Biochimica et Biophysica Acta, 628 (1980) 293—302

BBA 29192

COMPARATIVE STUDIES ON NATIVE AND ELASTASE-INACTIVATED α_1-PROTEASE INHIBITOR (α_1-ANTITRYPSIN)

J.S. BAUMSTARK [a,*], D.R. BABIN [a], C.T. LEE [a] and R.C. ALLEN [b]

[a] *Departments of Obstetrics and Gynecology, Biochemistry and Pathology, Creighton University School of Medicine, Omaha, NE 68131 and* [b] *Department of Pathology, Medical University of South Carolina, Charleston, SC 29401 (U.S.A.)*

(Received May 28th, 1979)

Key words: α_1-Antitrypsin; Elastase inactivation; Protease inhibitor

Summary

Purified α_1-protease inhibitor was reacted with a 1.3-fold molar excess of swine pancreatic elastase and incubated at 37°C for 30 min. Total inactivation of the inhibitor occurs under these conditions. Inactivated α_1-protease inhibitor was isolated from the reaction mixture by chromatography on DEAE-cellulose. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed the preparation to be homogeneous. The amino acid composition of native (M_r 54 000) and inactivated (M_r 49 000) α_1-protease inhibitor were practically identical; sialic acid residues were unchanged at 6 per mol. Carbohydrate analyses showed a net loss of 8 *N*-acetylglucosamine and 10 hexose residues per mol of inactivated inhibitor when compared to the native protein. The weight loss which occurs in α_1-protease inhibitor due to inactivation by elastase (54 000 vs. 49 000) is probably due primarily to the loss of carbohydrate residues. Electrophoresis of cyanogen bromide fragments in sodium dodecyl sulfate-urea-polyacrylamide gel produced nine distinct zones for the native inhibitor, eight of which were common to the inactivated protein. The missing zone in the elastase-inactivated inhibitor CNBr preparation was strongly periodic acid-Schiff positive, suggesting that the carbohydrate moiety of this glycopeptide is cleaved when the native protein is inactivated by elastase. Electrophoretic mobility determinations, at pH 8.6, gave $-5.5 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for the native protein and $-5.6 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for the inactivated inhibitor. The inactivated protein retained its ability to produce multiple zones upon isoelectric focusing with isoelectric points varying between 4.53 and 4.75 (av.

* To whom correspondence should be addressed.
Abbreviation: SDS, sodium dodecyl sulfate.

4.61). The average isoelectric point for the native inhibitor was 4.45 (4.38—4.51).

Introduction

The relationship between α_1-protease inhibitor deficiency and the development of emphysema [1] and cirrhosis of the liver [2,3] is well established.

Immunochemical studies carried out several years ago by this laboratory [4] revealed that approximately 23% of the α_1-protease inhibitor in human serum did not form an electrophoretically separable complex with elastase when the enzyme was present in excess of equivalence. More recently, quantitative experiments have revealed that the apparently unreactive α_1-protease inhibitor observed previously (the 23% portion) had indeed reacted with the enzyme and that the product, inactivated α_1-protease inhibitor, was the result of an extremely fast reaction with elastase [5]. In an 1.3-fold molar excess of elastase (37°C) about 50% of the α_1-protease inhibitor is immediately inactivated, while the remainder forms an electrophoretically separable complex with the enzyme [5]. Further, the α_1-protease inhibitor moiety of the complex is also degraded ($k = 2.1 \cdot 10^{-3}$ s^{-1}) under these conditions to the inactivated inhibitor; total inactivation of α_1-protease inhibitor occurs within 30 min at 37°C [5].

The present report describes the preparation of inactivated α_1-protease inhibitor and comparative studies with the native inhibitor with regard to amino acid and carbohydrate composition, cyanogen bromide cleavage, electrophoretic mobility, and isoelectric focusing.

Preliminary accounts of portions of this work have been published [6,7].

Materials and Methods

Elastase (EC 3.4.21.11) was isolated from Trypsin 1-300 (Nutritional Biochemicals, Freehold, NJ), a dried preparation of swine pancreas, as previously described [8]. α_1-Protease inhibitor (Pi MM phenotype; Ref. 9) * was isolated from human serum as previously described [5]. Antielastase antibody was produced in rabbits essentially as described by McIvor and Moon [11]. Antihuman α_1-protease inhibitor antibody was obtained from Behring Diagnostics.

