α-1-ANTITRYPSIN: STRUCTURAL RELATIONSHIPS OF THE SUBSTITUTIONS OF THE S AND Z VARIANTS

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1. Introduction

A deficiency of the plasma protein α-1-antitrypsin causes a predisposition to lung disease. This is of particular importance to Northern Europeans some 10% of whom are mixed heterozygotes for the normal (M) and a deficient (Z or S) allele [1]. These alleles have been shown to be due to single amino acid substitutions: in the mildly deficient S protein of a glutamic acid by a valine [2] and in the severely deficient Z protein of a different glutamic acid by a lysine [3–5]. The Z defect is of particular interest as it apparently prevents secretion of the protein which consequently accumulates in the liver cell. Two mechanisms have been suggested [6]. The molecular lesion could prevent cleavage of a secretory propeptide or it could disrupt an attachment site of a carbohydrate side-chain essential for secretion. We report here further structural studies of the amino-terminus and mutation areas of the antitrypsin molecule, carried out to examine these proposals and alternative mechanisms that may be responsible for the deficiency associated with the Z and S variants.

2. Methods

α-1-Antitrypsin was isolated from human plasma as in [2]. Sequence of the amino-terminal portion was determined using liquid phase Edman degradation in an updated Beckman 890 B sequenator. PTH amino acids were identified by gas chromatography and by amino acid analysis of back-hydrolysed products. The 'C' cyanogen bromide fragment was prepared and isolated by isoelectric focussing as in [3], with the exception that prior desialylation and denaturation of the antitrypsin was carried out by heating at 80°C in 0.5 M H_2SO_4. Ampholines and urea were removed by gel filtration on Sephadex G-50.

Sequence determination of the C-fragment was carried out [2] using tryptic peptides and also peptides from digests with thermolysin, chymotrypsin or *Staphylococcus aureus* V8 protease. Enzyme to substrate ratios of 1:30 were used with digestion at pH 8.6 in 0.05 M NH_4HCO_3 buffer. The peptides were eluted from paper peptide maps as in [2,5,7]. Electrophoresis was carried out at pH 6.5 with, as appropriate, subsequent electrophoresis at pH 2.1. Marker amino acids and grid plots were used [8] to determine peptide mobility and hence correlate molecular weight and charge. Ascending chromatography was carried out in the upper phase of isoamyl alcohol/pyridine/water (6:6:7). Amides were assigned from peptide and degradation-peptide mobilities. Overlaps were determined using chymotryptic digests and partial acid hydrolysates (0.5 M acetic acid, 14 h at 110°C) of the whole fragment. The molecular weight of the C-fragment was determined by SDS–acrylamide gel electrophoresis. Glycopeptides were identified by their characteristic chromatographic and electrophoretic mobilities. Full details of all the procedures used will be published [9,10].

Secondary structure of the C-fragment was predicted using the computer program of A.D. McLachlan based on principles similar to those in [11].

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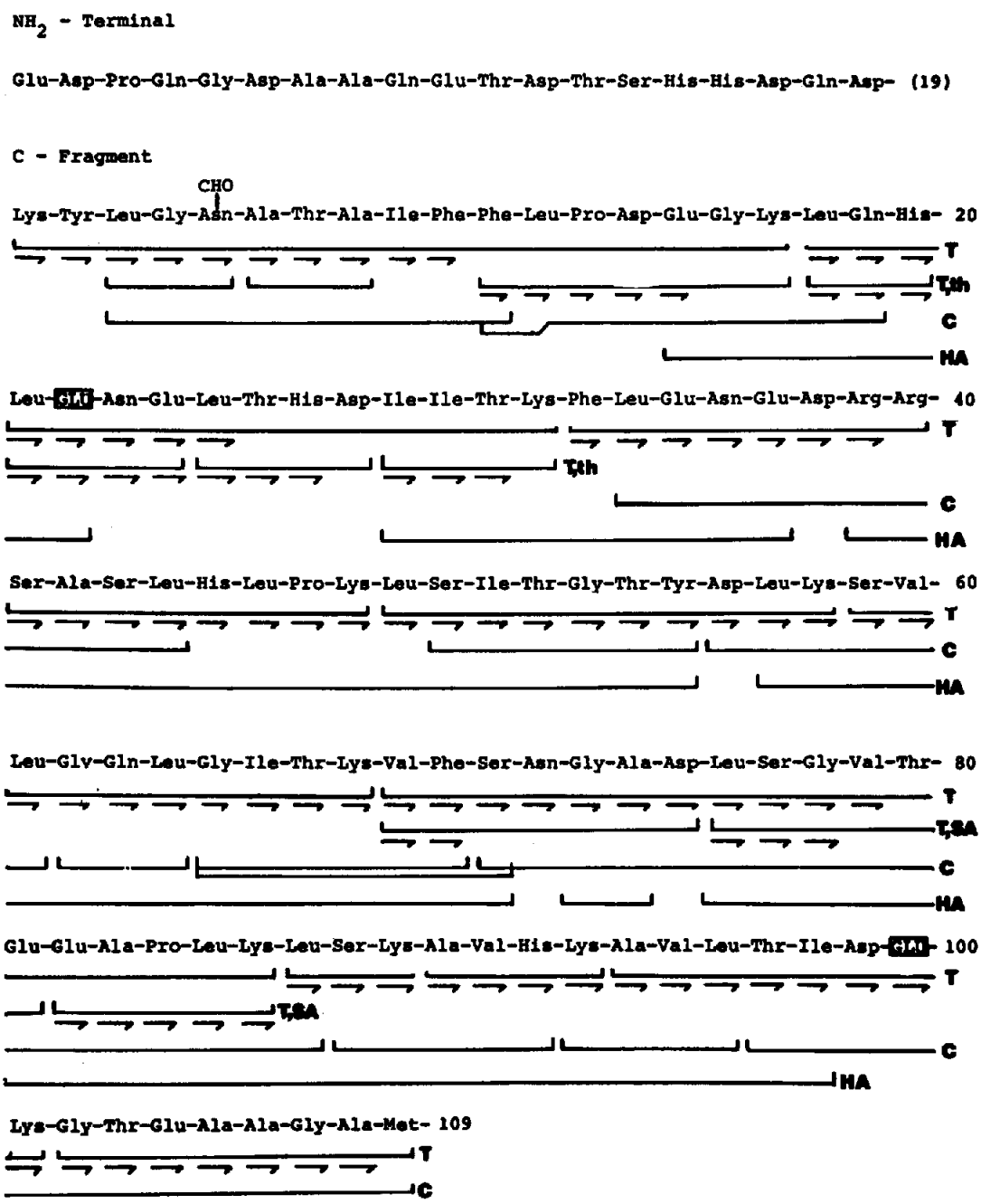

