Brief Definitive Report

CHARACTERIZATION OF AN AMYLOID FIBRIL PROTEIN FROM SENILE CARDIAC AMYLOID*

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Senile cardiac amyloidosis is a form of primary amyloidosis affecting mainly the heart (1, 2), but small deposits are often found in pulmonary vessels and sometimes in vessels of other organs such as the pancreas or the kidneys. Senile cardiac amyloidosis has been found in 12.5% of autopsied persons, and after the age of 90 such a high frequency as 50% has been reported (2). Although much more frequent than other types of systemic amyloidosis, this diagnosis is only rarely established before death and no studies have been reported on the nature of senile cardiac amyloid. In the present paper we have characterized, from five different patients with senile cardiac amyloidosis (SCA), by immunological and immunochemical techniques an amyloid fibril protein, A_{SCA}, which appears to be unique to this type of amyloid, and different from other types of amyloid proteins so far characterized.

Materials and Methods

Amyloid Tissues and Isolation of Amyloid Fibrils. Heart tissues rich in amyloid were from one female and four male patients [H. L. 74 yr (kindly provided by Dr. Hans Nordgren, Lund); 84, 82 yr; 120, 90 yr; 335, 85 yr; and 402, 89 yr]. The patients had all suffered from heart insufficiency or atrial fibrillation but amyloidosis was never suspected. The autopsies revealed massive amyloidosis of the heart (weights, 400–950 g) and small deposits of amyloid in pulmonary, renal, and in one case also of liver vessels.

Amyloid fibrils were isolated as described before (3, 4), with the exception that the main part of the amyloid was found in the top of the residual pellet after centrifugation and was less soluble than other amyloid fibrils in water. Previously characterized primary and secondary amyloid fibril proteins were used as controls. Degraded amyloid (DAM) was made by treatment with 0.1 M NaOH (3).

Immunological Technique Methods. Rabbit antisera were made against DAM preparations of cardiac amyloid fibrils (H. L.) in two rabbits and against amyloid protein AA, various amyloid proteins of immunoglobulin origin, and an amyloid-related high molecular weight protein (void volume peak material) (4, 5). Antisera were absorbed with pooled human serum.

Gel Filtration. Amyloid fibrils (15 mg/ml) were dissolved in 6 M guanidine HCl in 0.1 M Tris/HCl buffer, pH 8.0, with 0.1 M dithiothreitol. After centrifugation the clear solutions were gel filtered through a 2.6 × 70 cm Sepharose 6 B column equilibrated and eluted with 5 M guanidine/HCl. Pooled fractions were desalted through a short Sephadex G 25 column equilibrated with 10% formic acid or by exhaustive dialysis against water. The major subunit protein was purified further by rechromatography through a 1.6 × 100 cm Sephadex G 150 column equilibrated with 5 M guanidine/HCl (4, 5).

Electron Microscopy. Precipitates occurring after dialysis of fractions from the separations

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were suspended in water and placed on Formvar coated copper grids and air dried after the excess had been blotted off. The specimens were negatively contrasted with 2% phosphotungstic acid, pH 5.3, and studied in a Jeol JMC 100 electron microscope at 80 kV (6).

Results

Antigenic Analysis of DAM Fibrils. The anti-amyloid H. L. antiserum obtained by immunization with the DAM preparation of amyloid fibrils of the senile amyloidosis H. L. showed one line of precipitation with DAM H. L. and the DAM preparations of the four other senile cardiac amyloids (84, 120, 335, and 402). The precipitation lines fused completely (Fig. 1*a*). In contrast, no precipitation was obtained when the anti-amyloid H. L. antiserum was tested against DAM preparations of amyloid fibrils purified from the heart of a young patient with primary amyloidosis or a panel of DAM preparations of primary, myeloma-associated or secondary amyloids, nor did these preparations inhibit the reaction between DAM preparations from the cardiac amyloid fibrils and the anti-DAM H. L. antiserum. Similarly, normal human serum or an extract from normal heart treated as the DAM preparations, gave no reaction or inhibition.

Furthermore, antiserum against protein AA and several amyloid fibril proteins of immunoglobulin light chain origin did not react with any of the five DAM preparations of the senile cardiac amyloids (Table I). On the other hand, the high molecular weight material (4, 5) was present in all five cardiac amyloids as they also were in the amyloid preparation from the heart of the younger patient with primary amyloidosis. This high molecular weight protein which probably is a secondary protein in relation to the amyloid process can be isolated from almost all types of amyloids and may represent a normal protein of vessel walls (unpublished observation).

