Volume 233, number 1, 100–104 FEB 05911 June 1988

The amino acid substitution in albumin Roma: 321 Glu→Lys

M. Galliano, L. Minchiotti, P. Iadarola, G. Ferri, M.C. Zapponi and A.A. Castellani

Dipartimento di Biochimica, via Taramelli 3B, Università, 27100 Pavia, Italy

Received 5 April 1988

Albumin Roma is an electrophoretically slow moving genetic variant of human serum albumin found in 22 unrelated families. The protein was isolated from the serum of a healthy, heterozygous subject. Analysis of CNBr fragments by isoelectric focusing allowed us to localize the mutation to fragment CNBr IV (residues 299–329). This fragment was isolated on a preparative scale by RP-HPLC and subjected to tryptic digestion. Sequential analysis of two abnormal tryptic peptides, purified by RP-HPLC, revealed that the variant arises from the substitution of glutamic acid 321 by lysine. This amino acid replacement, probably resulting from a point mutation in the structural gene, causes a change in the net charge of +2 units which is in keeping with the decreased electrophoretic mobility of the native protein.

Human serum albumin; Genetic variant; Isoelectric focusing; HPLC; Amino acid sequence

1. INTRODUCTION

Human serum albumin polymorphism, known as bisalbuminemia, is an asymptomatic condition expressed with a frequency of about 0.0003 in individuals of Caucasian origin [1]. More than one hundred genetic variants had been detected during routine clinical electrophoresis and classified as slow or fast with respect to the electrophoretic behaviour of the normal protein [2–4]. The structural characterization of the mutants carried out in several laboratories has, so far, allowed identification of fourteen different amino acid substitutions within the albumin molecule and three in the prohexapeptide, which account for more than two dozen differently named alloalbumins [5–8]. An extensive clinical and genetic survey, carried out by the Italian Committee for Standardization of Hematology and Laboratory Methods (CISMEL), has brought up to fourteen electrophoretically different abnormal albumins of Italian origin to the light [9], four of which have been already characterized in our laboratory [6].

This paper reports the molecular defect of a variant named Roma [2], found in 22 unrelated families in Italy, which moves more slowly than normal albumin on cellulose acetate electrophoresis at pH 8.6 [9]. The results reported here show that albumin Roma is a new mutant arising from the substitution of glutamic acid 321 by lysine.

Correspondence address: M. Galliano, Dipartimento di Biochimica, via Taramelli 3B, 27100 Pavia, Italy

2. MATERIALS AND METHODS

2.1. *Albumin purification*

A serum sample, obtained from a healthy young man heterozygous for the variant, was supplied by Dr F. Porta (Ospedale di Circolo, Varese, Italy). Slow and normal albumins were separated on a DEAE-Sephadex column eluted with 0.12 M phosphate buffer, pH 5.75, as described in [6] and checked for purity by SDS-gel electrophoresis [10].

2.2. *Screening of CNBr fragments*

The whole reduced proteins were carboxymethylated as described by Swenson et al. [11] and cleaved with cyanogen bromide according to Gross [12]. The CNBr digests were compared by analytical isoelectric focusing performed on polyacrylamide gels as previously reported [13].

2.3. *Peptide purification*

CNBr fragments from albumin Roma were purified on a preparative scale by RP-HPLC on a Waters Associated liquid chromatograph according to the procedure described by Iadarola et al. [14]. The abnormal CNBr IV fragment was sub-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

jected to tryptic cleavage [6] and the digest was resolved by RP-HPLC on a μBondapak C-18 column (10 μm, 30 cm × 3.9 mm, i.d., Waters Assoc.) using the conditions described earlier [15]. Individual peaks were collected manually and vacuum dried.

2.4. *Structural studies*

Amino acid analyses were carried out by ion-exchange chromatography with post-column ninhydrin derivatization according to Moore [16] on a Kontron Chromakon 500 automatic analyzer. Manual dansyl-Edman degradation was performed according to the method of Gray [17]. The amino terminal dansylated amino acid residue was identified using the procedure of Woods and Wang [18] and the solvents described by Hartley [19].

