LOCATION OF THE ALBUMIN GAINESVILLE MUTATION IN THE N-TERMINAL QUARTER OF THE ALBUMIN MOLECULE

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1. Introduction

The nature and localization of the mutations responsible for the electrophoretic variants of human serumalbumin (HSA) have not been established so far, except in the case of the slow albumin B which is the most frequent variant among Europeans. It is due to a substitution of glutamic acid by lysine near the C-terminal of HSA at position 569 [1] in the numbering system of Behrens et al. [2].

Albumin Gainesville is another slow-variant of HSA which is very rare and has been described by Lau et al. [3], in a family of Irish descent. One identical case has been found in France and reported in [4]. Normal and slow albumins have been isolated from the serum of an heterozygote member of the French family. Both albumins have been degraded by cyanogen bromide. Study of the electrophoretic mobility of the fragments so obtained shows that the mutation responsible for the slow albumin is located in the N-terminal quarter of the albumin molecule.

2. Materials and methods

Serum was collected from a 73 year-old woman (Y) having a bisalbuminaemia. Identity between this case and the albumin Gainesville has been established by Weitkamp et al. [4].

Slow variant and normal albumins from the serum of Y were separated as described by Winter et al. [5] with some modifications. Four ml serum were dialyzed during 48 h against sodium phosphate buffer 0.15 M, pH 5.75 and chromatographied on a (1 × 90 cm) column of DEAE–Sephadex. Elution was carried out with the same buffer. The appropriate peaks were pooled, dialyzed against distilled water and lyophilized.

Serum Y, isolated slow variant and normal albumins were assayed by electrophoresis on cellulose acetate with Sebia apparatus in veronal sodium buffer 0.04 M, pH 9.2. Two μl serum diluted 1/4 in buffer and 2 μl albumin solution (20 mg/ml buffer) were used. The strips were stained with Ponceau S.

Slow variant and normal albumins from serum Y were degraded by cyanogen bromide and the fragments were separated by filtration on Sephadex G-100 as described by Meloun and Kusnir [6]. Fragment nomenclature is that of McMenamy et al. [7]. Fragment A was further purified and chromatographied on CM-cellulose CM-32 as described by Lapresle and Doyen [8].

Fragment A and mixture of fragments B and C were assayed by electrophoresis in polyacrylamide–agarose gel as described by Uriel [9] with plates from Industrie Biologique Française (Indubiose plates) in Tris–glycine buffer, pH 8.7. Ten μl solution of fragment A (20 mg/ml buffer) and 10 μl mixture of fragments B and C (40 mg/ml buffer) were used. The peptides were stained with Coomassie Blue.

3. Results and discussion

The DEAE–Sephadex chromatography of serum Y is shown in fig.1. Pooled and lyophilized slow variant and normal albumins were tested for purity by

Abbreviation: HSA, human serumalbumin

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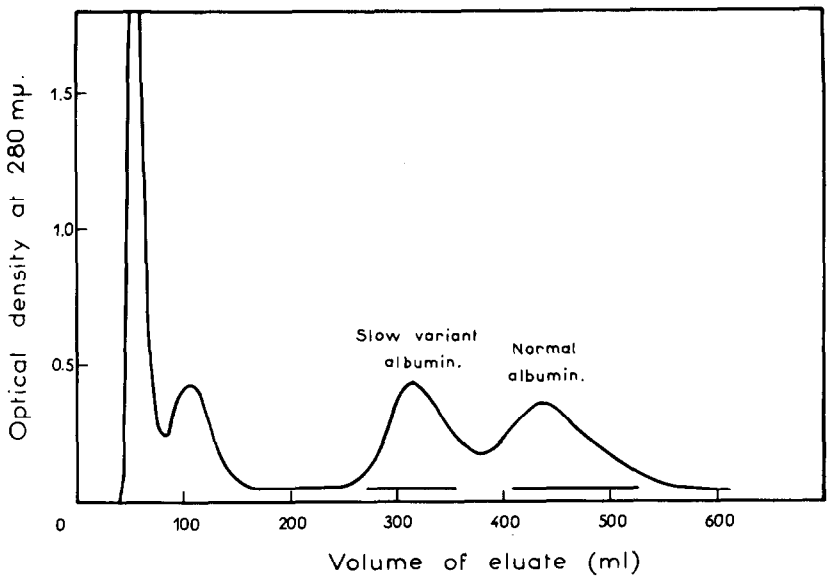

Fig.1. Chromatographic separation on DEAE–Sephadex of the slow-variant and normal albumins from the serum Y. Horizontal bars indicate regions that were pooled.

