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A new proalbumin variant: albumin Jaffna (–1 Arg→Leu)

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Albumin Jaffna is an electrophoretically slowly moving genetic variant of human serum albumin found in two members of a Tamil family from Jaffna (Northern Sri Lanka), both heterozygous for the abnormal protein. Sequential analysis of albumin Jaffna, purified from serum by ion exchange chromatography on DEAE Sephadex and Mono Q columns, revealed that this variant is a new abnormal proalbumin, arising from a –1 Arg→Leu substitution, which prevents the proteolytic removal of the N-terminal hexapeptide and allows the mutated proalbumin to enter the circulation. The presence of two additional positive charges is in keeping with the decreased electrophoretic mobility of albumin Jaffna, as well as with its isoelectric point of 5.01, determined by chromatofocusing on a Mono P column. The variant is selectively cleaved by trypsin in vitro, leaving leucine –1 as N-terminal residue.

Proalbumin; Genetic variant; N-terminal sequence; Chromatofocusing; Isoelectric point

1. INTRODUCTION

Albumin is synthesized by the liver cells in the form of a precursor, proalbumin [1], which has an additional highly basic hexapeptide Arg-Gly-Val-Phe-Arg-Arg attached at its N-terminal end [2]. This leader peptide is removed by a proteolytic event in the Golgi complex just before the protein enters the circulation [3]. Although the mammalian proalbumin converting enzyme has not yet been isolated, recent findings point to the role of a calcium dependent KEX2-like protease found in hepatic secretory vesicles [4,5]. However, a critical requirement for the excision of the propeptide is the presence of the Arg-Arg sequence at the cleavage site in the precursor molecule. In fact the substitution of one of this pair of basic residues prevents the proteolytic removal of the mutated propeptide, as demonstrated by the existence of circulating variants, proalbumin Christchurch (–1 Arg ⟶ Gln) [6], proalbumin Lille (–2 Arg ⟶ His) [7] and proalbumin Takefu (–1 Arg ⟶ Pro) [8].

In a continuing structural study of genetic variants of human serum albumin, we have established that albumin Jaffna, a genetic variant found in a 60 year old man, a Tamil from Jaffna (Northern Sri Lanka) [9] is a new type of proalbumin, arising from a –1 Arg ⟶ Leu substitution. The molecule shows the same susceptibility to limited tryptic cleavage as proalbumins Christchurch and Lille [7,10], but differs with respect to the great instability of its N-terminal hexapeptide.

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2. MATERIALS AND METHODS

2.1. *Albumin purification*

A 4 ml serum sample was dialyzed against 0.11 M phosphate buffer, pH 5.75 and chromatographed on a DEAE Sephadex column (1.3 × 100 cm) eluted with the same buffer [11]. The fractions containing albumin were pooled, concentrated and desalted on an Amicon apparatus equipped with a PM-30 membrane.

Further purification of albumin Jaffna was achieved by anion exchange chromatography using the Pharmacia FPLC system equipped with a mono Q (HR 5/5) column developed with a linear gradient of 0–200 mM NaCl in 20 mM piperazine·HCl buffer, pH 5.8.

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The material eluted under each peak was desalted by gel filtration on a PD 10 column.

2.2. *Structural studies*

Amino acid analyses were carried out by ion exchange chromatography with post column ninhydrin derivatization according to Moore [12] on a Kontron Chromakon 500 automatic analyzer. N-terminal residues were identified as dansyl derivatives according to Hartley [13]. Automated sequence analysis 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. [14].

2.3. *Screening of CNBr fragments*

Whole reduced slow and normal albumins were carboxymethylated and cleaved with CNBr as previously reported [15]. The fragments were analysed by polyacrylamide gel isoelectric focusing performed in the presence of 8 M urea in the pH interval 2.5–8 [16].