3. RESULTS AND DISCUSSION

Normal and Roma albumins were purified by ion-exchange chromatography: the abnormal protein eluted earlier than the normal one in agreement with its electrophoretic mobility observed on cellulose acetate at pH 8.6. The two homogeneous proteins have the same N-terminal residue, Asp, and essentially an identical molecular mass, 66.5 kDa, while comparative amino acid analyses do not show any significant differences. The normal and variant albumins, after carboxymethylation and cleavage with CNBr, were compared by analytical gel isoelectric focusing in the pH range 2.5–8 (fig.1). The band corresponding to fragment CNBr IV of albumin Roma is not present in the normal position and appears to comigrate with one of the bands corresponding to fragment CNBr III, with a change in isoionic point of about 0.4 pH units. This finding suggests that the mutation is localized in the region between residues 299 and 329 and consists of a double charge substitution, an acidic residue being replaced by a basic one.

Purification of the modified CNBr IV fragment on a preparative scale was achieved by RP-HPLC: comparing the elution profile reported in fig.2 with that obtained from normal albumin in the same experimental conditions [14], only CNBr IV behaves differently, showing a decreased retention time of 2 min with respect to the corresponding normal fragment. N-terminal and amino acid analyses were performed on all the purified CNBr fragments and, as expected, the amino acid compositions of all peptides, except CNBr IV, were identical to the corresponding ones from the normal protein. The amino acid analysis of CNBr IV, reported in table 1, shows the presence of an additional lysine and the lack of a glutamic acid residue, in agreement with the increased isoelectric point of the homogeneous modified fragment (4.75 for the homoserine C-terminal form, corresponding to a ΔpI of about +0.4 pH units). A

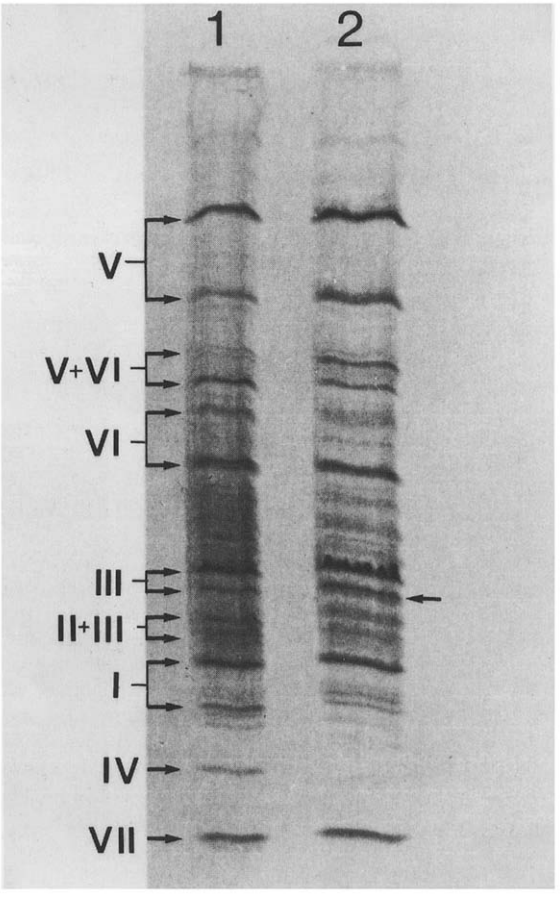

Fig.1. Isoelectric focusing of CNBr fragments from normal (lane 1) and Roma (lane 2) carboxymethylated albumins. Peptides were resolved in the pH range 2.5–8 in the presence of 8 M urea. CNBr fragments were originally identified by focusing each peptide purified by HPLC under the same conditions [14] and are numbered according to their order in the known sequence of human serum albumin [20]. Fragment CNBr II has not been identified, probably owing to its high solubility and low dye affinity. Each fragment, except the C-terminal CNBr VII, may have two charge forms, owing to the homoserine-homoserine lactone equilibrium. Microheterogeneity, mostly in the case of larger fragments, is probably due either to partial cleavage or oxidation of unreacted cysteines or deamidation. The arrow on the right marks the abnormal CNBr IV fragment: its position has been confirmed by focusing the homogeneous fragment in the same conditions.

