Amyloid Fibril Proteins: Proof of Homology with Immunoglobulin Light Chains by Sequence Analyses

Abstract. *The sequences of the 35 and 36 amino-terminal amino acids of two purified amyloid fibril proteins have been determined. Results indicate that these two proteins are derived from homogeneous immunoglobulin light chains of variable region subgroup $V_{\kappa I}$. The relation between amyloidosis and immunoglobulins is thus more firmly established.*

Amyloidosis is a disease characterized by the extracellular deposition of a microscopically homogeneous fibrillar protein in various tissues leading to morbidity and eventual death when extensive infiltration of the involved organs (such as heart and kidney) occurs. Deposition of amyloid in man may occur alone, or in association with a number of disease states such as multiple myeloma, rheumatoid arthritis, and tuberculosis. Extensive investigations have been carried out in an attempt to determine the nature and pathogenesis of the fibrillar deposits (*1*).

Human amyloid fibrils have been shown to have a characteristic "cross-β" pleated sheet conformation by x-ray diffraction (*2*) and a similar (*3*), but occasionally variable, appearance (*4*) by high-resolution electron microscopy. Techniques have been devised for the concentration of amyloid fibrils from involved tissues (*5*), thus making it feasible to isolate and purify them in order to analyze chemically their major protein constituent. A method for the fractionation of denatured amyloid fibril concentrates was developed and applied to the purification of amyloid fibril proteins from the organs of six patients dying of amyloidosis. A description of the clinical and pathologic findings in these patients and the results of studies of the amino acid composition, the amino-terminal amino acid, molecular weight, and peptide maps of these purified amyloid proteins have been reported (*6*, *7*). Two proteins from different patients had aspartic acid as the amino-terminal residue and were, therefore, suitable for direct amino acid sequence analysis. The sequence of the first seven residues of amyloid X have been reported (*8*). We have now extended the sequence information for amyloid X and report a partial sequence for amyloid VIII-b (*7*), the putative monomer of amyloid VIII.

Suspensions of homogenates of amyloid tissue were subjected to differential centrifugation, and the amyloid fibrils were concentrated (*5*). The isolate of concentrated amyloid fibils (as verified by electron microscopy) was further purified after denaturation by sequential gel filtration on Sepharose 4B and Sephadex G-100 columns with *5M* guanidine hydrochloride in *1N* acetic acid (*6*, *7*), and the major protein fraction was completely reduced and alkylated. The purified reduced and alkylated protein of each amyloid fibril preparation gave a single band when subjected to electrophoresis on 0.1 percent sodium dodecyl sulfate (SDS) polyacrylamide (10 percent gels) at *p*H 7.1 (*6*). If the amyloid fibril concentrate from differential centrifugation was taken as the starting material, purification by gel filtration led to an approximate loss of 30 percent of protein content.

Sequences of 4 mg of amyloid X and 6 mg of amyloid VIII-b were determined with an automatic amino acid sequencer (Beckman model 890) according to the method of Edman and Begg (*9*). The thiazolinone derivatives obtained from the sequencer were converted to the phenylthiohydantoins of the amino acids and identified by means of liquid-gas chromatography (*10*). Yields of the first amino acid were approximately 50 percent of theoretical, a result similar to that found with many other extensively reduced and alkylated homogeneous proteins.

The amino-terminal sequences of these two proteins (*11*) are compared in Table 1 with that of Ker, a Bence Jones κ protein of variable region subgroup $V_{\kappa I}$ (*12*). Ker and amyloid VIII-b are identical for 29 of the 30 residues for which they can be compared. The sequence of Ker beyond step 30 has not been published, and because of technical problems there is uncertainty concerning the residues at positions 31 and 34 in the sequence of amyloid VIII-b and in positions 22, 26, 30, 34, and 35

Table 1. Sequence analyses of amyloid protein X and VIII-b as compared to the sequence of the prototype $V_{\kappa I}$, Ker (*12*). Variant residues are underlined; undetermined or equivocal residues are indicated by brackets.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ker	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
Amyloid X	Asp	Ile	Gln	Met	Thr	Gln	Ser	Ala	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
Amyloid VIII-b	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Ker	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	Asp	Ile	Lys						
Amyloid X	Val	Ile	Ile	[ ]	Cys	Glx	Ala	[ ]	Glx	Asx	Ile	[ ]	Pro	Tyr	Leu	[ ]	[ ]	Tyr
Amyloid VIII-b	Val	Thr	Ile	Thr	Cys	Gln	Ala	Ser	Gln	Asx	Ile	Gly	[ ]	Tyr	Leu	[ ]	Trp	

in the sequence of amyloid X. Amyloid X is also homologous with the other two proteins, although it does differ from them in at least two of the first 24 positions.

