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THE N-TERMINAL SEQUENCE OF ANTILEUKOPROTEASE ISOLATED FROM BRONCHIAL SECRETION

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Antileukoprotease (ALP) was isolated from bronchial secretion. The amino acid composition as well as the N-terminal sequence were determined. No homology with other low molecular weight proteinase inhibitors was found.

Since the discovery of Laurell and Eriksson (1) that hereditary alpha$_1$-protease inhibitor (alpha$_1$-PI, α_1-antitrypsin) deficiency is associated with the early appearance of pulmonary emphysema, it has been recognized that proteolytic enzymes and their inhibitors play an important role in the pathogenesis of this disease. This view is supported by the observation of many investigators that intratracheal administration or aerosol inhalation of proteolytic enzymes, especially those with elastolytic properties, can induce emphysema in animals (2,3, 4). It has been suggested that endogenous elastases, derived from leukocytes and macrophages, play a role in the development of emphysema and other destructive lung diseases such as adult respiratory distress syndrome (5-8). There are several elastase inhibitors in bronchial mucus including alpha$_1$-PI and acid stable low molecular weight protease inhibitors (antileukoprotease and specific anti-elastase) (9, 10, 11).
Antileukoprotease (ALP) which is locally produced by serous cells of the bronchial glands and by non-ciliated cells of the bronchiolar epithelium,

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accounts for at least 80% of the total inhibiting capacity of bronchial mucus against granulocyte proteases (12,13,14).

In this paper we present part of the N-terminal sequence of ALP.

MATERIALS AND METHODS

Purification of ALP - ALP was purified from perchloric acid treated mucoid sputum by an immunoadsorbent column. The immunoadsorbent was prepared by coupling the immunoglobulin fraction, isolated by $(NH_4)_2$ SO4 precipitation from a rabbit anti-ALP antiserum, to Affigel-10 (Bio-Rad Laboratories, Richmond, USA). The monospecific anti-ALP antiserum was prepared as described previously (12). ALP from 150 ml acid treated sputum was bound to 25 ml immunoadsorbent by a batch procedure. After an overnight incubation, the slurry was filtered and poured into a column (1.5 x 14 cm). After washing with phosphate buffered saline, ALP was isolated by elution with 0.1M citric acid, 0.3M NaCl pH 2.8. The eluted ALP sample was neutralized immediately with solid Tris. Purified ALP from 10 batches were pooled, concentrated and applied on the top of a Sephadex G-75 column (Pharmacia Fine Chemicals Inc. Uppsala, Sweden: 1.7 x 95 cm) equilibrated with 0.01M Tris, 0.04M NaCl pH 7.6. Inhibitor containing fractions were pooled, dialyzed against distilled water and stored at -20°C.

Electrophoretic methods - Dodecyl sulphate electrophoresis was performed according to Laemmli (15). For calibration of the gels, Trasylol, Cytochrome C, human Lysozyme and Soybean Trypsin Inhibitor were used. Samples of 20 µg protein were used for analyses.

Amino acid analysis - Amino acid analysis was performed on a Beckman Multichrom B analyzer, following hydrolysis of the protein in 6 N HCl at 110°C for 24, 48 and 72 hr.

Sequence determination - For the manual sequence determination the method of Chang et al. (16) was used.
The amino terminal part of the intact protein (60 nmol) was sequenced on a Beckman Model 890C sequencer, with 0.25 M Quadrol buffer programme. Phenylthiohydantoin amino acids were identified using High Performance Liquid Chromatography (17).

RESULTS AND DISCUSSION

Purified ALP showed one homogeneous peak on Sephadex G75 and a single band with a molecular weight of approximately 14,000 on dodecyl sulphate electrophoresis (fig. 1).

The amino acid composition is similar to the one published previously by us (12) and only slightly different from the one of Ohlsson et al. (18) (table 1). No histidine was present whereas the protein is rich in lysine, proline and cysteine.

Figure 2 shows the N-terminal region of ALP. The amino acids at positions 10 and 18 probably are cysteine residues.

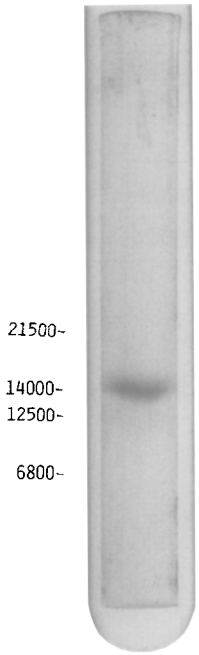

Fig. 1

SDS analysis of ALP purified from bronchial secretion by immuno adsorbent chromatography. Molecular weights of marker proteins are indicated.

A comparison of the presented ALP sequence with sequenced parts of human seminal plasma inhibitor (HUSI-I) as published by Fritz et al (19) did not show any similarities. However, biochemical characteristics and antigenic identity do suggest that HUSI-I and the bronchial inhibitor are identical molecules. An explanation for this discrepancy might be that the sequenced HUSI-I molecule has been degraded during the isolation procedure as was suggested by the authors themselves. So, the N-terminal part of the native HUSI-I molecule could be missing in the sequenced molecule. Another explanation might be that ALP from bronchial secretion and HUSI-I are different molecules.

Homology between the ALP sequence as presented here and sequences of other low-molecular-weight protease inhibitors were not found (20, 21). However, no definite conclusions can be drawn untill the complete sequence of ALP has been elucidated.

Table 1

Amino acid composition of antileukoprotease purified from bronchial secretion

Amino Acid	Molar ratio		Mol %[a]	Mol %[b]
	Experimental values[a]	Nearest integer[a]		
Lysine	9.7	10	7.8	13.1
Histidine	0.2	0	0.0	0.0
Arginine	4.8	5	3.9	4.5
Tryptophan	n.d.	n.d	n.d.	0.0
Aspartic Acid	11.0	11	8.8	9.5
Threonine[1]	5.6	6	4.5	3.6
Serine[1]	8.9	9	7.1	5.8
Glutamic Acid	11.8	12	9.5	5.5
Proline	14.2	14	11.4	10.8
Glycine	10.0	10	8.0	8.9
Alanine	6.9	7	5.5	3.7
Cysteine[2]	12.8	13	10.3	14.8
Valine	6.6	7	5.3	6.9
Methionine	3.8	4	3.0	3.3
Isoleucine	3.8	4	3.0	1.3
Leucine	8.2	8	6.6	4.7
Tyrosine	2.4	2	2.0	1.9
Phenylalanine	4.1	4	3.3	1.8

a. From this study, mean of two determinations of the same preparation.

b. Ohlsson et al (18)

n.d. not determined

1. Values for threonine and serine were obtained by extrapolation to zero hydrolysis time.

2. Cysteine residues were determined by acid hydrolysis in the presence of 0.3M dimethylsulfoxide (22).

The N-terminal sequence of ALP can be used to predict part of the ALP m-RNA sequence followed by synthesis of an oligonucleotide ALP probe. This would be the first step in the isolation of the ALP gene. These and

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                    (5)                 (10)
TYR-GLY-LYS-PRO-PHE-LYS-ALA-GLY-VAL- X -PRO-PRO-LYS-

    (15)                (20)                (25)
LYS-LYS-ALA-GLN- X -LEU-ARG TYR-LYS-ILE-PRO-GLU-GLU-
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Fig. 2

The amino terminal region of antileukoprotease from bronchial secretion. Amino acids which are underlined are also manually sequenced. Amino acids at positions 10 and 18 are probably cysteine residues.

studies on the active site of ALP are subject of future research in our laboratory.

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