The molecular defect of albumin Tagliacozzo: 313 Lys→Asn

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Albumin Tagliacozzo is a fast-moving genetic variant of human serum albumin found in 19 unrelated families. The protein was isolated from the serum of a heterozygous healthy subject. Analysis of CNBr fragments by isoelectric focusing allowed us to localize the mutation to CNBr fragment IV (residues 299–329). This fragment was isolated on a preparative scale and subjected to tryptic digestion. Sequential analysis of the abnormal tryptic peptide, purified by RP-HPLC, revealed the variant was caused by 313 Lys→Asn substitution, probably due to a point mutation in the structural gene. The lack of a lysine residue accounts for the electrophoretic behavior of albumin Tagliacozzo.

(Human serum) Albumin Genetic variant Isoelectric focusing HPLC Amino acid sequence

1. INTRODUCTION

Over 20 genetic variants of human serum albumin have been identified, based on their differing electrophoretic mobilities [1] which can be either faster or slower than that of normal albumin. So far, the molecular abnormalities responsible for nine of these variants have been elucidated [2–10]. Here, we have investigated the molecular defect of albumin Tagliacozzo, a fast-running variant which has recently been found in 19 unrelated families in Abruzzo (Italy). As reported by Zepponi et al. [11] this variant possesses the same electrophoretic mobility as albumin Reading [12] when compared on cellulose acetate electrophoresis at pH 8.6.

The results reported here show that albumin Tagliacozzo arises from 313 Lys⟶Asn substitution.

2. MATERIALS AND METHODS

2.1. *Albumin purification*

Serum was collected from a young healthy male heterozygous for the variant, which accounted for 50% of the total albumin, and processed as described by Winter et al. [2]. Homogeneity of the protein was checked by gradient gel electrophoresis [13].

2.2. *Screening of CNBr fragments*

Whole reduced fast and normal albumins were carboxymethylated [14] and cleaved with CNBr [15]. CNBr fragments were analyzed by polyacrylamide gel isoelectric focusing performed in the presence of 8 M urea in the pH intervals 2.5–8 and 2.5–4 as described [8].

2.3. *Isolation of CNBr IV from albumin Tagliacozzo*

The unreduced protein was carboxyamidomethylated at Cys 34, cleaved with CNBr and the four fragments, named A–D according to McMenamy et al. [16], were separated by Sephadex G-100 gel filtration followed by ion-exchange chromatography [17]. Fragment A (residues 299–585) is composed of four subfragments (CNBr IV, V, VI, VII) held together by S–S bonds. CNBr IV was further purified, after reduction of disulfide bridges, by ion-exchange

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chromatography on a CM-cellulose column [16].

2.4. *HPLC separation of CNBr IV tryptic peptides*

About 75 nmol of CNBr IV fragment were submitted to tryptic digestion according to Swenson et al. [14]. The separation of tryptic peptides was performed at room temperature by RP-HPLC on a Waters Associated liquid chromatograph [6].

2.5. *Structural studies*

N-terminal amino acids were identified as dansyl derivatives [18]. Amino acid analyses were carried out by ion-exchange chromatography with post-column ninhydrin derivatization according to Moore [19] on a Kontron Cromakon 500 automatic analyzer. Automated sequence analysis of the modified peptide was performed in a Beckman 890 M spinning cup liquid phase sequenator using the fast-protein Quadrol program with precycled polybrene.

The PTH-amino acid derivatives were identified by RP-HPLC on a Beckman Ultrasphere ODS column according to Pucci et al. [20].

3. RESULTS AND DISCUSSION

Fast variant and normal albumins were assayed for homogeneity by gradient gel electrophoresis, N-terminal analysis and amino acid composition. The two proteins have an identical molecular mass, 66.5 kDa, and the same N-terminal residue, Asp, while comparative amino acid analyses failed to show any significative difference.

