Localization of the amino acid substitution site in a fast migrating variant of human serum albumin

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Albumin Mi/Fg is an Italian genetic variant of human serum albumin arising from a Lys → Glu substitution which has been located in a CNBr fragment (CNBr VII) corresponding to the -COOH terminal portion of the molecule [(1984) J Chromatogr 298, 336–344] Tryptic peptides of CNBr VII from normal and Mi/Fg albumin have been purified by reverse-phase high-performance liquid chromatography (RP-HPLC) and submitted to comparative structural studies The amino acid sequence of the tryptic peptide of Mi/Fg variant that differs from the corresponding fragment of the normal serum albumin shows that the Lys → Glu substitution responsible for this variant is located at postion 573 This region of the albumin molecule is involved in the binding of long chain fatty acids

Human serum albumin *Genetic variant* *Tryptic peptide* *HPLC* *Amino acid sequence* *Point mutation*

1. INTRODUCTION

Genetic variants of human serum albumin have been identified on the basis of their different electrophoretic mobility which can be either faster or slower than that of normal serum albumin [1,2]. Although a very large number of variants have been reported [1–3], little information has been obtained, so far, about the structural basis of their genetically determined variation. Only two variants have been characterized with respect to amino acid sequence: albumin Oliphant in which glutamic acid 570 is replaced by lysine [4], and albumin Mexico 2 that rises from replacement of aspartic acid 550 by glycine [5].

Albumin Mi/Fg is a fast variant which has been found in one homozygous and several heterozygous subjects within the same Italian family: it is similar in electrophoretic mobility to the Gent albumin, but different from the Naskapi fast variant which has never been found in Italy [6] We have previously shown [7] that a Lys→Glu substitution which occurs in the COOH terminal portion of the molecule (residues 549–585), is responsible for the Mi/Fg fast variant. The present results state that Lys 573 is replaced by glutamic acid.

2. EXPERIMENTAL

2.1. *Sample preparation*

Human normal serum albumin and Mi/Fg variant have been isolated from serum of a normal subject and, respectively, of a 15-year-old boy homozygous for Mi/Fg albumin, according to Winter et al. [4], and comparatively processed. Carboxymethylation, cyanogen bromide cleavage, HPLC separation of CNBr fragments and amino acid analysis of the two proteins have been carried out as in [7]. CNBr VII from normal and Mi/Fg albumins have been submitted to tryptic digestion, according to Swenson et al. [8]: after 16 h at room temperature, the digest was brought to pH 2 by addition of trifluoroacetic acid (TFA).

2.2. *LC apparatus*

Reverse-phase HPLC was developed on a Waters Associates (Milford, MA, USA) liquid chromatograph. The system consisted of two M6000 pumps, a U5K sample injector, a model 680

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automated gradient controller and a variable wavelength detector (Japan Spectroscopic Co., Tokyo). The separation was carried out on a μ-Bondapak C-18 column, 10 μm particle size, 30 cm × 3.9 mm (Waters Associates).

2.3. *Procedure*

Sample (200 μl), corresponding to 50 nmol tryptic digest, was separated on the μ-Bondapak C-18 column equilibrated with 0.05% aqueous trifluoroacetic acid (solvent A). Elution of peptides was achieved by use of a 60 min linear gradient from 0 to 50% of CH_3CN containing 0.05% TFA (solvent B) at a flow rate of 2 ml/min. All runs were performed at room temperature; individual peaks were collected manually and vacuum-dried. Peptides eluted under each peak have been identified by amino acid and N-terminus analyses, and are designated by arabic numerals starting from the N-terminal of the CNBr VII fragment. Amino acid analysis was performed according to Dévényi [9]; N-terminal amino acid was identified as the dansyl derivative [10]

3 RESULTS AND DISCUSSION

Amino acid analyses of CNBr fragments allowed us to state that the amino acid substitution responsible for Mi/Fg variant is located in CNBr VII, corresponding to residues 549–585 of the albumin sequence: amino acid composition reported in table 1 shows that CNBr VII from Mi/Fg variant contains a glutamyl instead of a lysyl residue.

