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Finnish hereditary amyloidosis

Amino acid sequence homology between the amyloid fibril protein and human plasma gelsoline

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Amyloid fibrils were isolated from the kidney of a patient with Finnish hereditary amyloidosis. After solubilization of the fibrils in guanidine-HCl, fractionation by gel filtration, and purification by reverse-phase high-performance liquid chromatography, a homogeneous amyloid protein with an apparent M_r of 9000 was obtained. The protein was subjected to enzymatic digestion by trypsin and endoproteinase Lys-C. The amino acid sequences were determined for 6 of the released peptides and they were all found to be identical to the reported, deduced primary structure of human plasma gelsoline in the region of amino acids 235–269. The results show that the amyloid fibril protein in Finnish hereditary amyloidosis represents a new type of amyloid protein that shows amino acid sequence homology with gelsoline, an actin-modulating protein.

Finnish hereditary amyloidosis; Familial amyloid polyneuropathy; Amyloid protein; Gelsoline

1. INTRODUCTION

Familial amyloid polyneuropathy (FAP) syndromes are autosomal dominant disorders characterized by extracellular deposits of fibrillar protein with crossed β-pleated sheet conformation and a clinical syndrome of polyneuropathy [1]. The Finnish type of hereditary amyloidosis (FAP type IV) is characterized by a systemic disease with a distinct clinical picture involving progressive cranial neuropathy, corneal lattice dystrophy and distal sensorimotor neuropathy [2]. More than 300 cases have been recorded in the Finnish population [3]. In contrast to FAP type I and FAP type II syndromes which have been shown to be caused by specific variant transthyretin molecules that deposit in tissues as amyloid [4–10], the biochemical nature of the amyloid fibril protein in FAP type IV has remained unresolved [3,11]. Here we report the isolation and purification of kidney amyloid in Finnish hereditary amyloidosis and show amino acid sequence homology of the major M_r 9000 amyloid protein with the inner portion of human plasma gelsoline.

2. MATERIALS AND METHODS

2.1. *Materials*

Amyloid fibrils were isolated from the kidney of a 71-year-old man with Finnish hereditary amyloidosis. The amyloid tissue sample and control non-amyloid renal tissue sample, were obtained at autopsy and kept at −70°C until processed. Congo-red staining and microscopy under polarized light revealed amyloid in the glomerular capillaries and to a slighter extent in larger blood vessels and in the interstitium in the case of amyloidosis, whereas the control renal tissue sample was negative with respect to amyloid.

Sephadex G-100 was from Pharmacia (Uppsala, Sweden), trypsin-TPCK was from Sigma Chemical Co. (St. Louis, MO, USA), and endoproteinase Lys-C Sequence Grade from Boehringer Mannheim (Mannheim, FRG). The chemicals as well as other materials needed for sequencing and high-performance liquid chromatography (HPLC) were of Protein Sequencing Grade and were obtained from Applied Biosystems Ltd. (Warrington, UK). Polyacrylamide gel electrophoresis was performed in 15–17% gels, as described [12].

2.2. *Isolation and purification of amyloid proteins*

Using a modification of the original procedure of Pras et al. [13], 61.7 g of amyloid renal tissue was homogenized in 0.9% NaCl, 200 ml, followed by centrifugation at 12000 rpm for 30 min. The supernatant was discarded and the pellet then washed, repeating the procedure twice with 0.9% NaCl, twice with 0.1 M phosphate-buffered saline, and 6 times with 0.9% NaCl. The final sediment was homogenized in distilled H_2O, 100 ml each time, 15 times. The supernatants were lyophilized and subjected to Congo-red staining. The 5 last washings were all positive. Supernatant 12, with a high content of amyloid fibrils, was chosen for further purification. The lyophilized sample was denatured in 6 M guanidine-HCl buffered to pH 8.5 with 0.5 M Tris-HCl containing 0.002 M EDTA and 20 mM dithiothreitol. The sample was incubated at 37°C for 3 h and kept at room temperature overnight. After centrifugation, the supernatant, containing 69 mg protein, was chromatographed on a Sephadex G-100 column (1.5 × 98 cm) using 5 M guanidine-HCl with 1 M acetic acid as eluent. The pooled fraction of the retarded peak was purified on reverse-phase HPLC (Beckman, System Gold, Programmable Solvent Module 126, SP 8450, UV/VIS detector, Spectra Physics Merck Hitachi D-2500 Chromato-Integration) using a Vydac C_{18} column and a 10–80% acetonitrile gradient in 0.1% trifluoroacetic acid.

