PROALBUMIN LILLE, A NEW VARIANT OF HUMAN SERUM ALBUMIN

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1. Introduction

Genetic variants of human serum albumin were first described in [1] as bisalbuminemia, i.e., the presence of two serum albumin components on electrophoresis of the human serum at alkaline pH; one of the two components has the same mobility as normal serum albumin, the other, corresponding to the variant, has a mobility faster or slower than the normal component. Although a very large number of variants have already been described, their classification has been mainly established from criteria of electrophoretic mobility in different systems [2–5] and little information has been obtained about their structural abnormality. At present, the structures of only two variants have been identified: the first is the one of albumin B (albumin Oliphant [6]) in which glutamic acid in position 570 (numbering of [7]) is replaced by a lysine residue; the second is proalbumin Christchurch [8,9], which is a circulating proalbumin variant containing an abnormal propeptide sequence: Arg–Gly–Val–Phe–Arg–Gln, instead of: Arg–Gly–Val–Phe–Arg–Arg. This abnormal sequence is not cleaved off in the hepatocyte as is the normal propeptide. We report here the structure of a new variant of serum albumin characterized by an abnormal propeptide: Arg–Gly–Val–Phe–His–Arg, that appears not cleaved in vivo, but is easily cleaved in vitro by incubation with trypsin.

2. Materials and methods

Electrophoresis of the serum samples were performed on Cellogel (Sebia, Issy les Moulineaux) strips (5.7 × 14 cm) in 0.01 M veronal 0.05 M Na-veronal–0.06 M Tris buffer (pH 8.6) and in 0.031 M Na-acetate, 0.004 M EDTA (pH 5.0).

Isolation of the variant and normal serum albumins was performed by preparative electrophoresis on cellogel RS strips (5 × 24 cm) as follows: 1 ml serum was mixed with 0.05 ml solution of 0.5% bromophenol blue in electrophoresis buffer, and 0.2 ml were applied to the cellogel strip equilibrated in veronal–Tris buffer (pH 8.6). After electrophoresis (5 h at 300 V) the bands of normal and variant serum albumins, already stained with bromophenol blue, were cut off and eluted with distilled water. The eluates from 8–10 different electrophoresis runs were pooled, desalted on Sephadex G-25-fine equilibrated in 0.01 M ammonium carbonate buffer (pH 8.4) and freeze-dried. Controls of purity were performed by electrophoresis on cellogel at pH 8.6 and by SDS–polyacrylamide gel electrophoresis according to [10].

Treatment of the serum with trypsin was performed by adding 0.05 ml of a 0.1% solution of TPCK-trypsin (Worthington Biochemicals) in 0.1 M ammonium carbonate buffer (pH 8.0) to 0.1 ml serum. After incubation for 2 h at 37°C, the serum was directly examined by electrophoresis on Cellogel at pH 8.6. Incubation of the isolated variant with trypsin was achieved as for serum using a 20 mg/ml solution of variant in 0.1 M ammonium carbonate buffer and a ratio of trypsin to protein of 0.2% (w/w).

N-Terminal amino acid determinations were performed on the normal and variant albumins by dansylation in SDS according to [11]. Dansyl amino acids were identified by bidimensional chromatography on silica-gel thin layers according to [12].

N-Terminal amino acid sequence of the isolated variant (7 mg) was determined by automatic Edman degradation in a Beckman 890C Protein Sequenator using 1 M quadrol buffer program. Phenylthiohydantoin derivatives were identified by high-pressure liquid

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chromatography on C18 micro-Bondapack (Waters Associates) [13] and by gas-chromatography [14].

3. Results and discussion

Bis-albuminemia was discovered in a subject originating from the centre of France and family studies showed that the abnormality was inherited (Salmon, personal communication). No clinical manifestation was found associated with the presence of bis-albuminemia. Electrophoresis at pH 8.6 showed that the variant had a slower mobility than normal albumin (fig.1A), and that almost equal amounts of normal and variant albumins were present. Electrophoresis at pH 5.0 (fig.1B) showed that the abnormal albumin migrated slower then another variant already studied in our laboratory and found identical to proalbumin Christchurch (Y. A. et al. unpublished).

Incubation of the serum with trypsin led to a complete disappearance of the variant on electrophoresis at pH 8.6 (fig.2A). The isolated variant was found >95% pure by cellulose acetate electrophoresis (see fig.2B); SDS–polyacrylamide gel electrophoresis showed that it had the same apparent relative molecular mass (M_r) as normal serum albumin (not shown). Tryptic digestion of the isolated variant induced a complete change in its electrophoretic mobility that became identical to normal serum albumin (fig.2B). After this cleavage no significant change of M_r was observed, thus suggesting that trypsin had split a small peptide sequence at the N- or C-terminal ends of the molecule. N-Terminal amino acid analyses identified in the variant an arginine residue instead of aspartic acid found in normal albumin. N-Terminal amino acid sequence of the variant (fig.3) indicated the presence of an abnormal sequence that can be interpreted as the one of an abnormal proalbumin with an arginine in position-2 replaced by an histidine residue. Such an amino acid substitution can be explained by a single nucleotide replacement in the codons for arginine (replacement of CGU or CGC by CAU or CAC). The presence of an histidine residue explains the difference of mobility at pH 5.0 between proalbumin Lille and