The chromatographic patterns of tryptic peptides obtained from normal and Mi/Fg CNBr VII are given in figs 1,2. Comparison of the elution profiles shows the absence of $T_4 + T_5$ peptide in Mi/Fg CNBr VII tryptic digest. The $T_4 + T_5$ peptide obtained from normal albumin CNBr VII

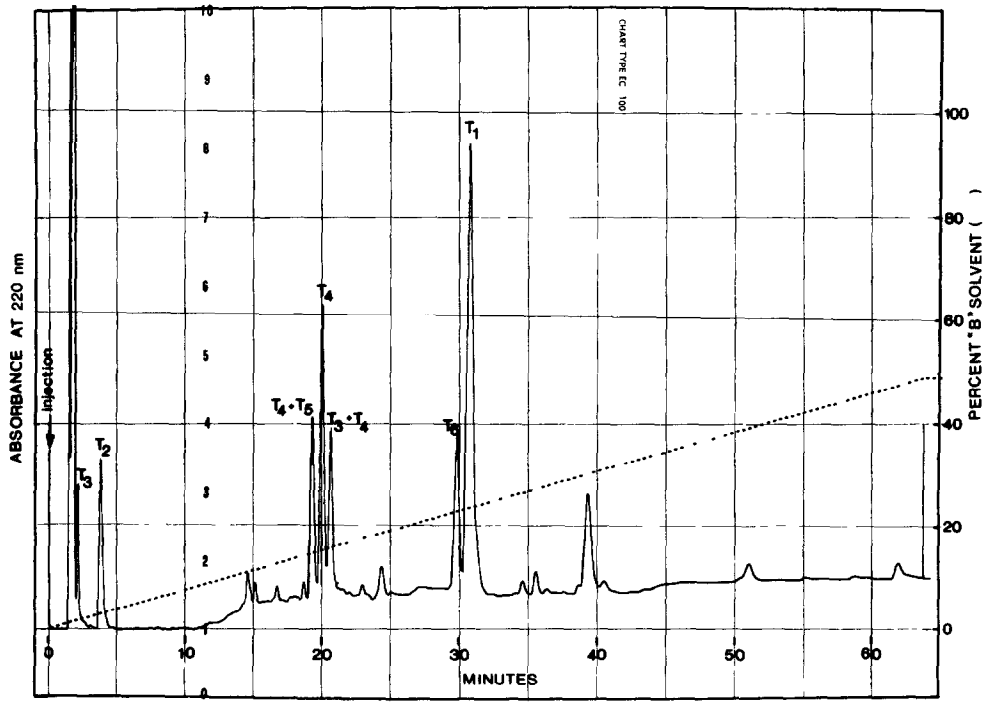

Fig.1. Elution pattern of tryptic digest of CNBr VII fragment obtained from normal human serum albumin The mixture was dissolved in 0.05% aqueous TFA, pH 2 (solvent A) and 200 μl (corresponding to about 50 nmol digest) was injected onto a μ-Bondapak C-18 column (30 cm × 3.9 mm), equilibrated with solvent A. The elution was performed at room temperature and a 60-min linear gradient from 0 to 50% acetonitrile containing 0.05% TFA (solvent B) was employed as indicated. Flow rate: 2 ml/min. Absorbance range: 0.64 full scale

Table 1

Amino acid composition of CNBr VII fragments isolated from normal and Mi/Fg albumins

Amino acid	Amino acyl residues[a]	
	Normal CNBr VII	Mi/Fg CNBr VII
Lys	4 95	3 95
Asp	4 02	4.06
Thr[b]	1.03	0.95
Ser[b]	1.05	1 09
Glu	5 01	6 03
Gly	2 05	2 08
Ala	7 81	7 79
Val[c]	2 03	1 97
Leu	3.03	2 99
Phe	2 81	2.88
Cys[d]	2 79	2 79
NH_2 terminus	Asp	Asp

[a] The values are the means of two independent determinations
[b] Corrected for destruction during acid hydrolysis
[c] Corrected for slow release during acid hydrolysis
[d] Determined as *S*-carboxymethylcysteine

Table 2

Amino acid composition of $T_4 + T_5$, T_4 and T_{4m}[a] fragments from normal and Mi/Fg albumins

Amino acid	Amino acyl residues[b]		
	Normal		Mi/Fg T_{4m}
	$T_4 + T_5$	T_4	
Lys	1 95	0.97	0 95
Thr[c]	0.94	0 95	0 97
Glu	3.00	3.01	3 98
Gly	1.08	1.08	1.07
Ala	1 01	1.00	1.02
Phe	1 02	1.01	1 02
Cys[d]	0.88	0.89	0.90
NH_2 terminus	Glu	Glu	Glu

[a] T_{4m}. modified peptide
[b] The values are the means of two independent determinations
[c] Corrected for destruction during acid hydrolysis
[d] Determined as *S*-carboxymethylcysteine

tryptic digest differs in the amino acid composition from T_4 peptide only in an additional lysyl residue and it obviously originated from a partial uncleavage of the Lys–Lys bond at Lys 573 (table 2).