Characterization of the Purified Amyloid Proteins. The amounts of amyloid fibril material from two patients were sufficient for further analysis. Their elution patterns upon gel filtration were almost identical with a high initial peak, a broader intermediate area, and a small but distinct retarded second peak (Fig. 1*b*). In contrast, preparations from nonamyloidotic hearts gave a similar large and broad initial peak and an intermediate area, but the second small and retarded peak was absent. This second peak material, only obtained from the senile amyloidotic hearts, corresponded to a mol wt of about 6,000 daltons when estimated by mobility in sodium dodecyl sulfate electrophoresis (7).

When tested against anti-DAM H. L. antiserum in immunodiffusion, the 6,000 mol wt proteins of amyloids H. L. and 335 each showed one line of precipitation, which fused completely with the lines obtained with the DAM preparations (Fig. 1*a*). Some material which reacted with anti-DAM H. L. was also detected in the broad intermediate area of the gel filtration but not in the first peak. The amino acid compositions of the subunit proteins of the amyloids H. L. and 335 were almost identical and differ significantly from that of protein AA (Table II).

Additional Experiments. Additional experiments were performed to study whether the isolated cardiac amyloid fibril proteins could form fibrils in vitro. The precipitate obtained when a solution of the pure subunit protein was dialyzed against water showed an ultrastructure of irregular, fine fibrils, with a

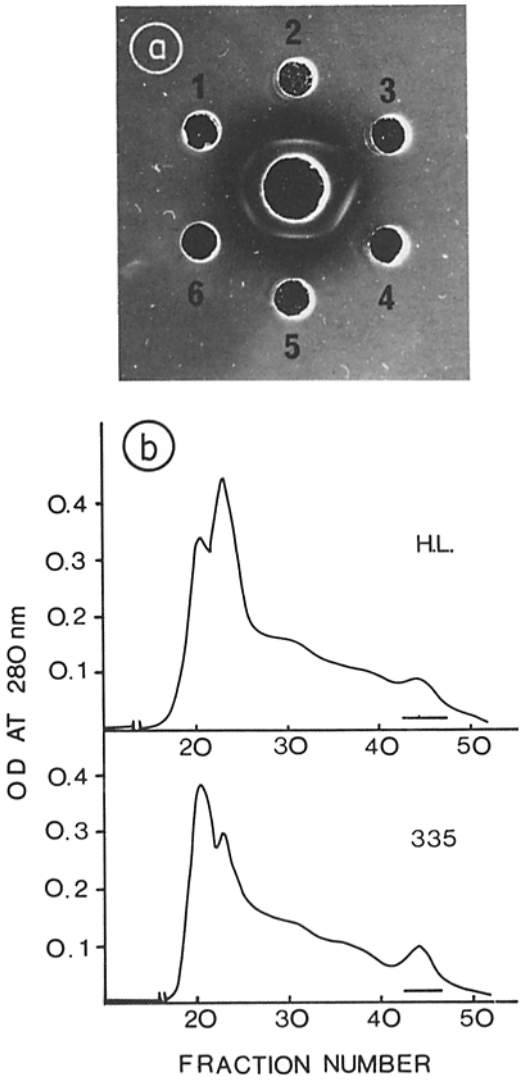

FIG. 1. (*a*) Double immunodiffusion with anti-DAM H. L. in central well. 1 and 4, protein subunit of H. L. (peak II in Fig. 1 *b*); 2, DAM 402; 3, DAM of material extracted from normal heart; 5, DAM H. L.; 6, DAM 335. (*b*) Elution profiles of DAM H. L. and DAM 335, from a Sepharose 6B column (2.6 × 70 cm). Elution buffer was 5 M guanidine/HCl as described in Materials and Methods. Horizontal bar in second small peak indicates fractions pooled for antigenic and immunochemical characterization of senile cardiac amyloid fibril subunit.

width of about 40 Å. The fibrils were vawy and not as rigid as native amyloid fibrils. The precipitate had affinity to Congo red but did not show green birefringence.