To localize the molecular abnormality to a specific region of the polypeptide chain, CNBr digests of the reduced and carboxymethylated albumins were compared by analytical gel isoelectric focusing in the pH interval 2.5–8. As shown in fig.1 all the CNBr fragments bar one behave identically: the band corresponding to CNBr IV from the variant focuses at a pH lower than the normal, in the proximity of CNBr VII. This finding suggests that the substitution site of the abnormal albumin is located between residues 299 and 329. The p*I* of the modified fragment, measured in the pH interval 2.5–4, differs from that of the normal one by about 0.25 pH units, thus suggesting, according to Ui [21], that a basic residue, a Lys, since CNBr IV contains no Arg or His, is substituted by a neutral residue.

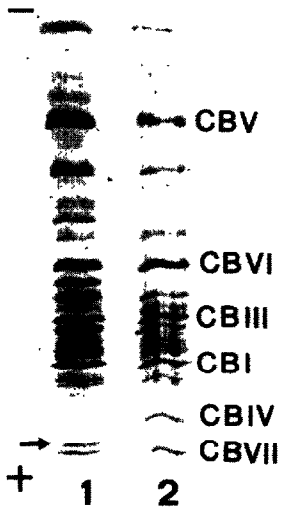

Fig.1. Resolution of alkylated CNBr fragments of albumin Tagliacozzo (lane 1) and normal albumin (lane 2) in the pH interval 2.5–8. CNBr fragments were originally identified by focusing under the same conditions each peptide purified by HPLC [25] and are numbered according to their order in the known sequence of human serum albumin [2]. Fragment CNBr II has not been identified, probably owing to its high solubility and low dye affinity. Overloading of the gel was necessary in order to detect smaller peptides. The arrow indicates the abnormal fragment.

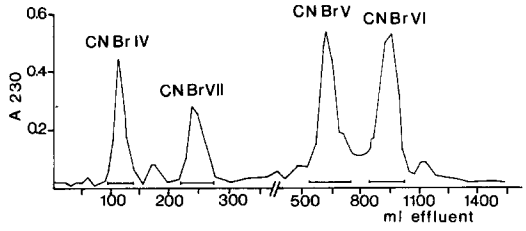

Fig.2. CM-cellulose ion-exchange chromatography of reduced fragment A from albumin Tagliacozzo. Peptides were eluted with a three-solution gradient: 0.033 M H_3PO_4/H_2PO_4 (1000 ml) against 0.01 M H_3PO_4/H_2PO_4, 0.10 M NaCl (1000 ml) against 0.10 M H_3PO_4/H_2PO_4, 0.3 M NaCl (1000 ml), pH 2.7, according to McMenamy et al. [16]. The identity of the peptides was confirmed by amino acid analysis.

Table 1

Amino acid composition of the abnormal CNBr fragments from albumin Tagliacozzo

	Fragment A		CNBr IV	
	Taglia-cozzo[a]	Normal[b]	Taglia-cozzo[a]	Normal[b]
Lys	29.9	31	2.0	3
His	6.1	6	–	–
Arg	10.2	10	–	–
Cys[c]	15.7	16	0.9	1
Asp	24.2	23	6.1	5
Thr[d]	15.9	16	–	–
Ser[d]	11.8	12	1.9	2
Glu	39.2	39	2.1	2
Pro	14.2	14	2.1	2
Gly	6.1	6	1.1	1
Ala	27.8	28	5.0	5
Val[e]	26.2	26	3.0	3
Met[f]	2.6	3	0.8	1
Ile[e]	3.0	3	–	–
Leu	30.3	30	3.1	3
Tyr	9.7	10	0.9	1
Phe	14.1	14	2.0	2
Trp	n.d.	–	n.d.	–

[a] Compositions are given in residues/mol. The values are the means of three independent determinations
[b] From sequence [22]
[c] Determined as *S*-carboxymethylcysteine
[d] Corrected for destruction during acid hydrolysis
[e] Corrected for slow release during acid hydrolysis
[f] Present as homoserine

n.d., not determined

To achieve the purification of CNBr IV from albumin Tagliacozzo on a preparative scale, fragment A was isolated from fragments B–D as reported in section 2. As expected, comparative amino acid analyses of the purified fragments allowed us to conclude that only fragment A is modified in the abnormal protein: its composition, reported in table 1, shows the presence of an additional Asp residue and the lack of a Lys residue.

