α-1-ANTITRYPSIN: SEQUENCE OF THE Z VARIANT TRYPTIC PEPTIDE

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1. Introduction

Some 4% of Northern Europeans are mixed heterozygotes for the normal (M) and the variant (Z) allele of the plasma protein α-1-antitrypsin [1]. The Z protein has a decreased electrophoretic mobility at pH 8.6 but is of particular medical interest because of its severely depressed levels and associated predisposition to lung damage. We report here the sequence of the variant tryptic peptide of the Z protein, the sequence being in agreement with the amino acid composition [2] and compatible with the substitution [3] noted by previous workers.

2. Methods

The normal, M, α-1-antitrypsin was isolated from individual donors by thiol-disulphide interchange [4] using a glutathione–Sepharose column. The final step was chromatography on DEAE–Sephadex A-50, at pH 8.6, using a linear sodium chloride gradient (0.1 M–0.4 M) in 0.1 M Tris–HCl buffer.

The variant, Z, protein was obtained from homozygous donors but because of the greatly decreased plasma concentration, a modified technique was used for its isolation. The plasma was first taken to 50% saturation with ammonium sulphate and the supernatant chromatographed on DEAE–Sephadex A-50 as described for the M, protein. The fractions containing antitrypsin were passed through a blue dextran–Sepharose column [5] to remove any remaining albumin and then applied to a glutathione–Sepharose column. Traces of haptoglobin were removed by gel filtration on Sephadex G-100. Purity was checked by immunoelectrophoresis of each preparation at a concentration of 10 mg/ml against polyvalent antisera and by agarose gel electrophoresis at pH 8.6. A final check was made by amino-terminal residue determinations [6].

The M and Z proteins were aminoethylated and then heated for one hour at 80°C in 0.05 M H_2SO_4 to denature the protein and remove sialic acids. Tryptic digestion was performed with trypsin, at pH 8.6, for 2 h at 37°C in 0.1 M NH_4HCO_3 with an enzyme to substrate ratio of 1:30. The pH 6.5 soluble peptides were chromatographed in 1% acetic acid on a column (1.6 × 70 cm) of Sephadex G-25 fine. A division of the fractions was made into two groups, those that eluted before, and those that eluted after, 1.5 void volumes. Peptide maps were prepared by simultaneous electrophoresis of the two corresponding groups of M and Z peptides, at pH 6.5, and then re-running neutral peptides, at pH 2.1. Marker amino acids and grid plots were used [7] to determine peptide mobility and hence correlate their molecular weight and charge. Ascending chromatography was carried out in the upper phase of isoamyl alcohol/pyridine/water (6:6:7). Amino acid analysis was performed on a Durrum analyser following hydrolysis of the peptide in 6 M HCl at 110°C for 18 h. Amino acid sequences were determined by the dansyl-Edman technique [8].

3. Results and discussion

The tryptic digest of the aminoethylated antitrypsin contained some 60 soluble peptides. The division of the peptides into two groups by gel filtration and the subsequent re-electrophoresis of their neutral peptides, greatly simplified interpretation.