All the peptides of Mi/Fg CNBr VII tryptic digest are eluted with the same retention times as the corresponding ones from the normal CNBr VII. However, amino acid analysis of T_{4m} reported in table 2 shows that the peptide contains the Lys→Glu substitution. These results, together with the lack of $T_4 + T_5$ in tryptic digest of Mi/Fg CNBr VII, support the suggestion that Mi/Fg albumin originates by replacement of Lys 573 by glutamic acid.

This conclusion has been unequivocally proved by sequential analysis: dansyl Edman degradation of T_{4m} carried out according to Gray [11] gives the following result which accounts for residues 565–574 of the albumin sequence in which Lys 573 is substituted by glutamic acid:

565 574
Glu-Thr-Cys-Phe-Ala-Glu-Glu-Gly-Glu-Lys

These findings allow us to conclude that Mi/Fg albumin, as Oliphant and Mexico 2 variants, originates from a point mutation in the structural gene: the 573 codon, AAA in normal albumin [12] must be changed in GAA, which encodes for glutamic acid. The Glu-Lys interchange found in Oliphant and Mi/Fg variants is also the most frequently occurring among the abnormal haemoglobins [13] which are the group of variant proteins thus far most thoroughly examined.

It is not known, at present, if a single amino acid replacement may modify the functional properties of albumin variants. However, it seems of interest to stress that all the amino acid substitutions so far characterized [4,5] occur in the same region of the molecule in which the high affinity binding site of long chain fatty acids is located [14]. Chemical modifications of aminoacyl residues such as Lys 525 [15] have been reported to affect considerably the affinity of albumin for some ligands; thus it seems likely that an amino acid replacement in this region may similarly produce some functional modifications in albumin variants.

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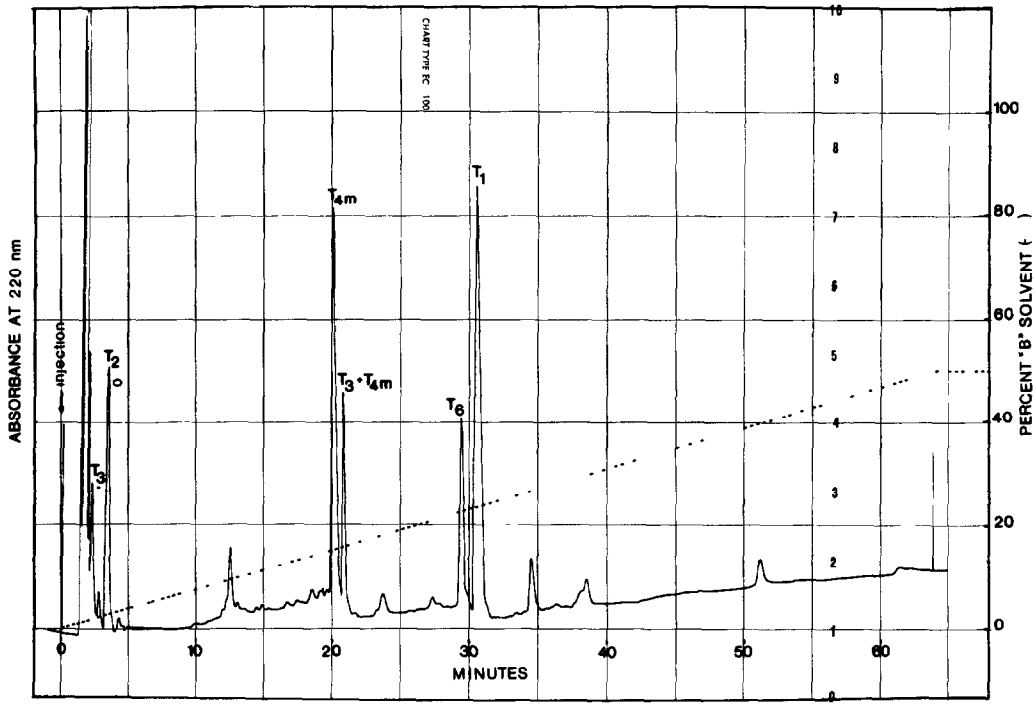

Fig.2. Elution pattern of tryptic digest of CNBr VII fragment from Mi/Fg variant obtained under the conditions used in fig.1

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