johnson, D., and Pannell, R. (1976) Biochem. J. 157, 301-306.
12. Travis, J., Garner, D., and Bowen, J. (1978) Biochemistry 17, 5647-5651.
13. Morii, M., Odani, S., Koide, T., and Ikenaka, T. (1978) J. Biochem. 83, 269-277.
14. Gadek, J.E., Fells, G.A., and Crystal, R.G. (1979) Science 206, 1315-1316.