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A NEW FORM OF AMYLOID PROTEIN ASSOCIATED WITH CHRONIC HEMODIALYSIS WAS IDENTIFIED AS β_2-MICROGLOBULIN

F. Gejyo[a], T. Yamada[a], S. Odani[b], Y. Nakagawa[a], M. Arakawa[a], T. Kunitomo[c], H. Kataoka[c], M. Suzuki[d], Y. Hirasawa[d], T. Shirahama[e], A.S. Cohen[e] and K. Schmid[f]

Departments of [a]Internal Medicine and [b]Biochemistry, Niigata University School of Medicine, Niigata 951, Japan

[c]Basic Research Laboratories, Toray Industries, Inc., Kamakura 248 , Japan

[d]Shinrakuen Hospital, Niigata 950-21, Japan

[e]Arthritis Center and [f]Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

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Amyloid fibrils were isolated from amyloid-laden tissue obtained from a chronic hemodialysis patient with carpal tunnel syndrome. After solubilization in guanidine HCl, a significant amount of the protein was located in a homogeneous low molecular weight fraction. The protein was found to be identical to β_2-microglobulin, with regard to its molecular weight of 11,000, amino acid composition and 16 amino-terminal amino acids: Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala-Glu-. These results demonstrate that the amyloid associated with chronic hemodialysis contains as major component a new form of amyloid fibril protein that is homologous to β_2-microglobulin.

An increased incidence of carpal tunnel syndrome (CTS) in patients on long-term hemodialysis treatment has recently been reported (1-5). Amyloid deposition has been observed in the perineural tissue of the median nerve in the majority of the patients who had been operated for CTS (6-8). In such patients amyloid deposition has also been found in various other tissues indicating systemic involvement of the chronic hemodialysis-associated amyloidosis (1-3, 5-9).

Several different forms of amyloid have so far been characterized by chemical analysis, especially amino acid sequencing, and have been related to the clinical classification of amyloidosis (10-13). Protein AL which is of immunoglobulin origin was identified as the amyloid fibril protein of primary and myeloma-associated amyloidosis. Protein AA that is considered to be a

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derivative of the acute phase protein SAA is present in secondary amyloid. $A_{prealbumin}$ constitutes amyloid fibrils of familial amyloidotic polyneuropathy and a certain type of senile cardiac amyloidosis. $A_{precalcitonin}$ is the amyloid protein of medullary carcinoma of the thyroid. Gamma trace is the identified protein of hereditary amyloidotic cerebral angiopathy, and a "novel" protein is present in the cerebrovascular amyloid in Alzheimer's disease. However, the chemical and immunologic nature of the hemodialysis-associated amyloid remains to be clarified.

The purpose of this paper is to report our investigation on the identification of the fibril protein of the hemodialysis-associated amyloid as β_2-microglobulin.

MATERIALS AND METHODS

Patient. A 48-year old woman with end stage renal disease secondary to chronic glomerulonephritis had received hemodialysis treatment for 5 h three times a week for 13 years using a disposable hollow fiber dialyzer consisting of cuprophane membrane. During the past year the patient developed CTS, and on a carpal tunnel release operation an amyloid-rich block of tissue was obtained. Judging from Congo-red staining and green birefringence under polarized light, the amyloid accounted for over 90% of the tissue.

Isolation of amyloid fibrils. Using a modification of the procedures of Glenner et al. (14) and Pras et al. (15), 500 mg of the amyloid tissue were homogenized in 3.0 ml of 0.1 M phosphate buffered saline, pH 7.1, at 4°C. The homogenate was then centrifuged at 13,000 rpm for 30 min in a refrigerated centrifuge. The supernatant was discarded and the sediment was several times more subjected to the same procedure until the absorbance of the supernatant solution at 280 nm was less than 0.05. The final sediment was homogenized in 3.0 ml of dist. water and centrifuged at 8,000 rpm for 30 min. This procedure was repeated twice, and the supernatants were combined and lyophilized. The high content of amyloid in the lyophilized preparation was confirmed by Congo-red staining.