Preparation of inactivated α_1-protease inhibitor. As was indicated above, α_1-protease inhibitor is converted to inactivated α_1-protease inhibitor in the presence of a 1.3-fold molar excess of elastase in 30 min at 37°C. Accordingly, 48 mg (2 ml) of purified α_1-protease inhibitor was mixed with 30 mg (20 ml) of elastase and incubated at 37°C for 30 min. Both reactants were dissolved in 25 mM diethylbarbiturate buffer, pH 8.6. The reaction mixture was cooled, and subjected to chromatography on a 2.1 × 80 cm column of DEAE-cellulose equilibrated with 10 mM potassium phosphate buffer, pH 8.3. The columns were prepared and operated as previously described [12,13]. Following the

* For a discussion of the protease inhibitor system see Fagerhol [10].

collection of four holdup volumes of effluent (800 ml) a 2 × 400 ml gradient was added. Chamber 1 contained 10 mM potassium phosphate buffer, pH 8.3, and the limit buffer was 0.5 M KH_2PO_4.

Chemical determinations. Amino acid compositions were determined using the method of Moore and Stein [14] after hydrolysis in vacuo for 24 h at 110°C in 6 M HCl. Total hexose was determined using the anthrone procedure of Scott and Melvin [15], glucosamine by the method of Boas [16], and sialic acid by the thiobarbituric acid method of Warren [17].

Cyanogen bromide cleavage. Native and inactivated α_1-protease inhibitor were reduced with 2-mercaptoethanol and alkylated with iodoacetamide according to Crestfield et al. [18]. The alkylated proteins were dialyzed against eight successive changes of distilled water, lyophilized and subjected to CNBr cleavage, in 70% formic acid, as described by Steers et al. [19]. The CNBr fragments were subjected to SDS-polyacrylamide gel electrophoresis in 12.5% gels (ratio acrylamide: *N*, *N'*-methylene-bis-acrylamide = 10 : 1) containing 8 M urea as described by Swank and Munkres [20]. The gels were stained with Coomassie Brilliant Blue R250 (Bio-Rad Laboratories) and with periodic acid-Schiff reagent as described by Zacharius et al. [21]. To avoid possible artifactual staining of noncarbohydrate-containing CNBr fragments the SDS was removed to prior to periodic acid-Schiff staining as suggested by Glossmann and Neville [22].

Electrophoretic mobility. This parameter was determined, relative to the free solution of mobility of human albumin, as previously described [4]. The method involves immunoelectrophoresis and the use of specific antibody. Distances migrated from the origin(s) were measured with a Nikon Microcomparator (10× objective) [4].

Electrophoresis. Polyacrylamide gel isoelectric focusing was carried out using the LKB 2117 Multiphor in a pH 4—6 gradient essentially as described by Allen et al. [23] For the determination of the pH gradient, twenty-two 5 × 30 mm segments of gel were cut (anode to cathode) and placed in 5 ml of boiled distilled water (screw cap tubes). Following incubation at 20°C overnight the pH was determined and plotted as a function of distance from the cathode.

Immunoelectrophoresis was carried out as previously described [4]. SDS-polyacrylamide gel electrophoresis in 7.5% gel was carried out according to Weber and Osborn [24].

Results

Preparation of inactivated α_1-protease inhibitor. Fig. 1 illustrates the chromatogram obtained when purified native α_1-protease inhibitor was reacted with a 1.3-fold molar excess of elastase and the reaction mixture subjected to chromatography on DEAE-cellulose. Excess elastase (peak a) emerged with the breakthrough volume and the second peak is inactivated α_1-protease inhibitor.

