AMINO ACID SUBSTITUTION Glu→Lys IN α_1-ANTITRYPSIN PiZ

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1. Introduction

α_1-Antitrypsin (α_1-AT) is the major protease inhibitor (Pi) in plasma. More than 20 alleles contribute to the genetic polymorphism [1]. Most alleles give normal α_1-AT concentration in plasma. The PiZ allele is consistent with reduced α_1-AT level in plasma and decreased electrophoretic mobility at pH 8.6.

An amino acid substitution, lack of sialic acid or loss of a complete carbohydrate chain have been proposed as explanation for the atypical electrophoretic mobility. We have earlier reported that the Z-protein contains 1–2 residues less sialic acid but almost normal content of the other carbohydrates [2]. Fingerprint and amino acid analysis of a corresponding CNBr-fragment of 63 amino acids from M and Z protein give clear evidence for an amino acid substitution, glutamic acid to lysine. The carbohydrates are attached to the same CNBr-fragment and it is still obscure how this amino acid substitution can interfere with glycosylation and completeness of the carbohydrate chain.

2. Materials and methods

α_1-Antitrypsin was prepared from individual blood donors through thiol–disulfide interchange method [3]. Sialic acid was removed by circulating the M and Z protein through a neuraminidase (Vibrio cholera, Behringwerke) conjugated Sepharose 4-B column for 24 h at room temperature. Cyanogen bromide degradation was performed according to Steers et al. [4]. Thin-layer electrofocusing in polyacrylamide was performed as described earlier [5]. Electrofocusing in flatbed with Sephadex G-75 was done as described by Radola [6]. In both cases the gels contain 6 M urea passed through a mixed resin Amberlite MB-1 just before use. The larger CNBr-fragments were separated on an Ultrogel ACA-54 (LKB-Sweden) column. Corresponding fragments C from both proteins were digested with thermolysin for 3 h at 55°C in 0.05 M NH_4HCO_3. Electrophoresis of the digest was carried out in pyridine–acetate buffer pH 3.6 on Whatman 3 MM paper. Chromatography was performed in the pyridine–isoamylalcohol system [7]. Amino acid analysis was performed on a JEOL 5AH amino acid analyser on material hydrolysed in 6 M hydrochloric acid at 110°C for 24 h in evacuated and sealed pyrex tubes.

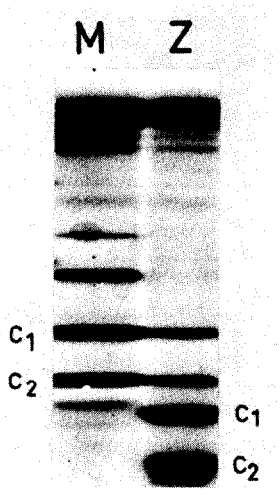

Fig.1. Thin-layer electrofocusing of CNBr fragments from α_1-antitrypsin PiM and PiZ. Ampholyte range pH 3.5–10, 6 M urea. Sialic acid is eliminated before CNBr degradation (Anode at the top).