2.3. *Peptide fragmentation and separation on reverse-phase HPLC*

The amyloid protein was alkylated and desalted and repurified on

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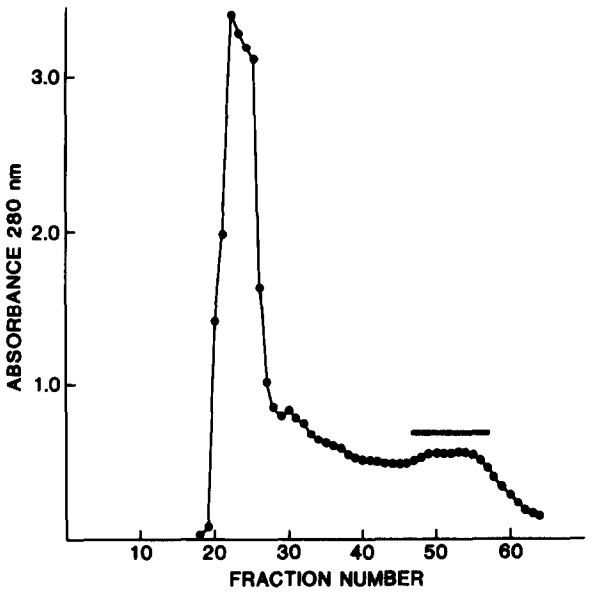

Fig.1. Gel filtration on Sephadex G-100 of extracted renal amyloid. The elution was performed with 5 M guanidine-HCl in 1 M acetic acid. The retarded peak containing the monomeric amyloid proteins was pooled as indicated.

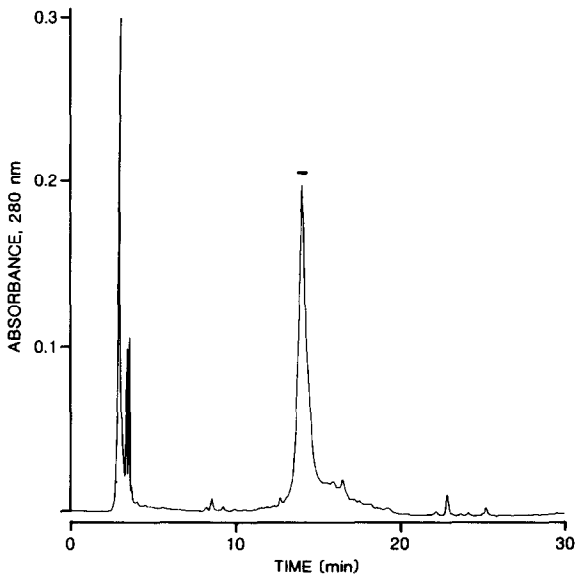

Fig.3. Reverse-phase HPLC of the isolated amyloid protein. Column, Vydac C_{18}; solvent system, a linear gradient of acetonitrile (0–80%) in 0.1% trifluoroacetic acid.

HPLC. Tryptic digestion was carried out in 1% ammonium bicarbonate; incubation with 3% (w/w) of trypsin for 2 h at 37°C was followed by another addition (3%) of the protease and continued incubation overnight at 37°C. The released peptides were separated by reverse-phase HPLC using a Vydac C_{18} column equilibrated with 0.1% trifluoroacetic acid in water, with a linear gradient of acetonitrile (0–60%) in 60 min. Digestion with endoproteinase Lys-C was carried out in 25 mM Tris-HCl buffer, pH 8.5; incubation with 3% (w/w) of enzyme for 2 h at 37°C was followed by another addition of the protease (3%) and continued incubation overnight at 20°C. The released peptides were separated by HPLC as described above.

2.4. *Amino acid sequence analysis*

The sequence analysis was performed by automated Edman degradation using a modified Applied Biosystems 477A/120A on-line pulsed liquid phase/gas sequencer in the gas phase mode. The NBRF Swiss-PROT sequence database 11.0, June 1989, was used for computer search of sequence homologies.