Fragment A was then reduced, carboxyamidomethylated and its components were separated by ion-exchange chromatography (fig.2). CNBr fragments V, VI and VII have the same amino acid composition as the normal ones while CNBr IV showed the same difference detected in the whole fragment A (table 1).

Table 2

Amino acid composition and N-terminal residues of tryptic fragments of CNBr IV from variant Tagliacozzo[a]

	HPLC peaks (from fig.3)			
	1	2[c]	3[c]	4
Lys	0.99			0.98
CM-Cys				0.91
Asp	1.05	1.03	1.01	4.03
Ser				1.95
Glu	1.06			1.03
Pro				2.00
Gly		1.05	1.04	
Ala	2.02			3.07
Val		1.02	1.01	2.00
Leu		1.04	1.05	2.03
Tyr	0.88			
Phe		1.01	1.02	1.00
Homoserine		0.85	0.87	
N-terminal	Asx	Asx	Asx	Pro
Position in the sequence[b]	318–323	324–329	324–329	299–317

[a] Values are determined based on the calculated average of nmol/residue
[b] According to Dugaiczyk et al. [22]
[c] The presence of two peptides with the same amino acid compositions is probably due to the homoserine-homoserine lactone equilibrium

The modified fragment was then treated with trypsin and the digest was resolved by RP-HPLC (fig.3). Each peptide was characterized by amino acid and N-terminal analyses (table 2). Peak 4 shows an abnormal amino acid composition corresponding to the sequence 299–317 with the lack of the Lys 313 and the presence of an additional Asp residue. Sequential analysis of the abnormal peptide gives the following results: Pro-Ala-Asp-Leu-Pro-Ser-Leu-Ala-Ala-Asp-Phe-Val-Glu-Ser-Asn-Asp-Val-Cys-Lys thus demonstrating that albumin Tagliacozzo is due to a Lys⟶Asn replacement in position 313 of the sequence.

This lack of one positive charge is consistent with the higher anodic mobility of the native protein at pH 8.6 as well as with the observed isoelectric point of CNBr fragment IV.

The amino acid replacement found in albumin Tagliacozzo can be accounted for by a single base substitution in the structural gene: codon 313,