Fractionation of the amyloid protein by HPLC. The lyophilized sample was denatured in 70 µl of 6 M guanidine HCl buffered to pH 7.6 with 0.1 M Tris-HCl containing 0.01 M EDTA. The solution was gently stirred at room temperature for 12 h. The denatured amyloid was then chromatographed on a TSK-GEL G3000SW column (0.75 x 60 cm) using a Hitachi Model 638-30 liquid chromatograph. HPLC was carried out at 25°C with 4 M guanidine HCl buffered to pH 7.6 with 0.1 M Tris-HCl containing 0.01 M EDTA. The flow rate was 0.5 ml/min, and the effluent was monitored at 280 nm. The major fraction containing amyloid protein was dialyzed against dist. water at 4°C and then lyophilized.

Molecular weight determinations by HPLC and SDS-PAGE. The same column and conditions employed for fractionation were used for the molecular weight determination of the protein as reported by Ui (16). The standard proteins used in these experiments are listed in the legends of Fig. 1. SDS-Polyacrylamide gel electrophoresis (PAGE) was performed according to a modification (17) of the method of Laemmli (18) using 1.0 mm thick gradient (8-18%) slab gel. Appropriate standard proteins were also included in this experiment.

Amino-terminal amino acid sequence analysis. The amino-terminal sequence was determined using a gas/liquid phase model 470 A sequencer (Applied Biosystems Inc.). The obtained PTH-amino acids were identified by HPLC with the aid of an Unisil Q C_{18} column and a gradient elution system of acetonitrile in sodium acetate buffer, pH 4.6.

RESULTS

The HPLC pattern of the denatured amyloid fibril protein revealed a major symmetrical and two minor peaks (Fig. 1). The molecular mass of the protein (not reduced and alkylated) of the major peak was estimated to be 9,800 Da using HPLC. SDS-PAGE electrophoresis of the protein (reduced) of the major peak revealed a main band with an apparent M_r of 11,000 and a few minor bands that accounted for a small percentage of the total protein. The protein present in the mentioned peak was used for the subsequent analyses without