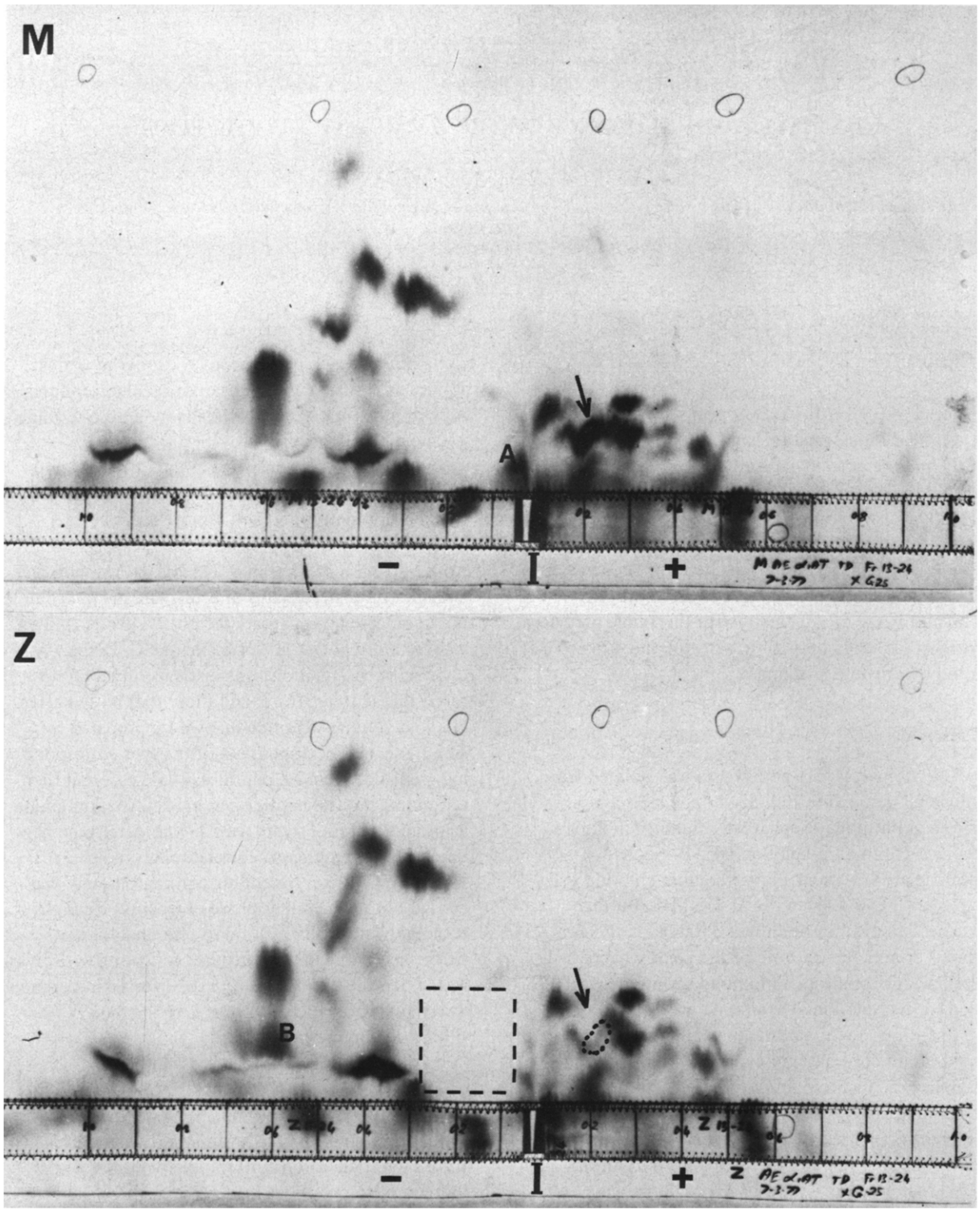

Fig.1

Four maps were obtained for each protein, each map having only 10–30 peptides. Careful comparisons were made of repeated maps of each of the M and Z fractions. The two sets of maps were identical except for one consistent difference noted in the maps of the high molecular weight fractions (fig.1). An acidic peptide present in the M map was absent from the Z map. This peptide was eluted, its sequence determined as in fig.2, and its composition confirmed by amino acid analysis. The results leave no doubt that the peptide is the same as that whose amino acid composition was determined by Yoshida et al. [2]. Not only is the composition the same, but the sole glutamic acid is in a position where its neighbouring aspartic acid residue would inhibit extra tryptic cleavage [9] on mutation of the glutamic acid to lysine, as observed by Yoshida et al. [2].

The puzzling feature is the apparent absence of the mutant peptide from the Z map. The position that this peptide should occupy on the map can be calculated with some certainty. However, it is not present in the calculated position nor is there an extra peptide present in any position on the map. It is tempting to consider this absence in terms of alternative mutations, but it is more likely to be due to one of those quirks that still leave some unpredictability in protein chemistry. It cannot be explained by the obvious considerations such as altered solubility or chymotryptic cleavage. A possible, though unlikely [10] explanation is that under the conditions used here for tryptic digestion, cleavage may have been inhibited at both the lysines of the new peptide by the preceding aspartic acid residue.

Otherwise, the peptide maps provide strong evidence for the common sequence of the M and Z proteins, further support for this being provided by our unpublished finding of identical amino acid composition in tryptic peptides representing some fifty residues. Our overall conclusion is that the Z protein, like the common S variant [11], is allelic with the M and is likely to have a primary abnormality explicable by the one amino acid substitution.

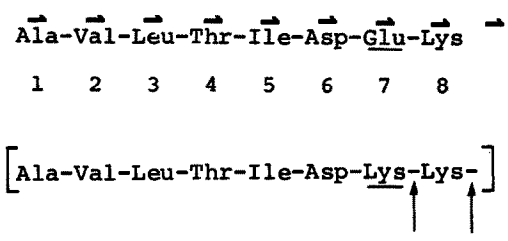

Fig.2. The amino acid sequence of the affected peptide, Dansyl-Edman steps indicated by (⟶). An extra step confirmed the lysine as the carboxy-terminal residue. From peptide mobility, residues 6 and 7 must be in the acid form. The corresponding predicted mutant peptide is shown in brackets. The major tryptic cleavage point would be after the second lysine with only minor splitting at the penultimate lysine. The mobilities of peptides resulting from either cleavage can be calculated but neither appears on the map of the Z protein (fig.1).

Acknowledgements

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Fig.1. Maps of acidic and basic tryptic peptides of the 'high molecular weight' group. The upper, M, antitrypsin, map shows the position of the acidic peptide (arrowed) missing in the Z map. The outlined box on the Z map is the position that would be occupied by the expected mutant peptide with a glutamate to lysine substitution (see fig.2). Other differences in the maps are due to technical variations as with B (chromatography artefact) or A (incomplete excision of neutral peptides).