The fractions comprising the leading edge (peak b; shoulder) and the hatched portion of the peak (peak c) were pooled, dialyzed against three successive 17 l vol. of distilled water (2°C) and lyophilized. Neither fraction (peaks b and c) reacted with anti-elastase antibody indicating the absence of the α_1-protease inhibitor-elastase complex. The amino acid compositions as well as the electro-

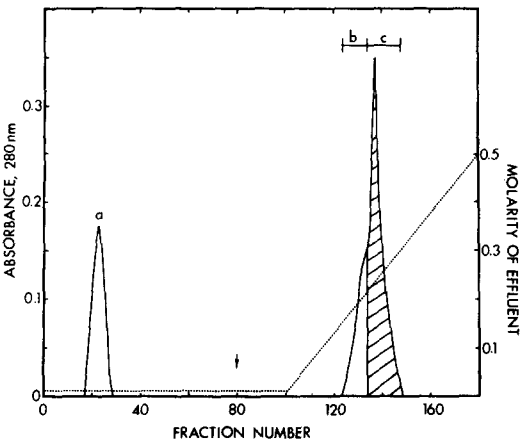

Fig. 1. Separation of inactivated α_1-protease inhibitor from an α_1-protease inhibitor-elastase reaction mixture. a, elastase; b and c, inactivated α_1-protease inhibitor. Column: DEAE-cellulose, 2.1 X 80 cm; fraction volume, 10 ml; flow rate, 50 ml/h; gradient (arrow) was begun at fraction 80. See text for details.

phoretic mobilities were identical and both preparations produced single zones with identical mobilities when subjected to SDS-polyacrylamide gel electrophoresis. The shoulder (peak b) comprised about 27% of the total protein and could be either artifactual or could represent an intermediate in the inactivation reaction. Under any circumstances, like peak c, it did not inhibit elastase. The fraction comprising the hatched portion of the peak (Fig. 1, peak c) was used for the determinations reported below.

TABLE I

AMINO ACID COMPOSITION OF NATIVE AND ELASTASE-INACTIVATED α_1-PROTEASE INHIBITOR

Based on 47 leucyl residues per mol (54 000 g) [25]. Native is an average of the five determinations and inactivated an average of three determinations. n.d., not determined.

	Native (Residues/mol)	Inactivated (Residues/mol)
Lysine	35.2 (35)	34.0 (34)
Histidine	12.8 (13)	12.3 (12)
Arginine	7.4 (7)	8.0 (8)
Aspartic Acid	46.0 (46)	48.0 (48)
Threonine	28.8 (29)	28.7 (29)
Serine	22.2 (22)	24.0 (24)
Glutamic Acid	54.8 (55)	54.7 (55)
Proline	16.8 (17)	14.5 (15)
Glycine	24.0 (24)	27.0 (27)
Alanine	25.4 (25)	25.6 (26)
Half-cystine	n.d.	n.d.
Valine	24.6 (25)	27.0 (0)
Methionine	8.8 (9)	9.0 (9)
Isoleucine	18.8 (19)	19.7 (20)
Leucine	47.0 (47)	47.0 (47)
Tyrosine	7.0 (7)	7.7 (8)
Phenylalanine	28.0 (28)	27.7 (28)
Tryptophan	n.d.	n.d.

TABLE II

CARBOHYDRATE COMPOSITION OF NATIVE AND ELASTASE-INACTIVATED α_1-PROTEASE INHIBITOR

Native and inactivated are an average of two determinations. Values obtained on an additional preparation of native and inactivated α_1-protease inhibitor for total hexose and sialic acid were: native, 24 hexose residues/mol and seven sialic acid residues/mol; inactivated, 16 hexose residues/mol and six sialic acid residues/mol.

	Native (Residues/mol, 54 000 g)	Inactivated (Residues/mol, 49 000 g)	Residues, net change	Residue weight x net change
N-Acetylglucosamine	13.9 (14)	6.0 (6)	−8	−1624
Hexose	24.1 (24)	14.1 (14)	−10	−1620
Sialic acid	5.6 (6) *	6.4 (6)	0	0
		Total:	−18	−3244

* Other values reported for this residue and recalculated on the basis of 54 000 g/mol are Chan et al. [26], 9.0; Roll et al. [27], 8.4; Travis et al. [28], 1.9; Yu and Gan [29], 5.9; Kress and Laskowski, [30], 8; Jeppsson et al. [34], 6.3. Values for *N*-acetylglucosamine and total hexose reported here do not differ appreciably from those reported by the above investigators.