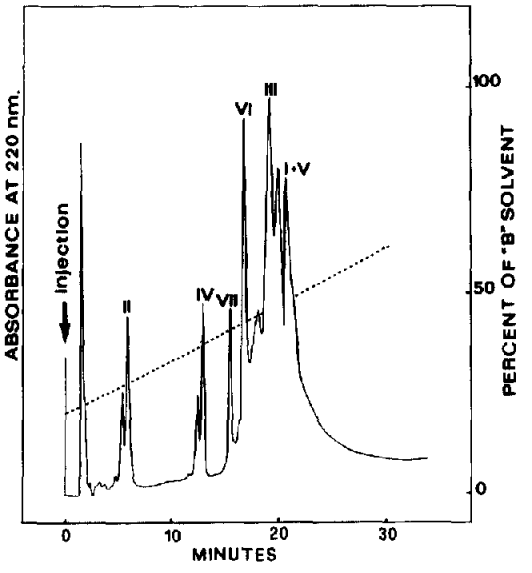

Fig.2. RP-HPLC elution profile of CNBr fragments from carboxymethylated Roma albumin. The digest (120 nmol) was dissolved in 0.05% aqueous trifluoroacetic acid (solvent A) and 100 μl were injected into a Vydac C-18 column equilibrated with 80% solvent A and 20% acetonitrile-2-propanol (2:1, v/v) containing 0.05% trifluoroacetic acid (solvent B). The elution was performed at room temperature, using a 30 min linear gradient from 20% to 60% of solvent B. Flow rate, 2 ml/min; absorbance range, 1.28 full scale.

Glu ⟶ Lys substitution also accounts for the HPLC behaviour of the abnormal CNBr IV: in fact, this kind of replacement, found in another genetic variant of human serum albumin named Verona (570 Glu ⟶ Lys), decreases the retention time of the modified CNBr fragment to the same extent [6].

The purified CNBr IV fragment from albumin Roma was then cleaved with trypsin and the digest was resolved by RP-HPLC (fig.3). Each of the six peaks obtained was characterized by N-terminal and amino acid analyses. The results, reported in table 2, account for the whole amino acid content of the modified CNBr IV. On the basis of their amino acid composition and N-terminal residues peptides 2 and 6 fit the normal sequence between residues 299 and 317 and peptides 4 and 5 correspond to the sequence 324–329. Fragments 1 and 3, which revealed an abnormal amino acid content, were submitted to manual dansyl-Edman degradation. Fragment 3 shows the sequence Asx-Tyr-Ala-Lys corresponding to residues 318–321 except for

Table 1

Amino acid composition of CNBr IV fragments from normal and Roma albumins

Amino acid	Normal CNBr IV[a]	Roma CNBr IV[b]
Lys	3	4.05
Cys	1	0.91[c]
Asp	5	5.09
Ser	2	1.97
Glu	2	1.08
Pro	2	2.02
Gly	1	1.07
Ala	5	5.03
Val	3	2.99
Met	1	0.85[d]
Leu	3	3.04
Tyr	1	0.90
Phe	2	2.00

[a] From sequence in [20]
[b] Compositions are given in residues/mol. The values are the mean of three independent determinations
[c] Determined as *S*-carboxymethyl-cysteine
[d] Present as homoserine

the substitution of glutamic acid 321 by lysine. Fragment 1 is a dipeptide, Ala-Lys, corresponding to sequence 322–323, which arises from the tryptic cleavage occurring at lysine 321.

The reported data state that albumin Roma is a new variant arising from a 321 Glu ⟶ Lys substitution. Although no further sequence analysis was undertaken, the finding that only CNBr IV behaves differently, when considering isoelectric focusing patterns, HPLC retention times and amino acid compositions of the CNBr fragments, rules out the possibility that additional substitutions may occur in other regions of the molecule. The Glu ⟶ Lys mutation of albumin Roma corresponds to a single base alteration in the structural gene: codon 321, GAG in normal albumin [20], is probably changed in AAG encoding for lysine. This change causes a double charge substitution which is in keeping with the decreased mobility of the native protein at pH 8.6. However, the same amino acid replacement located in a different region of the molecule causes albumin Verona (570 Glu ⟶ Lys) to migrate more slowly than albumin Roma when compared in the same electrophoretic conditions [21]. This different electrophoretic behaviour may reflect several factors, such as differences in the pK_a values, degree to which the modified residues are