2.4. *Chromatofocusing*

The isoelectric points were determined by chromatofocusing on a mono P column HR 5/20 on the Pharmacia FPLC System according to Hutchinson and Matejtschuk [17].

2.5. *Limited tryptic digestion*

The conditions for tryptic hydrolysis were essentially those of Quinn et al. [2]. After digestion for 1 h at 37°C, the mixture was equilibrated in 20 mM piperazine·HCl buffer, pH 5.8, on a PD 10 column and resolved on mono Q column as described in section 2.1.

3. RESULTS AND DISCUSSION

Normal and Jaffna albumins have been purified by ion exchange chromatography (fig.1) and, as expected on the basis of the electrophoretic behaviour [9], the variant elutes ahead of the normal protein. The serum content in the two proteins is unequal, the variant accounting for about one third of the total albumin amount. The proteins were assayed for homogeneity by SDS gel electrophoresis which shows in both cases the presence of a single band with a molecular weight approximately equal to that of normal albumin (66500). Their amino acid composition reveals that the slow variant differs from the normal protein by its higher arginine, glycine, valine, phenylalanine and leucine content. While the N-terminal residue of normal albumin is aspartic acid, in the peak of the abnormal protein we found 3 different amino acids: arginine, phenylalanine and aspartic acid. We therefore resolved peak J by anion exchange chromatography on a mono Q column (fig.2) into

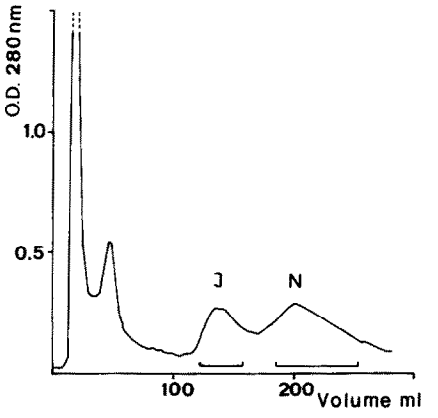

Fig.1. Chromatographic separation of normal (N) and Jaffna (J) albumins on a DEAE Sephadex column eluted with 0.11 M phosphate buffer, pH 5.75.

3 components, 1, 2 and 3. These fractions account respectively for 40%, 15% and 45% of the total protein content and are characterized by the N-terminal residues arginine, phenylalanine and aspartic acid. They were subsequently submitted to automatic sequence analysis for 10 cycles yielding the single N-terminal portion of normal albumin in the case of fraction 3 and a different primary structure, together with a background of the normal sequence, in the other two cases. The predominant component of peak 1 gave Arg-Gly-Val-Phe-Arg-Leu-Asp-Val-His-Lys, while that of peak 2 gave Phe-Arg-Leu-Asp-Ala-His-Lys-Ser-Glu-Val. From these data we conclude that albumin Jaffna is a

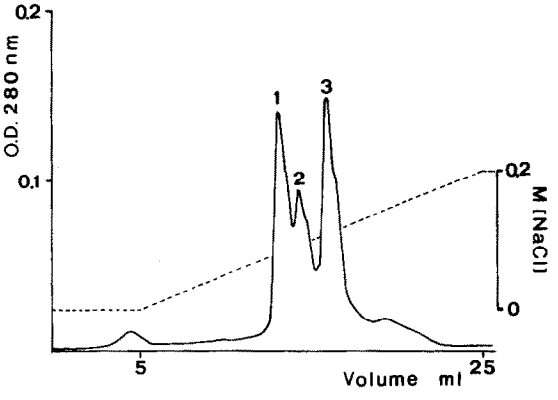

Fig.2. FPLC of the components of peak J from the DEAE Sephadex chromatography on a mono Q column. Elution was performed by a linear gradient of 0–200 mM NaCl in 20 mM piperazine·HCl buffer, pH 5.8. Flow rate: 1 ml/min.

new proalbumin variant arising from a −1 Arg ⟶ Leu substitution, which prevents the cleavage of the propeptide and allows the proalbumin to leave the liver cell intact.