Amino acid and carbohydrate composition of native and elastase-inactivated α_1-protease inhibitor. These results are presented in Tables I and II. It is apparent from the results presented in Table I that when the amino acid composition is calculated on the basis of 47 leucyl residues and a molecular weight of 54 000 [25], there is little or no difference in the compositions of native and inactivated α_1-protease inhibitor. The carbohydrate content of native and inacticated α_1-protease inhibitor are presented in Table II. Table II shows that inactivated α_1-protease inhibitor possesses eight fewer residues of *N*-acetylglucosamine and 10 less hexose residues than the native inhibitor. The sialic acid content remained the same at six residues per mol. The net weight loss due to carbohydrate was 3244 or about 6% of the total mass of α_1-protease inhibitor.

Cyanogen bromide cleavage. The results obtained when native α_1-protease inhibitor and inactivated α_1-protease inhibitor were reduced and alkylated * followed by CNBr cleavage * and electrophoresis in SDS-urea-polyacrylamide gel is shown in Fig. 2. The results show that nine principal fragments were obtained when native α_1-protease inhibitor was cleaved by CNBr (Fig. 2B) and this is in accord with the number of methionine residues in α_1-protease inhibitor (Table I). Carbohydrate appears to predominate in zones 1 and 9 (Fig. 2A, periodic acid-Schiff (reagent) stain) since only light staining was observed with Coomassie Blue (Fig. 2B). Eight of the nine zones of native α_1-protease inhibitor were present also in the inactivated α_1-protease inhibitor sample (Fig. 2C and D). Zone 9 of native α_1-protease inhibitor (Figs. 2A and B) was not present in

* Amino acid analyses of reduced and alkylated native and inactivated α_1-protease inhibitor showed the absence of cystine and the presence of carboxymethyl-cysteine. Similarly, when the mixtures of CNBr fragments from carboxamidomethylated α_1-protease inhibitor and inactivated α_1-protease inhibitor were analyzed, methionine was shown to have been converted to homoserine and its lactone.

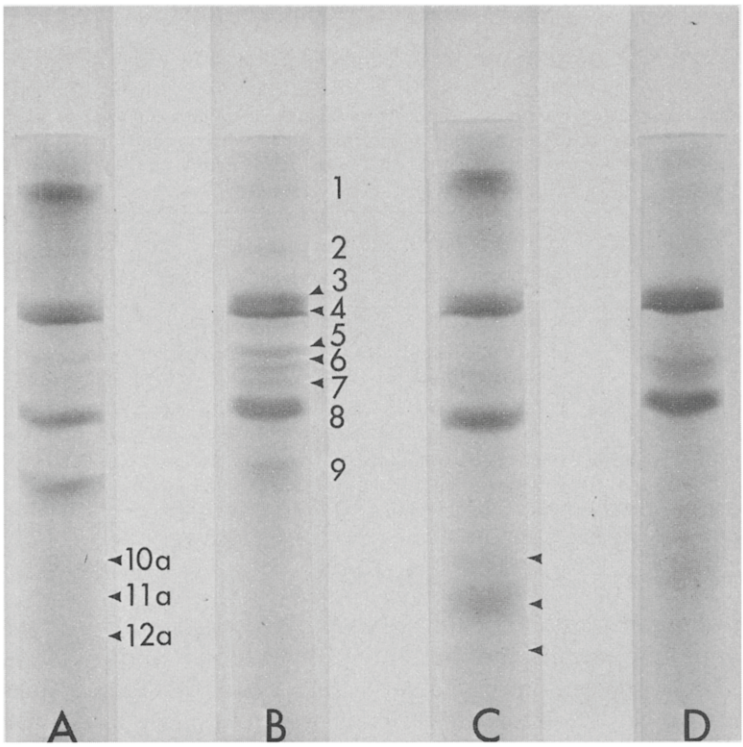

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel (12.5% in 8 M urea) electrophoretograms of cyanogen bromide cleavage products derived from native and elastase-inactivated α_1-protease inhibitor. A and B, native α_1-protease inhibitor. C and D, elastase inactivated α_1-protease inhibitor. A and C, stained with periodic acid-Schiff reagent; C and D, stained with Coomassie Brilliant Blue R250. Gel dimensions, 5 × 100 mm; samples are equivalent to 60 μg of original protein; 14 h at 2.1 mA/gel followed by 5.5 h at 4.2 mA/gel. Note that zone nine is missing in the digest of inactivated α_1-protease inhibitor (C and D). See text for further details.

the inactivated α_1-protease inhibitor preparation indicating that a methionine-containing peptide, which also contains an appreciable amount of carbohydrate (Table II), was cleaved when α_1-protease inhibitor was inactivated by elastase. Three lightly staining additional periodic acid-Schiff-positive zones (10a, 11a, and 12a) were present in both preparations (Figs. 2A and C).