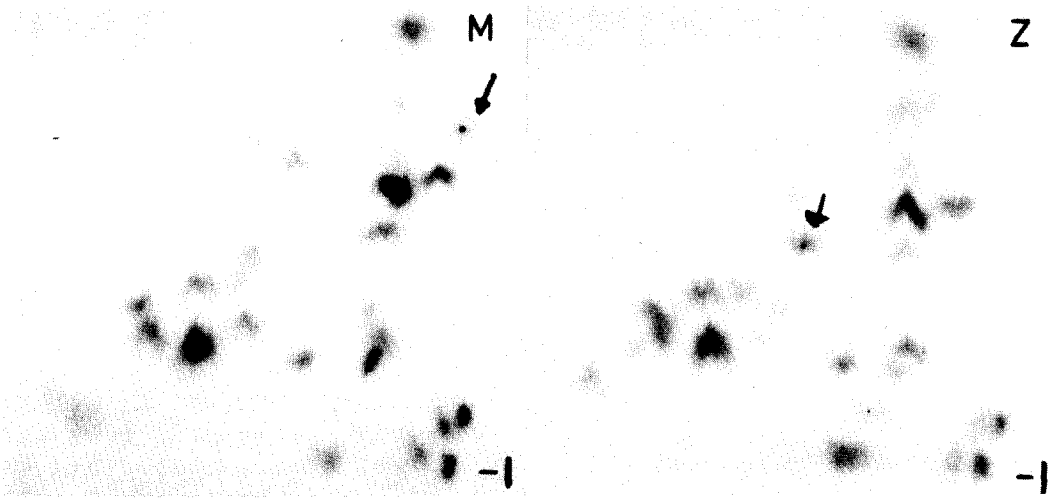

Fig.2. Fingerprints of thermolysin digest of CNBr-fragment C from α_1-antitrypsin PiM (left) and PiZ (right). The arrows indicate the substituted peptide.

Table 1
Amino acid composition of CNBr fragment C from α_1-AT PiM and Z[a]

Amino acid	Residues	M	Residues	Z
Lysine	5.1	(5)	6.1	(6)
Histidine	2.0	(2)	2.0	(2)
Arginine	0.8	(1)	0.8	(1)
Half-cystine	–	(0)	–	(0)
Aspartic acid	5.8	(6)	5.8	(6)
Threonine	4.3	(5)	4.2	(5)
Serine	3.8	(4)	3.8	(4)
Glutamic acid	6.0	(6)	5.2	(5)
Proline	1.8	(2)	2.1	(2)
Glycine	4.6	(5)	4.9	(5)
Alanine	5.6	(6)	5.6	(6)
Valine	2.7	(3)	2.8	(3)
Methionine (Homoserine)	0.6	(1)	0.6	(1)
Isoleucine	2.9	(3)	2.7	(3)
Leucine	9.8	(10)	9.6	(10)
Tyrosine	0.9	(1)	0.9	(1)
Phenylalanine	2.9	(3)	2.7	(3)
Tryptophan[b]	–	(0)	–	(0)
		63		63

[a] The values denote the average of three determinations on each fragment after 24 h hydrolysis, calculated relative to 2 residues of histidine.
[b] Determined spectrophotometrically.

3. Results and discussion

Complete removal of sialic acid after extensive neuraminidase treatment still gives different p*I* of the proteins suggesting an amino acid substitution [2]. We have therefore degraded the proteins with CNBr to search for differences in fragments between the normal M-protein and the deficient Z-protein. We have isolated eight fragments extending over a range in size from 4 amino acids to a glycopeptide of 12 000 daltons.

Fig.1 illustrates the pattern of larger CNBr-fragment separated on thin-layer polyacrylamide electrofocusing at a gradient of pH 3.5–10. Neuraminidase digestion has also been performed to eliminate the micro-heterogeneity caused by sialic acid [2]. The C fractions represent the third peak after void volume in a gel filtration run on Ultrogel ACA-54. The same elution profile is obtained on both CNBr-degraded M and Z-protein. The double banding of fragments is explained by the C-terminal amino acids. Both homoserine and homoserine lactone are obtained as a result of the CNBr degradation. Gel filtration followed by preparative electrofocusing with a gradient from pH 3.5–10 in 6 M urea give pure fragments with a single lysine as N-terminal amino acid. The isolated C_1 and C_2 fragments give identical amino acid composition. The fragments contain 63 amino acids and glucosamine.

Fig.2 shows fingerprints of C-peptides after thermolysine digestion. An acidic peptide in the M-fragment is more basic in the Z-fragment. Amino acid analysis of the fragments (table 1) shows significant difference only in content of glutamic acid and lysine indicating a substitution of glutamic acid or glutamin in M-protein to lysine in the Z-protein. The amino acid substitution for the S-protein is located in the same CNBr-fragment as indicated by the electrofocusing technique but has not been identified by us. Owen and Carrel have recently reported a glutamic acid to valine substitution for the S-protein [8]. They isolated the corresponding tryptic peptides from the complete protein directly from fingerprints. The electrofocusing pattern of C-fragments in our system gives clear evidence for a shift of two charge units for asialo-Z-protein and one charge unit for asialo-S-protein compared to asialo-M-protein. This fact together with the change in fingerprint pattern is in favour of a glutamic acid to lysine substitution for the Z-protein.

Acknowledgements

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