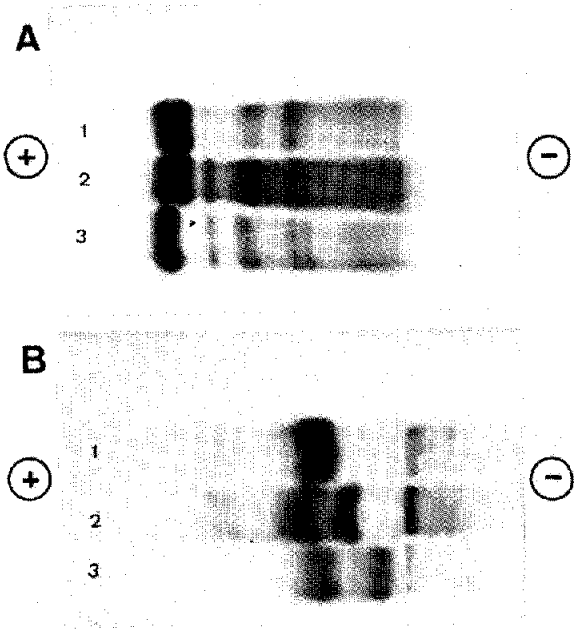

Fig.1. (A) Electrophoresis of the serum on cellogel in veronal–Tris buffer (pH 8.6): (1) serum with proalbumin Christchurch; (2) serum with proalbumin Lille; (3) normal human serum.

(B) Electrophoresis of the serum in acetate EDTA buffer (pH 5.0): (1) normal human serum; (2) serum with proalbumin Christchurch; (3) serum with proalbumin Lille.

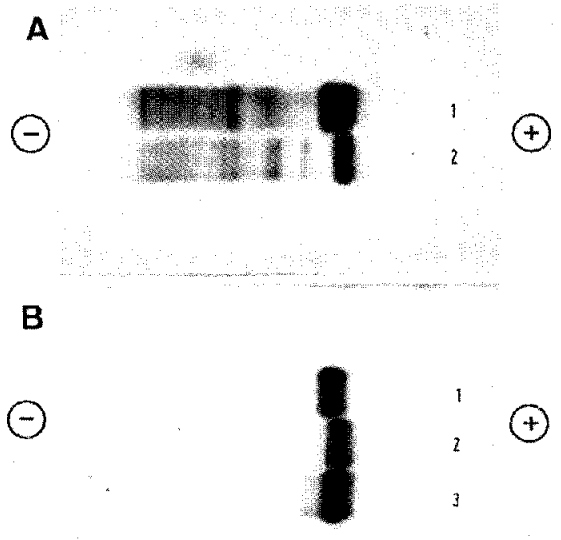

Fig.2. (A) Electrophoresis of the serum with proalbumin Lille after incubation with trypsin (electrophoresis on Cellogel at pH 8.6): (1) untreated serum; (2) serum incubated 2 h at 37°C with trypsin.

(B) Electrophoresis of the isolated variant after incubation with trypsin (electrophoresis on cellogel at pH 8.6): (1) isolated variant; (2) isolated variant incubated 2 h at 37°C with trypsin (enzyme/protein ratio 0.2%); (3) normal serum albumin.

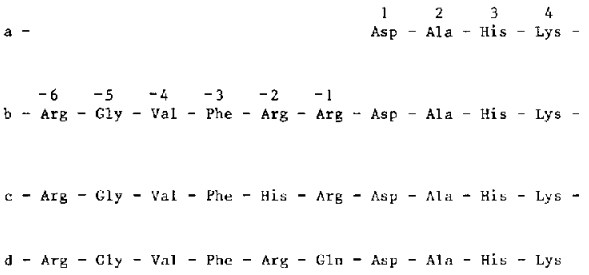

Fig.3. Amino-terminal sequences of: (a) human serum albumin; (b) human proalbumin as presumed in [8]; (c) proalbumin Lille; (d) proalbumin Christchurch.

proalbumin Christchurch where arginine in position-1 is replaced by a glutamine. In the hepatocyte cleavage of the propeptide from normal proalbumin may be effected in the Golgi vesicles by a specific protease, possibly cathepsin B, that recognizes the Arg–Arg sequence [15]. In [8,9] it was therefore suggested that in proalbumin Christchurch the replacement of the second arginine by a glutamine led to an impossibility of cleavage by the cathepsin. Our results confirm this hypothesis as in proalbumin Lille the substitution of the first arginine by an histidine in the Arg–Arg sequence leaves the propeptide sequence uncleaved in vivo. However, our experiments show that this propeptide sequence is easily cleaved by trypsin, as observed for proalbumin Christchurch [9]. The presence of equal amounts of variant and normal serum albumins argues also against some regulatory effects of the propeptide on biosynthesis of human serum albumin [16].

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