Electrophoretic mobility. Side-by-side determinations were carried out with native and inactivated α_1-protease inhibitor. The results of four determinations (average) * gave $-5.5 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for native α_1-protease inhibitor and $-5.6 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for inactivated α_1-protease inhibitor showing that inactivated α_1-protease inhibitor is slightly faster, electrophoretically, and thus possesses a higher net negative charge density, at pH 8.6, than native α_1-protease inhibitor. Fig. 3 presents the results obtained when native and inactivated α_1-protease inhibitor were subjected to immunoelectrophoresis and

* These values ranged from $-5.41 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} to $-5.52 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for the native inhibitor and from $-5.50 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} to $-5.76 \cdot 10^{-5}$ cm$^2 \cdot$ V$^{-1} \cdot$ s^{-1} for inactivated α_1-protease inhibitor. The means are significantly different ($t = 2.120$, $P > 0.01$).

diffused against anti α_1-protease inhibitor antibody. The results show the closeness in electrophoretic mobility and illustrate that the antigenic determinent(s) of α_1-protease inhibitor are retained in the inactivated α_1-protease inhibitor molecule (Fig. 3). The proteins also produced a reaction of 'identity' (complete coalescence) when subjected to double diffusion analysis in agarose gel.

Isoelectric focusing. Fig. 4A presents the results obtained when native α_1-protease inhibitor and inactivated α_1-protease inhibitor were subjected to isoelectric focusing in a pH 4—6 gradient. A scheme of the principal zones shown in Fig. 4A is presented in Fig. 4B. It is apparent that a cathodal shift in the inactivated α_1-protease inhibitor zones has occurred when compared with the parent native α_1-protease inhibitor (Fig. 4B). The heavier zones in the inactivated α_1-protease inhibitor pattern are probably representative of inactivated M4 and M6 zones (Fig. 4B).