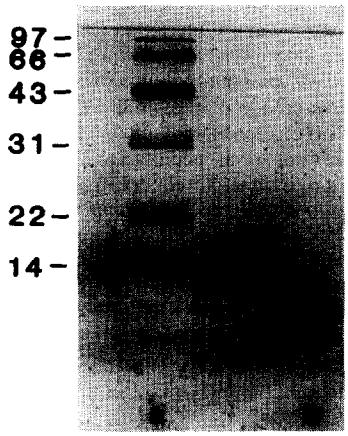

Fig.2. Polyacrylamide gel electrophoresis of the amyloid fraction (lane 'b'). Lane 'a' shows molecular mass markers ($M_r \times 10^{-3}$).

3. RESULTS

Fig.1 shows the gel filtration curve of the extracted renal amyloid. Gel electrophoresis of the retarded peak revealed two bands in the low molecular weight region (fig.2). These bands were absent in the gel electrophoretic runs of corresponding fractions of extracts from normal kidney. The amyloid protein fraction was then purified on reverse-phase HPLC and the major peak isolated (fig.3). The apparent molecular mass of the peptide was 9 kDa as judged from gel electrophoresis. Direct sequence analysis of the amino-terminus of the amyloid protein was not feasible. After alkylation, the protein was digested by trypsin and endoproteinase Lys-C and the released peptides sequenced. All the sequences determined were identical with the reported primary sequence of human plasma gelsoline in the region of amino acids 235–269 as shown in fig.4.

4. DISCUSSION

Several types of amyloid fibril proteins have been identified in the systemic forms of amyloidosis [14]. Under these conditions the amyloid proteins are composed of whole or fragmented plasma proteins. In primary and myeloma-associated amyloidosis, the amyloid protein is related to immunoglobulin light chains, in secondary amyloidosis to serum amyloid A protein, in haemodialysis-associated amyloidosis to β_2-microglobulin, and in the familial polyneuropathic amyloidosis to transthyretin (prealbumin). Recent biochemical studies of the transthyretin amyloid proteins in various FAP syndromes have shown that they

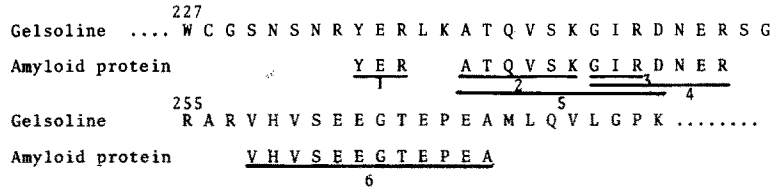

Fig.4. Amino acid sequences of proteolytic peptides 1–6 of amyloid fibril protein. Peptides 1–3, 5 and 6 were obtained by trypsin digestion and peptide 4 by endoproteinase Lys-C digestion. The region shown of the deduced primary structure of human plasma gelsoline is from [16], and the numbers 227 and 255 refer to the amino acid positions starting from the signal peptide.

are expressions of specific single amino acid alterations in the transthyretin molecules [5–10]. However, the biochemical nature of the amyloid fibril protein in the Finnish hereditary amyloidosis has remained unclear. The clinically different expression of the FAP syndrome in the Finnish form has raised the possibility that the fibril protein in this syndrome may have a unique structure. The results of this study indicate that the amyloid protein in Finnish hereditary amyloidosis represents a novel type of amyloid protein that shows amino acid homology with the inner portion of human gelsoline.

Gelsoline (M_r ~90000) is an actin-modulating protein found in a variety of mammalian tissues [15–17]. It binds actin monomers, nucleates actin filament growth, and severs actin filaments. Plasma gelsoline represents a slightly larger form (M_r ~93000) of gelsoline that is present in human plasma at a concentration of about 200 mg/l [18]. Both forms of gelsoline are derived by alternative transcriptional initiation sites and message processing from a single gene [16,17]. The function of circulating gelsoline has not been defined, but it may be essential for the clearance of actin filaments released into the bloodstream on extracellular space during tissue injury and cell senescence. The results of this study suggest that plasma gelsoline may be involved in the pathogenesis of a neurometabolic disease, the Finnish FAP type IV.

The M_r of the monomeric amyloid protein was of the order of 9000, thus representing a fragment of the putative plasma precursor, gelsoline. Whether the fragment contains a specific amino acid alteration with respect to the corresponding region of normal gelsoline, explaining a possible defect in the proteolytic processing of the molecule in Finnish FAP type IV, is not known at present. The amino acid sequences of all enzymatically derived peptides sequenced in this study were exactly homologous to the reported, deduced primary structure of human plasma gelsoline in the region of amino acids 235–269, leaving, however, gaps at positions 238, 239 and 253–257.

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