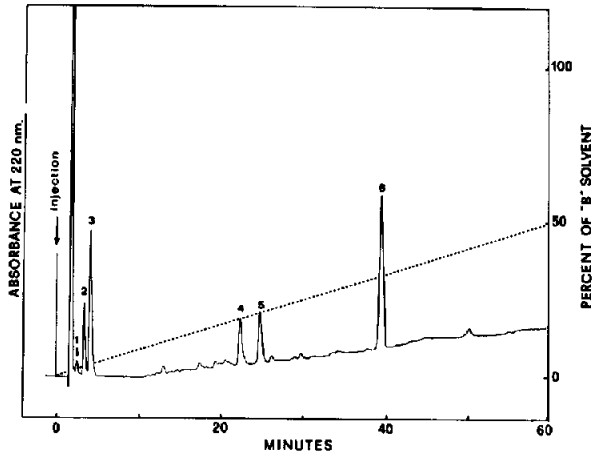

Fig.3. RP-HPLC elution profile of the tryptic digest of CNBr IV fragment from albumin Roma. A 200 μl portion (10 nmol) of the digest was applied to a μ-Bondapak C-18 column equilibrated in 0.05% aqueous trifluoroacetic acid (solvent A). Peptides were eluted with a 60 min linear gradient from equilibration buffer to 50% acetonitrile containing 0.05% trifluoroacetic acid (solvent B). Flow rate, 2 ml/min; absorbance range, 0.32 full scale. Peptides were detected by their absorbance at 220 nm. The numbers above the peaks refer to the elution position. Each peak was subjected to amino acid and N-terminal analyses (table 2).

exposed to the solvent and conformational changes due to the substitution. According to the theoretical model of the albumin molecule proposed by Brown and Shockley [22], glutamic acid 321 is located five residues away from the beginning of helix X of subdomain 2 C, in the outside portion of the trough-like structure of domain 2, while glutamic acid 570 is in the outer surface of helix 3 C Z, corresponding to the C-terminal end of the molecule: this region, which is free from the restrictions imposed by the disulfide bridges, probably allows for more flexibility and is less shielded from the solvent. Such an exposed position of the C-terminal end of the albumin molecule is in agreement with the fact that also in the fast variants characterized so far a charge substitution in this region has a greater effect on the electrophoretic mobility: albumin Mi/Fg (573 Lys → Glu) shows a faster anodic mobility with respect to albumin Naskapi (372 Lys → Glu) and Maku (541 Lys → Glu) [5,21], also albumin Vanves (574 Lys → Asn) moves faster than albumin Tagliacozzo (313 Lys → Asn) [6].

As in the case of other genetic variants, no clinical symptoms seem to be associated with the

Table 2

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

Amino acid	HPLC peaks (from fig.3)					
	1	2	3	4[c]	5[c]	6
Lys	1.01	1.02	1.03			1.01
CM-Cys		0.92				
Asp		1.02	1.03	1.03	1.01	2.02
Ser						1.97
Glu						1.03
Pro						2.00
Gly				1.06	1.04	
Ala	0.99		1.01			2.97
Val		1.04		1.03	1.04	0.99
Leu				1.03	1.03	2.03
Tyr			0.93			
Phe				1.00	1.01	0.98
Homoserine				0.85	0.87	
N-terminal	Ala	Asx	Asx	Asx	Asx	Pro
Position in the sequence[b]	322–323	314–317	318–321	324–329	324–329	299–313

[a] The values are determined based on a calculated average nmol/residue
[b] According to Minghetti [20]
[c] The presence of two peptides with the same amino acid composition is due to the homoserine-homoserine lactone equilibrium

presence of albumin Roma in all the observed subjects. Although subdomain 2 C is thought to be the primary steroid-binding site and also a binding site for long chain fatty acids, glutamic acid 321 is not directly involved in these bindings [22]; thus, it seems likely that the 321 Glu ⟶ Lys substitution, which is conservative with respect to the hydrophilicity parameters [23], does not affect the transport functions of albumin Roma.