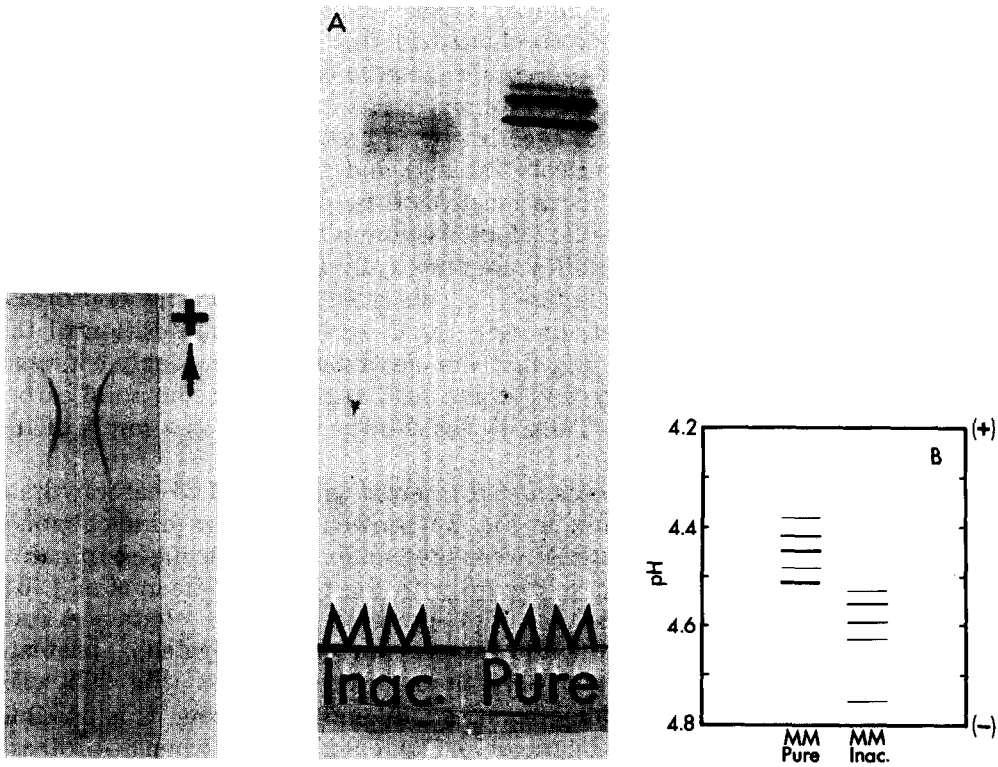

Fig. 3. Immunoelectrophoretic patterns of native and inactivated α_1-protease inhibitor. Top well, native α_1-protease inhibitor; bottom well, inactivated α_1-protease inhibitor; protein concentration, 1.5 mg/ml; sample volume 1.5 µl. Diffused against anti α_1-protease inhibitor antibody (trough). 1% agarose in 25 mM diethylbarbiturate buffer, pH 8.6; 9.3 V · cm^{-1} for 1 h.

Fig. 4A. Polyacrylamide gel isoelectric focusing patterns (pH 4—6 gradient) of purified native α_1-protease inhibitor (MM, pure; 80 µg) and inactivated α_1-protease inhibitor (MM, Inac.; 100 µg). The cathode is at the bottom of the figure. Fig. 4B Isoelectric points and principal zone positions for the samples shown in Fig. 4A. Breadth of zones indicates relative protein concentration. Note cathodic focusing of inactivated α_1-protease inhibitor (MM, Inac.).

Discussion

We had previously shown that elastase-inactivated α_1-protease inhibitor possessed a molecular weight of 49 000 [5]. This amounted to a loss of approx. 5000 daltons as a result of the exposure of native α_1-protease inhibitor (M_r 54 000) to a 1.3-fold molar excess of elastase.

Since a discrepancy of some 5000 daltons in the two molecules was demonstrated [5] it was somewhat of a surprise, initially [6], to find that their amino acid compositions (Table I) were practically identical when based on a molecular weight of 54 000 and 47 leucyl residues [25]. Since we were reasonably certain that the molecular weight of inactivated α_1-protease inhibitor was not 54 000 but 49 000 (from at least 25 SDS-polyacrylamide gel experiments) the amino acid composition of inactivated α_1-protease inhibitor was recalculated on the basis of the latter molecular weight. These calculations showed a net loss of 37 residues amounting to a residue weight of 4244 g/mol. This weight corresponds closely to the 5000 dalton loss which occurred when native α_1-protease inhibitor was reacted with a 1.3-fold molar excess of elastase (54 000 vs. 49 000) [5]. Other experiments conducted in this laboratory, however, have failed to demonstrate amounts of peptide material which would account for a loss of 37 residues. For this reason we are reasonably certain that the amino acid compositions of native and inactivated α_1-protease inhibitor are (within the limits of the method) identical (Table I) and that the weight loss which occurs when α_1-protease inhibitor is inactivated by elastase is due primarily to the loss of carbohydrate (Table II). Since the amino acid composition of native and inactivated α_1-protease inhibitor are practically identical it is obvious that the peptide bond cleaved by elastase (i.e., the reactive site on the α_1-protease inhibitor molecule) must be at, or near, the C- or N-terminal position of the α_1-protease inhibitor molecule and that the cleaved portion probably contains, in addition to asparagine, very few amino acid residues but the total carbohydrate component which, as Table II has shown, was lost as a result of the inactivation reaction.

The CNBr cleavage experiments are of interest with regard to carbohydrate content. For example, the results in Fig. 2A showed that four principal zones of carbohydrate-containing material were present in the CNBr digest of native α_1-protease inhibitor and this is in accord with the results of Chan et al. [26], who suggested that one molecule of α_1-protease inhibitor contained four oligosaccharide units. Our results also showed that the inactivated α_1-protease inhibitor CNBr digest contained only three principal zones (Fig. 2C) with zone 9 of native α_1-protease inhibitor (Fig. 2A and B) missing. It is possible therefore that the oligosaccharide moiety of zone 9 is cleaved when α_1-protease inhibitor is inactivated by elastase. It is also perhaps significant that the molecular weights of the oligosaccharide units designated glycopeptides II and III by Chan et al. [26], exclusive of the sialic acid residues, total 3239. This is almost exactly equivalent to the 3244 carbohydrate weight loss we observed in Table II for inactivated α_1-protease inhibitor.