Amyloids VIII-b and X are, therefore, portions of κ-type light chains of the $V_{\kappa I}$ variable region subgroup. The molecular weight of an intact light polypeptide chain is approximately 22,500. The molecular weights of 7,500 for amyloid X and 18,300 for amyloid VIII-b, as determined by both SDS polyacrylamide gel electrophoresis and on calibrated Sephadex G-100 columns (*6*), indicate that these proteins are not complete light chains. Since both proteins start with the appropriate amino-terminal sequence, it is possible that these represent light chain fragments with varying amounts of the constant portion degraded. Alternatively, amyloid may consist of light polypeptide chains having internal deletions of varying lengths affecting variable regions, constant regions, or both, such as those reported in proteins from patients with "heavy chain disease" (*13*).

The losses on purification and the yield on sequence analysis (when compared with known homogeneous light chains) indicate that the proteins sequenced are the major components of amyloid and are not minor contaminants. Since only 30 percent of the protein content of the fractionated amyloid fibril concentrate is lost in purification and since sequence analysis and disc-gel electrophoresis give evidence of the presence of a single monomeric component in the final preparation, the possibility that nonspecifically absorbed homogeneous immunoglobulins accounted for 70 percent of the amyloid fibril concentrate would appear highly unlikely. The major protein component of these amyloid preparations is, therefore, a portion of an immunoglobulin polypeptide chain.

Observations and speculations concerning the possible relation between "antibodies" and amyloid deposits were made initially by Magnus-Levy (*14*) and Apitz (*15*). Experimental support for these speculations was later provided by the studies of Vasquez and Dixon (*16*) and Mellors and Ortega (*17*). Osserman *et al.* (*18*) analyzed the urines and serums of a large series of amyloid patients, and using more modern concepts of immunoglobulin structure they concluded that gamma globulins, particularly light chains, were directly involved in the amyloid infiltrates. Our data prove that in at least some cases, the major protein component of the fibrils of amyloid deposits are portions of light polypeptide chains.

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Plasmodium berghei: Enhanced Protective Immunity after Vaccination of White Rats Born of Immune Mothers

Abstract. *Young white rats born of immune mothers had a significantly higher level of immunity to* Plasmodium berghei *after immunization with a nonliving antigen than either unvaccinated littermates or vaccinated rats born of normal nonimmune mothers.*

Partial protective immunity to *Plasmodium berghei* can be conferred by passive transmission of maternal antibody (*1*) and by artificial active immunization with a nonliving antigen (*2*). The combined effect of these two factors has not been studied before. Data presented here suggest that vaccination of white rats born of immune mothers confers a significantly higher level of protective immunity than vaccination of rats born of normal mothers.

An inbred strain of Wistar white rats and the NYU-2 strain of *P. berghei* constituted the host-parasite system in my investigation. An initial study on the relation of age and weight to pathophysiology revealed that, in this system, 50 to 80 percent of rats weighing 75 g (about 28 days old) to 100 g (about 34 days old) would die of acute infection characterized by high parasitemia and hepatorenal damage. In my study, animals were vaccinated when they weighed 50 g (about 21 days old), and the challenging infective inoculum was given at 75 g. This procedure was

Table 1. Mortality rates, average peak parasitemias, and prepatent periods after challenge with *Plasmodium berghei* in vaccinated and unvaccinated young white rats born of immune and normal nonimmune mothers.

Group		Mortality (%)	Average peak parasitemia (% infected erythrocytes)	Average prepatent period (days)
Description	No.			
Unvaccinated progeny of normal mothers	51	70	50 ± 13.6	1.2
Unvaccinated progeny of immune mothers	47	19	32 ± 14.1	3.2
Vaccinated progeny of normal mothers	40	40	41 ± 12.2	1.3
Vaccinated progeny of immune mothers	35	0	8 ± 5.3	4.2