Discussion

Senile systemic amyloidosis appears to affect especially the heart. Other deposits are small and mostly not symptom giving (1, 2). Furthermore, as shown

TABLE I
Reaction Pattern of Senile Cardiac Amyloid with Different Amyloid Antisera

	Antiserum against:				
	AA	A_λIV	A_λV	A_{SCA}	V_o
Senile cardiac amyloid					
DAM preparation (H. L., 84, 120, 335, 402)	–	–	–	+	+
Peak I* (H. L. and 335)	–	–	–	–	+
Peak II (H. L. and 335)	–	–	–	+	–
Controls					
DAM secondary amyloid	+	–	–	–	+
DAM primary amyloid (A_λIV)	–	+	–	–	+
DAM primary amyloid (A_λV)	–	–	+	–	+

* Peak I is material from the initial high void volume peak and peak II is material from the last small peak indicated by horizontal bar in Fig. 1 *b*.

in this paper, the major subunit protein of senile cardiac amyloid is unique to this type of amyloid. Thus, the subunit proteins from the five senile cardiac amyloids were identical when tested against an antiserum to one of them. This antiserum failed to react with any other amyloids tested, with normal heart extracts, or normal serum. Furthermore, antisera against different other major amyloid proteins did not react with the unique senile cardiac amyloid fibril protein. Although a serum protein, SAA, (8–11) structurally closely related to protein AA (4, 5, 12) of secondary amyloidosis is found in increasingly high amount with age (10, 11), no protein AA-like material was found in senile cardiac amyloid. The possibility of light chain fragments which are often found in typical primary amyloidosis is not totally excluded. However, no cross-reactions have been encountered with a variety of antisera to light chains or other amyloids. It would also be rather unexpected to find the same immunoglobulin chain in the amyloid fibrils of five different patients only selected because they had SCA. Therefore, the senile cardiac amyloid protein subunit, termed protein A_{SCA}, appears to be a unique protein to this type of amyloidosis. It should be noted that tryptophane is present as in all other systemic amyloids (13).

The yield of the subunit protein A_{SCA} from the cardiac amyloid was only about 10%, probably because the protein is even more insoluble than the other amyloid proteins. The cardiac amyloid materials were also more contaminated with other proteins, possibly myofibrillar proteins, than other amyloids. The conclusion that the eluted protein subunit A_{SCA} is amyloid related is based on the comparison with the elution pattern of material extracted from normal hearts, where no similar peak was seen at gel filtration, and where no antigenic cross-reaction could be found with the amyloid subunit protein, A_{SCA}. The subunit protein, although immunologically and chemically different from other amyloid fibril proteins has a low molecular weight like other amyloid proteins. Finally, the amyloid protein A_{SCA} can make fibrils in vitro. The reason why the fibrils precipitated in vitro, although taking up Congo red, did not show green birefringence is unclear. However, as is seen with some other amyloid proteins, the formation of fibrils in vitro is difficult and such fibrils do not totally resemble the native amyloid fibrils, possibly because the molecular arrangement is not that of

TABLE II
*Amino Acid Composition of the Subunit Protein of Senile Heart Amyloid Fibrils from Two Patients**

	HL	335	AA
Asp	8.1	7.3	10.1
Thr	8.1	7.9	0.3
Ser	9.0	8.3	5.2
Glu	12.4	11.7	6.1
Pro	5.4	5.6	1.2
Gly	8.5	7.7	8.6
Ala	8.7	9.1	11.7
Cys/2‡	0.7	0.9	0.2
Val	7.8	7.0	1.1
Met	0.7	1.1	1.9
Ile	4.6	4.6	2.8
Leu	6.7	6.7	1.4
Tyr	4.3	4.6	4.0
Phe	3.4	3.9	6.5
His	3.1	3.3	1.9
Lys	4.2	6.1	2.1
Arg	4.3	4.2	7.9
Trp	ND§	ND§	1.3

* Values expressed as residues/100 residues found, as compared with protein AA.
‡ Determined as cysteic acid after oxidation.
§ ND, not determined, but typical absorption spectrum for tryptophan was recorded.

a perfect β-pleated sheet, typical of the native amyloid fibrils (14). The protein concentration is also of utmost importance for the in vitro formation of fibrils with properties of amyloid (10). Further studies are presently being performed to characterize the amyloid fibril protein A_{SCA} of SCA in more detail.

Summary

A protein, A_{SCA}, is isolated from amyloid fibrils extracted from heart tissue of five different patients with senile cardiac amyloidosis (SCA). The proteins of all five patients showed immunological identity when reacted with an antiserum raised against one of the proteins. In contrast, no reaction was obtained with antisera against a variety of other amyloid proteins. The antiserum against the subunit protein of senile cardiac amyloid did not react with any other amyloid preparations tested, nor with extracts of normal heart tissue. Thus, the subunit protein appeared to be unique to senile heart amyloid. The protein could form fibrils in vitro, had a mol wt of about 6,000 daltons and the amino acid compositions investigated in two cases showed extensive similarities but were clearly different from that of protein AA of secondary amyloid fibrils.

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