According to Chan et al. [26] all of the oligosaccharide chains of α_1-protease inhibitor contain sialic acid. Our results are difficult to explain in this regard, in that inactivated α_1-protease inhibitor possesses the same number of sialic acid

residues as native α_1-protease inhibitor, but significantly fewer *N*-acetylglucosamine and hexose residues (Table II). These results, in contrast to those of Chan et al. [26], suggest that not all of the oligosaccharide chains of native α_1-protease inhibitor contain sialic acid. Other evidence which supports the retention of sialic acid residues is the fact that our purified α_1-protease inhibitor possesses the same electrophoretic mobility as the α_1-protease inhibitor of whole serum and that the mobility of inactivated α_1-protease inhibitor was increased slightly rather than decreased. In this regard, it has been shown that even a partial cleavage of sialic acid residues in α_1-protease inhibitor results in the production of a more basic protein [31—33]. Thus our purified α_1-protease inhibitor must contain the full complement of sialic acid residues. It must also be emphasized that the reported values for the sialic acid residue in native α_1-protease inhibitor [26—30,34] varied from 1.9—9 per mol of protein (54 000 g/mol).

The electrophoretic and isoelectric focusing data (Fig. 4) further delineate perplexing differences in native and inactivated α_1-protease inhibitor. For example, one would expect that, since inactivated α_1-protease inhibitor possesses an electrophoretic mobility at pH 8.6 slightly more acidic than the parent native protein, zones equivalent to or slightly anodal to native α_1-protease inhibitor would result when inactivated α_1-protease inhibitor was subjected to isoelectric focusing in a pH 4—6 gradient. However, the results presented in Fig. 4 showed that inactivated α_1-protease inhibitor produced zones characteristic of a more basic protein whose average isoelectric point was 4.61 as opposed to an average isoelectric point of 4.45 for native α_1-protease inhibitor. Therefore, on the basis of the amino acid and carbohydrate analyses (Tables I and II) the behavior of inactivated α_1-protease inhibitor at its electric point(s), (Fig. 4; average isoelectric point = 4.61) must be due to loss of carbohydrate (Table II) which at this pH could result in a conformational change and apparent burial of negatively charged residues or the unmasking of residues with positive charge.

Elastase-inactivated α_1-protease inhibitor possesses many properties similar to the α_1-protease inhibitor found in globular inclusion bodies in the hepatocytes of α_1-protease inhibitor-deficient individuals [34]. These include a similarity in molecular weight, the retention of the antigenic determinent(s) of native α_1-protease inhibitor, loss of carbohydrate residues, and approximately the same amino acid composition as native α_1-protease inhibitor. The α_1-protease inhibitor found in the livers of α_1-protease inhibitor-deficient individuals is practically insoluble and contains few, if any, sialic acid residues [34]. By contrast, elastase-inactivated α_1-protease inhibitor is quite soluble and, as indicated above, possesses approximately the same number of sialic acid residues as the native protein. These similarities in the two proteins suggest that proteases may, in part, be involved in the conversion of native α_1-protease inhibitor to the inactive deglycosylated α_1-protease inhibitor which accumulates in the livers of α_1-protease inhibitor-deficient individuals.

In conclusion, the inactivation of α_1-protease inhibitor by elastase results in the production of a molecule of M_r 49 000. The polypeptide backbone of native α_1-protease inhibitor is apparently unaffected (Table I) by the inactivation reaction and the observed weight loss (54 000 vs. 49 000) which occurs

when native α_1-protease inhibitor is inactivated by elastase appears to be due primarily to the loss of carbohydrate residues (Table II).

Experiments to isolate and characterize the cleaved glycopeptide moiety of elastase-inactivated α_1-protease inhibitor, using preparative amounts of α_1-protease inhibitor, are currently in progress.

Acknowledgements

The authors are indebted to Ms. Linda Frisk, Ms. Kailas Patel and Ms. Patricia Pickton for excellent technical assistance and to Ms. Jan Boggs for typing the manuscript. This work was supported by grant HL15979 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, U.S.A.