Fig.1. Sequence data, human M α-1-antitrypsin. Amino-terminal sequence by sequenator; cyanogen bromide C-fragment by peptide analysis with positions assigned by dansyl-Edman steps (——→) and enzyme specificity. T, trypsin; C, chymotrypsin; th, thermolysin; SA, *S. aureus* protease; HA, partial acid hydrolysis. The S and Z substitutions occur in the C-fragment: S, 22 Glu → Val; Z, 100 Glu → Lys. A carbohydrate side-chain is attached at Asn 5.

3. Results and discussion

The sequence data is summarised in fig.1. The 109 residue sequence of the C cyanogen bromide fragment is compatible with both the overall amino acid composition and the determined mol. wt 11 500 and is in agreement with the sequence independently obtained by S. K. Chan (personal communication). As predicted [3], the C-fragment contains both the S and Z mutation sites. The positions of the S and Z substitutions at residues 22 and 100, respectively, were placed from [2,5] and by independent mapping of the variants. The presence was confirmed of a carbohydrate linkage point at Asn 5 of the C-fragment, as in [12]. No other glycopeptide was detected.

The computer prediction of secondary structure in the C-fragment showed the strongest features to be helical regions from Pro 13–His 27 and from Glu 81–Leu 96, and β-sheets from Ala 6–Phe 11 and Leu 64–Phe 70. This places the point of attachment of the carbohydrate side chain as just prior to a β-sheet region and strongly indicates that the S mutation (22 Glu → Val) has occurred on the hydrophilic face of an α-helical section. This last conclusion supports the proposal [2] that the observed lability of the S protein is due to the introduction of a hydrophobic residue on an external aspect of the molecule which would allow disadvantageous aggregation.

Neither the S nor Z mutation is in a position that should directly affect attachment of the carbohydrate side-chain at residue 5. An alternative proposal for the inadequate secretion of the Z protein is that its mutation may be at the site of cleavage of a secretory propeptide [6] or prepropeptide. Any such uncleaved peptide would necessarily have to be small and situated at the amino-terminus since the Z protein has no detectable increase in molecular weight and has the same, single, amino-terminal residue (Glu) as the normal protein [9]. The Z mutation is at least 119 residues from the amino-terminus which excludes the possibility that it is directly involved in the cleavage site of an amino-terminal peptide. These results, then, indicate that the S and Z mutations do not, at the primary sequence level, affect carbohydrate side-chain attachment or amino-terminal secretory peptide cleavage. Whether the conclusion is also true at the tertiary level will have to await crystallographic studies

One possibility as to the defect in the Z protein is suggested from the primary sequence. This is the appearance in the Z variant of the sequence Asp–Lys–Lys (residue 99–101) analogous to the earlier Asp–Arg–Arg (residue 38–40). Double basic residues are known to act as a molecular marker for propeptide cleavage [13] but the explanation of the Z deficiency may lie in the wider role that is suspected for double basic residues, e.g., as markers for degradation cleavage [14].

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