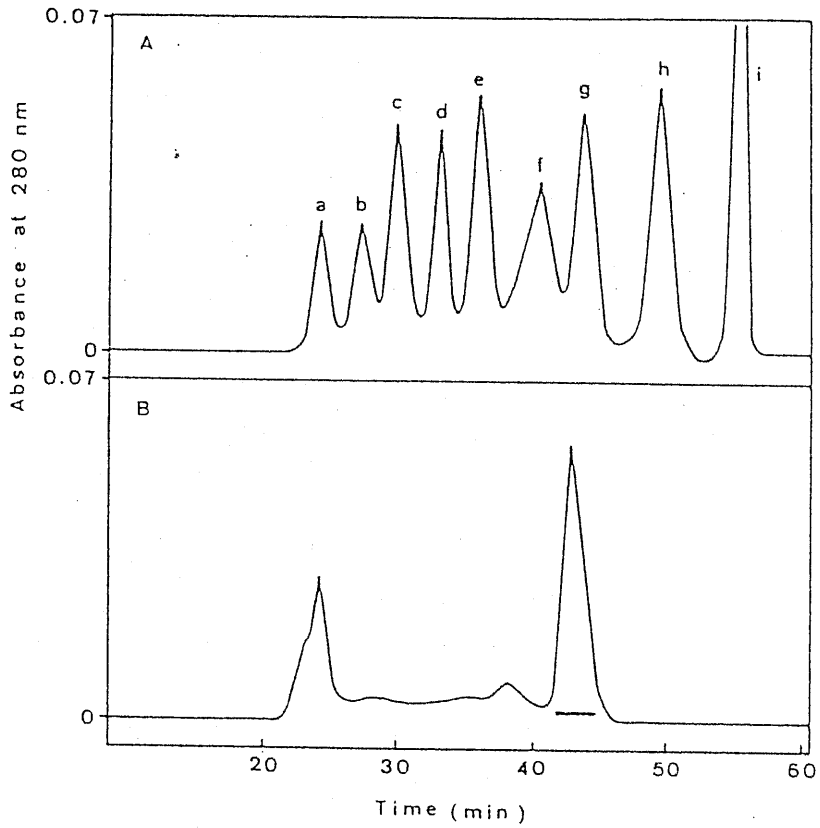

Fig. 1: The elution profiles of standard proteins (A) and amyloid fibril protein (B) obtained by HPLC using in both runs conditions described in Methods. Abbreviations used are: a, blue dextran; b, rat serum albumin (M_r 65,000); c, ovalbumin (M_r 44,000); d, carbonic anhydrase (M_r 29,000); e, soybean trypsin inhibitor (M_r 20,100); f, lysozyme (M_r 14,300); g, low molecular weight protease inhibitor (M_r 14,300); h, insulin, chains A and B (M_r 2,380, 3,420), and i, DNP-Alanine. The major fraction of the amyloid protein is indicated by the bar (B).

Table I

AMINO ACID COMPOSITION OF THE AMYLOID PROTEIN AND β_2-MICROGLOBULIN

Residue[a]	Amyloid protein	β_2-Microglobulin[c]
Asp	11.3	12
Thr	4.6	5
Ser	7.1	10
Glu	11.6	11
Pro	2.8	5
Gly	5.8	3
Ala	3.9	2
Cys	2.1	2
Val	6.7	7
Met	0.9	1
Ile	4.0	5
Leu	5.5	7
Tyr	4.0	6
Phe	5.3	5
Lys	4.9	8
His	4.6	4
Arg	3.9	5
Trp	ND[b]	2

[a] Expressed in residues per mole of protein.

[b] ND, not determined.

[c] Calculated from sequence data (18).

further purification because of the scarcity of the sample. Amino acid analysis (redist. HCl, 110°C, 24 h) (Table I) shows that the composition of the amyloid fibril protein is different from those of known amyloid fibril proteins (10-13). Noteworthy, however, is the similarity of this amino acid composition to that of normal human β_2-microglobulin (19), although higher amounts of Gly and Ala and lower amounts of Ser, Pro and Lys were noted.

Amino-terminal amino acid sequence determination of the amyloid protein afforded 16 residues. In the first step a few amino acids, of which Ile was the major one, were detected. However, in the second cycle, only Gln was present, and in each subsequent cycle only one major amino acid was found. The amino-terminal amino acid sequence of the amyloid protein could thus be identified as: Ile-Gln-Arg-Thr-Pro-Lys-Ile-Gln-Val-Tyr-Ser-Arg-His-Pro-Ala-Glu- which was completely identical to that of β_2-microglobulin (19).

DISCUSSION

The results of the present study have established the identity of the amyloid fibril protein in hemodialysis-associated amyloidosis as β_2-microglobulin. β_2-Microglobulin is a small protein (M_r 11,731) present in normal biological fluids (20). The amino acid sequence of this globulin showed that this protein is related to the IgG (19). It is well known that β_2-microglobulin accumulates in the blood of patients undergoing hemodialysis because the procedure can not remove this protein from the blood plasma (21, 22).

Carpal tunnel syndrome is known to be associated with some cases of AL (primary and myeloma-associated) amyloidosis and of familial amyloidosis (23, 24). It is of particular interest to note that the amyloid proteins of AL-amyloidosis and hemodialysis-associated amyloidosis are both related to immunoglobulins and both have a tendency to develop CTS.

On the basis of the present observations, one may postulate a concept of etiology of hemodialysis-associated CTS and amyloidosis as follows. As hemodialysis cannot remove the excess of β_2-microglobulin, an increased level of this protein results in the blood plasma over a long period of treatment. These conditions lead to the accumulation of the protein in tissues causing the formation of amyloid fibrils which have a relatively high affinity, as in the case of AL-amyloid, to the carpal tunnel area. Amyloid deposition in this area then creates compression of the median nerve, thus causing CTS.

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