Acknowledgements: This work was supported by grants from the Ministero della Pubblica Istruzione (Rome, Italy) and from Regione Lombardia (Milano, Italy). The authors wish to thank Dr K. Dyne for critically reading the manuscript and Mr A. Mortara for skilful technical assistance.

REFERENCES

[1] Tàrnoky, A.L. (1980) Adv. Clin. Chem. 21, 101–146.
[2] Weitkamp, L.R., Salzano, F.M., Neel, J.V., Porta, F., Geerdink, R.A. and Tarnoky, A.L. (1973) Ann. Hum. Genet. 36, 381–392.
[3] Weitkamp, L.R., McDermid, E.M., Neel, J.V., Fine, J.M., Petrini, C., Bonazzi, L., Ortali, V., Porta, F., Tanis, R., Harris, D.J., Peters, T., Ruffini, G. and Johnson, E. (1973) Ann. Hum. Genet. 37, 219–226.
[4] Schell, L.M. and Blumberg, B.S. (1977) in: Albumin Structure, Function, and Uses (Rosenoer, V.M. et al. eds) pp.113–141, Pergamon, New York.
[5] Takahashi, M., Takahashi, Y., Isobe, T., Putnam, F.W., Fujita, M., Satoh, C. and Neel, J.V. (1987) Proc. Natl. Acad. Sci. USA 84, 8001–8005.
[6] Minchiotti, L., Galliano, M., Iadarola, P., Stoppini, M., Ferri, G. and Castellani, A.A. (1987) Biochim. Biophys. Acta 916, 411–418.
[7] Hutchinson, D.W. and Matejtschuk, P. (1985) FEBS Lett. 193, 211–212.
[8] Sugita, O., Endo, N., Yamada, T., Yakata, M. and Odani, S. (1987) Clin. Chim. Acta 164, 251–259.
[9] Porta, F., Galliano, M., Warnsing, P., Galvani, G., Rossi, A. and Salvatore, S. (1988) Progr. Med. Lab., in press.
[10] Weber, K. and Osborne, M. (1969) J. Biol. Chem. 244, 4406–4412.
[11] Swenson, R.P., Williams, C.H., jr, Massey, V., Ronchi, S., Minchiotti, L., Galliano, M. and Curti, B. (1982) J. Biol. Chem. 257, 8817–8823.
[12] Gross, E. (1967) Methods Enzymol. 11, 238–255.
[13] Galliano, M., Minchiotti, L., Iadarola, P., Zapponi, M.C., Ferri, G. and Castellani, A.A. (1986) J. Biol. Chem. 261, 4283–4287.
[14] Iadarola, P., Ferri, G., Galliano, M., Minchiotti, L. and Zapponi, M.C. (1984) J. Chromatogr. 298, 336–344.
[15] Iadarola, P., Minchiotti, L. and Galliano, M. (1985) FEBS Lett. 180, 85–88.
[16] Moore, S. (1968) J. Biol. Chem. 243, 6281–6283.
[17] Gray, W.R. (1972) Methods Enzymol. 25, 333–344.
[18] Woods, K.R. and Wang, K.T. (1967) Biochim. Biophys. Acta 133, 369–370.
[19] Hartley, B.S. (1970) Biochem. J. 119, 805–822.
[20] Minghetti, P.P., Ruffner, D.E., Kuang, W.J., Dennison, O.E., Hawkins, J.W., Beattie, W.G. and Dugaiczyk, A. (1986) J. Biol. Chem. 261, 6747–6757.
[21] Lippi, U., Cappelletti, P., Signori, D., Cattapan, G., Calabrese, M., Graziani, M.S., Pattacini, L., Burlina, A. and Porta, F. (1983) J. Res. Lab. Med. 10, 281–288.
[22] Brown, J.R. and Shockley, P. (1982) in: Lipid-Protein Interactions (Jost, P.C. and Griffith, O.H. eds) vol.1, pp.26–68, John Wiley and Sons, New York.
[23] Hoop, T.P. and Woods, K.R. (1981) Proc. Natl. Acad. Sci. USA 78, 3824–3828.