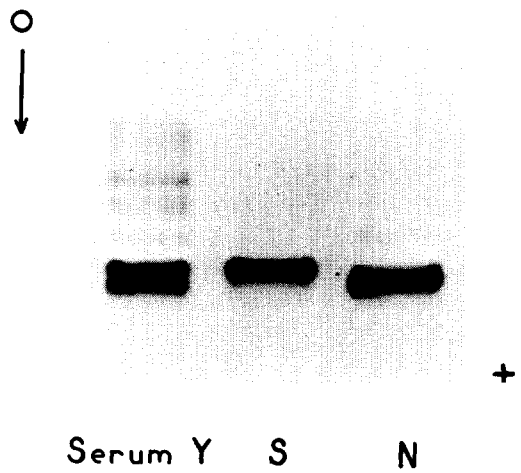

Fig.2. Cellulose acetate electrophoresis of serum Y and of the slow (S) and normal (N) albumins isolated from this serum. 14 V/cm, duration 75 min.

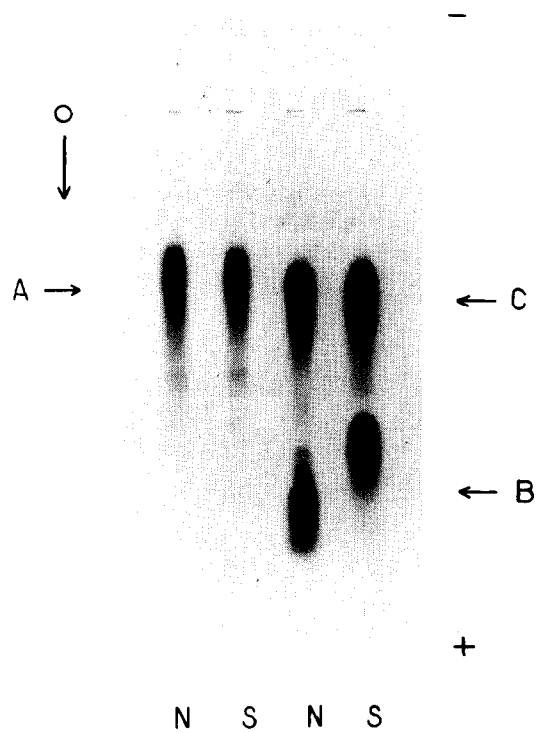

Fig.3. Electrophoresis in polyacrylamide–agarose gel of fragments A,B,C of normal (N) and slow (S) albumins isolated from serum Y. 10 V/cm, duration 3 h.

electrophoresis in cellulose acetate. Figure 2 shows that there is a very slight contamination of the normal albumin by the slow variant.

Degradation of human serum albumin by cyanogen bromide gives rise to three fragments which have been named by McMenamy et al. [7] B, C and A, from the N- to the C-terminal end of albumin molecule. Fragment A, which corresponds approximately to one half of the albumin molecule, is separated by gel-filtration on Sephadex G-100 from fragments B and C corresponding to approximately one quarter of the albumin molecule, and elute together.

Degradation by cyanogen bromide of slow-variant and normal albumins from serum Y gives rise in both cases to the three fragments A, B and C. Figure 3 shows the electrophoretic pattern in polyacrylamide–agarose gel of fragment A and the mixture of fragments B and C. Fragments A and C from the slow and normal albumins have the same mobilities whereas fragment B from the slow albumin has a slower mobility than fragment B from normal albumin. This demonstrates that the mutation responsible for the Gainesville albumin variant is located in the part of the albumin molecule corresponding to fragment B with the exception of the C-terminal methionine, i.e., in the first 122 residues of albumin, according to the sequence of Behrens et al. [2] or Meloun et al. [10].

Electrophoretic study of cyanogen bromide fragments of isolated variant and normal albumins from bisalbuminaemia can thus provide a first indication concerning the localization of the mutation.

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