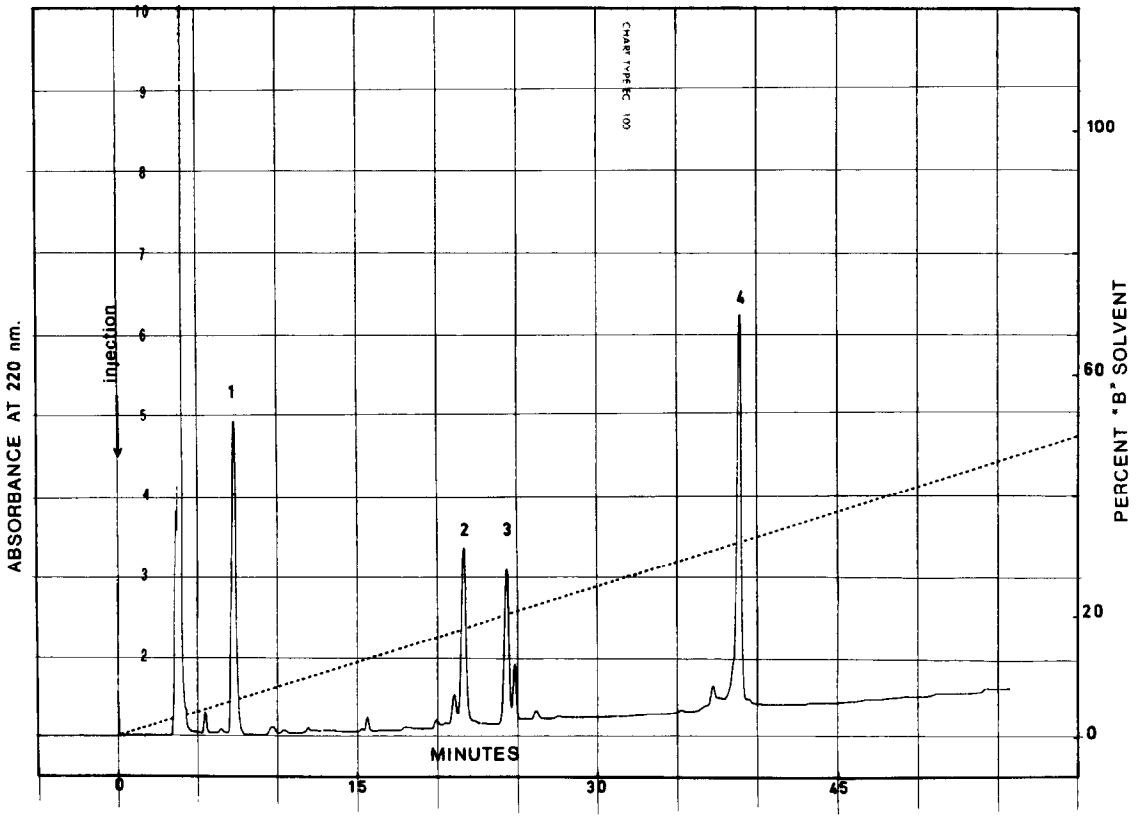

Fig.3. Reverse-phase high-performance liquid chromatography of the tryptic digest of CNBr IV from albumin Tagliacozzo. A 200 μl portion (15 nmol) of the tryptic digest was applied to an Aquapore RP-300 column (2.5 cm × 7 mm i.d.) obtained from Brownlee Labs equilibrated in 0.05% aqueous TFA (solvent A). Peptides were eluted with a 60 min linear gradient from equilibration buffer to 50% CH_3CN in 0.05% TFA (solvent B). The flow rate was 2 ml/min. Peptides were detected by their absorbance at 220 nm. The numbers above the peaks refer to the elution position. Each peak was submitted to amino acid and N-terminal analysis (table 2).

AAG in normal albumin [22], is probably changed in AAC or AAT both encoding for Asn. This kind of replacement, determined by a transversion, a purine being substituted by a pyrimidine, is one of the most frequently observed changes in hemoglobin variants, but Tagliacozzo is the first case among abnormal albumins.

Albumin Tagliacozzo has been reported to possess the same electrophoretic mobility first described by Tarnoky for albumin Reading [12], which was then found in several other variants with a wide geographical distribution: albumins New Guinea and Westcott, as reported by Weitkamp et al. [23], and albumin Cuneo, which has been found by Vacca et al. [24] in a family group in Piemonte.

Unfortunately, as the original Reading sample is still unavailable (Tarnoky, A.L., personal communication), it is impossible at present to state its structural relationship with albumin Tagliacozzo. The other sera are difficult to obtain and we processed only a small amount of albumin Cuneo in order to compare it with Tagliacozzo: their CNBr fragment patterns, analyzed by gel isoelectric focusing as described, are identical, thus suggesting that they arise from the same mutation.

In fact, as observed among other variant proteins, the same amino acid replacements may occur on multiple occasions: recently Putnam et al. [9] demonstrated that albumin Naskapi, found in the Naskapi Indians of Quebec, and albumin Mersin, found in Eti Turks of South Eastern Turkey, arise

from an identical amino acid change.

Thus it may be that some of these variants with the same electrophoretic mobility occurring in people of diverse origins possess identical structural properties.

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