Jaffna and normal albumins have been submitted to comparative analysis of the CNBr fragments by isoelectric focusing in the presence of 8 M urea (fig.3). The only fragment which behaves different in the two samples is CNBr I which in the case of the Jaffna variant focuses closer to the cathode than the normal counterpart (ΔpI = +0.25 pH units). This result shows that the molecular abnormality of this variant is only located in this fragment and that the rest of the albumin molecule is unaffected by chemical or proteolytic cleavage.

In order to further characterize the fractions and determine their isoelectric point, we also separated the components of peak J from the DEAE-Sephadex column by chromatofocusing on mono P column, under the conditions described by Hutchinson and Matejtschuk [17]. The elution profile (fig.4) shows the presence of 3 peaks, with an isoelectric point of 5.01, 4.90 and 4.80, accounting for 40%, 15% and 45% of the total albumin content, which correspond to albumin Jaffna, the N-terminal phenylalanine form and normal albumin respectively. The isoelectric point of albumin Jaffna (5.01) is identical with that of proalbumins of the Christchurch type, while it is lower than that of proalbumins of the Lille type (5.06) obtained under the same conditions. The presence of two additional positive charges in the propeptide of the variant matches the isoelectric point of both the native protein and its CNBr I fragment, as well as the electrophoretic mobility on cellulose acetate [9].

As susceptibility to limited tryptic cleavage in vitro is a characteristic of other proalbumins, such as Christchurch [10] and Lille [7], we submitted albumin Jaffna to tryptic digestion under the conditions described by Quinn et al. [2]. This induced a complete change in the chromatographic behaviour on Mono Q, with the retention time becoming identical to that of normal serum albumin (fig.5). After this cleavage no significant changes of molecular weight were observed, while N-terminal amino acid analysis identified in the variant a leucine residue instead of the aspartic acid found in normal albumin. When the limited tryptic digestion was performed on the DEAE-Sephadex peak J, we observed the complete disap-

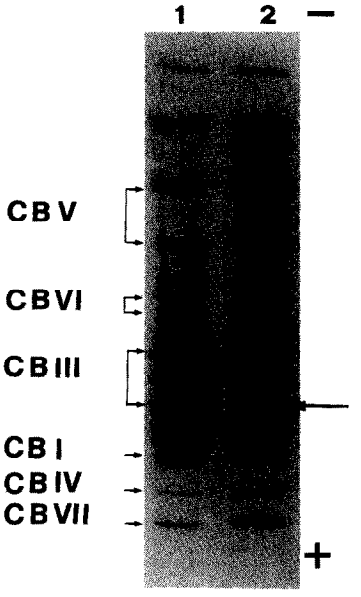

Fig.3. Isoelectric focusing of CNBr fragments from normal (lane 1) and Jaffna (lane 2) albumins. Peptides were resolved in the pH range 2.5–8 in the presence of 8 M urea. CNBr fragments are numbered according to their order in the known sequence of human serum albumin [19]. Each fragment, except the 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 I fragment.

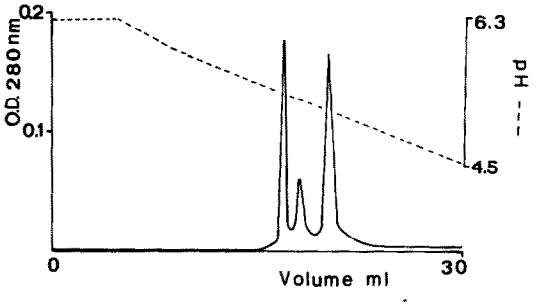

Fig.4. FPLC analysis of the components of peak J from the DEAE Sephadex chromatography (fig.2) by chromatofocusing on a mono P column. Aliquots (1 ml) of the mixture (1 mg/ml) in piperazine·HCl buffer (25 mM, pH 6.3) were applied to the mono P column equilibrated with this buffer. Elution was performed at 1 ml/min with Polybuffer 74 diluted 1:10 (v/v) in water (UHQ, Elgastad) which had been brought to pH 4.5 with HCl [17].