References

1 Laurell, C.B. and Eriksson, S. (1963) Scand. J. Clin. Lab. Invest. 15, 132—140
2 Sharp, H.L., Bridges, R.A. and Krivit, W. (1969) J. Lab. Clin. Med. 73, 934—939
3 Ishuk, K.G., Jenis, E.H., Marshall, M.L. and Bolton, B.H. (1972) Arch. Pathol. 94, 445—455
4 Baumstark, J.S. (1967) Arch. Biochem. Biophys. 118, 619—630
5 Baumstark, J.S., Lee, C.T. and Luby, R.J. (1977) Biochim. Biophys. Acta 482, 400—411
6 Baumstark, J.S. (1976) The α_1-antitrypsin-elastase interaction. A Workshop on Isolation, Characterization, and Mechanism of Action of Proteases and Antiproteases. N.H.L.B.I., N.I.H. Airlie, Virginia, February 9—11
7 Baumstark, J.S. (1979) Federation Proc. 38, 834
8 Baumstark, J.S. (1970) Biochim. Biophys. Acta 220, 534—551
9 Baumstark, J.S., Lee, C.T. and Luby, R.J. (1976) Biochim. Biophys. Acta 446, 287—300
10 Fagerhol, M.K. (1968) Series Haematol. 1, 153—161
11 McIvor, B.C. and Moon, H.D. (1962) J. Immunol. 88, 274—276
12 Baumstark, J.S. (1968) Arch. Biochem. Biophys. 125, 837—849
13 Baumstark, J.S., Laffin, R.J. and Bardawil, W.A. (1964) Arch. Biochem. Biophys. 108, 514—522
14 Moore, S. and Stein, W.H. (1963) Meth. Enzymol. 6, 819—831
15 Scott, T.A. and Melvin, E.H. (1953) Anal. Chem. 25, 1656—1661
16 Boas, N.F. (1953) J. Biol. Chem. 204, 553—563
17 Warren, L. (1959) J. Biol. Chem. 234, 1971—1975
18 Crestfield, A.M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622—627
19 Steers, E. Jr., Craven, G.R. and Anfinsen, C.B. (1965) J. Biol. Chem. 240, 2478—2484
20 Swank, R.T. and Munkres, K.D. Anal. Biochem. (1971) 39, 462—477
21 Zacharius, R.M., Zell, T.E., Morrison, J.H. and Woodlock, J.J. (1969) Anal. Biochem. 30, 148—152
22 Glossman, H. and Neville, D.M., Jr. (1971) J. Biol. Chem. 246, 6339—6346
23 Allen, R.C., Harley, R.A. and Talamo, R.C. (1974) Am. J. Clin. Pathol. 62, 732—739
24 Weber, K. and Osborn, M. (1975) The Proteins 1, 179—223
25 Heimberger, N., Haupt, H. and Schwick, H.G. (1971) in Proceedings of the International Research Conference on Proteinase Inhibitors (Fritz, H. and Tschesche, H., eds.), pp. 1—21, Walter de Gruyter, New York
26 Chan, S.K., Rees, D.C., Li, A.C. and Li, Y.T. (1976) J. Biol. Chem. 251, 471—476
27 Roll, D., Aguanno, J.J., Coffee, C.J., Glew, R.H. and Iammarino, R.M. (1978) Biochim. Biophys. Acta 532, 171—178
28 Travis, J., Johnson, D. and Pannel, R. (1974) in Bayer Symposium I 'Proteinase Inhibitors' (Fritz, H., Tschesche, H. and Greene, L.J., eds.), pp. 31—39, Springer-Verlag, New York
29 Yu, S.D' and Gan, J.C. (1977) Arch. Biochem. Biophys. 179, 477—485
30 Kress, L.F. and Laskowski, M. Sr. (1973) Prepaprative Biochem. 3, 541—552
31 Myerowitz, R.L., Handzel, Z.T. and Robbins, J.B. (1972) Clin. Chim. Acta 39, 307—317
32 Bell, O.F. and Carell, R.W. (1973) Nature 243, 410—411
33 Cox, D.W. (1975) Am. J. Hum. Genet. 27, 165—177
34 Jeppsson, J.O., Larsson, C. and Eriksson, S. (1975) N. Engl. J. Med. 293, 576—579