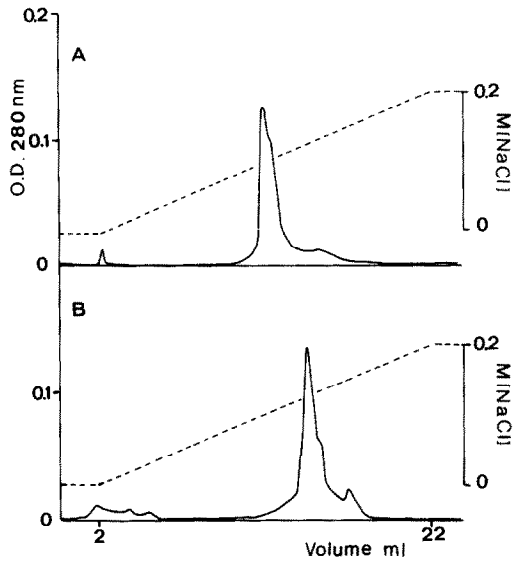

Fig.5. Limited tryptic digestion of proalbumin Jaffna. Purified albumin Jaffna was incubated with trypsin (1:5000, w/w) in 50 mM $K_2HPO_4 \cdot HCl$ buffer, pH 7.5, 50 μM $CaCl_2$ at 37°C, and digestion was stopped with soybean trypsin inhibitor [2]. The digests (0 h (A); 1 h (B)) were resolved by FPLC on a Mono Q column under the conditions described in fig.2. The same results were obtained when the digestion was performed on the components of peak J from the DEAE Sephadex chromatography (0 h (fig.2); 1 h (B)).

pearance of the first two peaks which were converted into the species with N-terminal leucine that coelutes with normal albumin (fig.5). These data show that albumin Jaffna, as well as the form with N-terminal phenylalanine, is selectively cleaved by trypsin after arginine −2, leaving leucine −1 as the N-terminal residue. This result differs from the findings of Tillyer et al. obtained under slightly different conditions [18].

The −1 Arg ⟶ Leu substitution found in the variant can be accounted for by a single base mutation in the structural gene: codon −1, CGA in normal proalbumin [19], must be changed to CTA encoding for leucine. Albumin Jaffna is the third variant substituted in this position: thus, 3 of the 4 possible single base changes in codon −1, which would result in detectable abnormalities, have been so far observed. They all occur in the second position of this codon. Further studies will be required to establish whether the apparent clustering of the mutations in the Arg-Arg sequence of the propeptide reflects the existence of hypermutable sites in the albumin gene or is instead due to the relative ease of identifying proalbumin variants. The net increase of at least two positive charges due to the presence of the propeptide, which is likely exposed to the solvent, could make these variants more easily detectable than others.

Although it has been reported that all the proalbumins are somewhat unstable, probably because the propeptide may be cleaved by one of the many serine proteases present in serum [8], the Jaffna variant is particularly labile: upon storage for two months at 4°C the initially nearly equal amount of the two albumins in fresh serum changes to 14% Jaffna and 86% normal [9]. This agrees with the proportion of albumin Jaffna compared with normal (1:2) that we found after the DEAE Sephadex chromatography performed on a serum sample stored for a long period. This purification step allowed us to obtain the variant proalbumin in a homogeneous and stable form in all cases so far examined in our laboratory, i.e. albumin Pollibauer [20] and several samples of the Lille and Christchurch type. Thus it is likely that albumin Jaffna, even after this isolation procedure, may undergo degradative processes, giving rise to both the N-terminal phenylalanine form and the normal protein. It is unclear whether this is due to a proteolytic cleavage or reflects a chemical instability of the N-terminal propeptide of this variant.

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