Antisera Specific for Human Amyloid Reactive with Conformational Antigens[1] (36504)

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Amyloid, a substance which is widely distributed in a large number of species, assumes a typical fibrillar appearance in tissues and in the isolated state. The recent observation by Glenner *et al.* (1) that the major constitutent of most if not all amyloid fibrils is a fragment of immunoglobulin light chains containing primarily the variable region represents a significant advance in our understanding of the chemical nature of amyloid. In the light of this finding it is difficult to explain several previously noted antigenic properties of amyloid fibrils and certain of their subunits (2–4). One of these, the failure of amyloid fibrils and subunits prepared either with 0.1 *M* NaOH or by reduction and alkylation to react with antisera to κ or λ light chains, may be in part due to the inaccessibility of these determinants in the completely or partially folded molecule and also due to the poor immunogenicity of the antigenic determinants of the variable region of the light chain. As a consequence antisera to light chains frequently react weakly or not at all with the v region of light chains. These possibilities are supported by the finding of Glenner *et al.* (5) that antisera, prepared against fully reduced and alkylated amyloid subunits, which presumably have most of the antigenic determinants readily accessible, react with either kappa or lambda Bence Jones proteins depending on the type of amyloid protein. The other apparent inconsistency is the ability to produce antisera to sodium hydroxide degraded amyloid (DAM) which fail to react with immunoglobulin light and heavy chains, but which react with isolated amyloid fibrils and DAM, and with amyloid deposits in tissues from many patients (6). This brief report presents evidence that DAM continues to have certain features characteristic of a fibrillar substance in the EM, but that these are lost following reduction and alkylation. The reaction of these antisera with intact DAM but not with the subunits produced by reduction suggests that these antisera may be directed against certain conformational features of the amyloid fibrils and thus supports their value as specific reagents to detect amyloid deposits in tissues.

Materials and Methods. Amyloid fibrils were isolated from tissues by methods previously described (7). Degraded amyloid (DAM) was prepared by treatment with 0.1 *M* NaOH for 1–3 hr, followed by dialysis against distilled water. Bence Jones proteins were purified from the urines of patients with multiple myeloma by precipitation with 50% saturated $(NH_4)_2SO_4$ followed by starch zone electrophoresis. Complete reduction and alkylation was carried out for 1 hr with 0.1 *M* 2-mercaptoethanol in Tris HCl buffer (pH 8.2) made 6 *M* with guanidine HCl followed by the addition of a twofold excess of iodoacetic acid for 1 hr. The material was then dialyzed against distilled water. Antisera to DAM, gamma, kappa and lambda chains are similar to those previously described (2, 8). Immunologic studies were done by double diffusion in agar (9).

For analysis by electron microscopy DAM in distilled water was added to an equal volume of 2% phosphotungstic acid at pH 5.5.

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TABLE I. Antigenic and Ultrastructural Properties of Native and Reduced and Alkylated (R & A) DAM and Bence Jones Proteins.

Material	Reactivity with		Fibrillar structure
	Anti-DAM	Anti-BJP	
DAM	+++	−	+
DAM (R & A)	−	−	−
BJP	−	+++	ND
BJP (R & A)	−	+	ND

A drop of this mixture was applied to a carbon-coated Formvar covered grid and allowed to dry. A Siemens Elmiskop I electron microscope equipped with a cooling device was used to view the specimens.

Results. Table I summarizes the results of the immunologic studies. DAM produced from amyloid by treatment with 0.1 *M* NaOH for 1–3 hr reacted with antisera to DAM. Following complete reduction and alkylation all reactivity was lost. Reduction and alkylation of 3 κ and 4 λ Bence Jones proteins under similar conditions generally did not completely eliminate reactivity with antisera to light chains. In most instances a large precipitate appeared and the reactivity decreased significantly but a precipitin line continued to be observed; only one κ protein failed to react in Ouchterlony analysis after complete reduction.

Figure 1a–c compare the structure of native amyloid fibrils with that of DAM prepared by treatment of the protein with 0.1 *M* NaOH for 1–3 hr. While amyloid fibrils are of indeterminate length, the longest filaments found in DAM did not exceed 180 mμ. The majority of filaments consisted of shorter fragments, with the shortest unit distinguishable above background grain measuring 105 Å. Lateral aggregation of filaments was still noticeable (arrow Fig. 1b) giving rise to short, stubby appearing fibrils which ranged in width from 105 to 400 Å. Although the smallest recognizable units were abundant in every DAM preparation, the impression was gained that the ratio of short to long fragments increased as the time of NaOH digestion was extended from 1 to 3 hr. However even in the 3 hr specimen some 150 mμ fibrils were still seen. After complete reduction and alkylation only amorphous material remains (4).

Discussion. Previous studies have demonstrated that native amyloid fibrils are poorly or nonimmunogenic, while partially degraded amyloid fibrils can induce antibodies specifically reactive with amyloid and nonreactive with Ig light chains which are now known to be a constituent of amyloid (2). The results of the present study demonstrate that DAM continues to consist of fragments of fibrils and suggest that some of its conformational determinants may be responsible for the observed immunologic reaction.

Since longitudinal fragmentation of the amyloid fibril appears to be the major morphologic alteration effected by NaOH, it seems conceivable that the crucial antigenic determinants are located at the end of the filaments rather than along their lateral aspect. If this were the case, prolonged digestion with NaOH might enhance the immunogenicity of DAM. Moreover, since native amyloid fibrils are not phagocytosed *in vitro* without prior opsonization the improved antigenicity of DAM could also be attributable to the small, nonfibrillar fragments which may be susceptible to phagocytosis and thus facilitate the afferent limb of the immune response. This hypothesis could be tested.

It appears likely that DAM, when presented to an animal as an immunogen, induces the synthesis of antibodies directed against certain conformational antigens of the molecule, and that antibodies to the primary amino acid sequence determinants are not produced until the molecule is completely unfolded by complete reduction and alkylation with disruption of the fibrillar structure. The concept that antibodies to native proteins are directed largely against conformational rather than sequential antigens has been reviewed by Kaminski (11) and Sela *et al.* (12). A related phenomenon has been clearly demonstrated recently in the striking cross reaction between reduced and alkylated hen lysozyme and reduced and alkylated human lysozyme and bovine α lactalbumin when

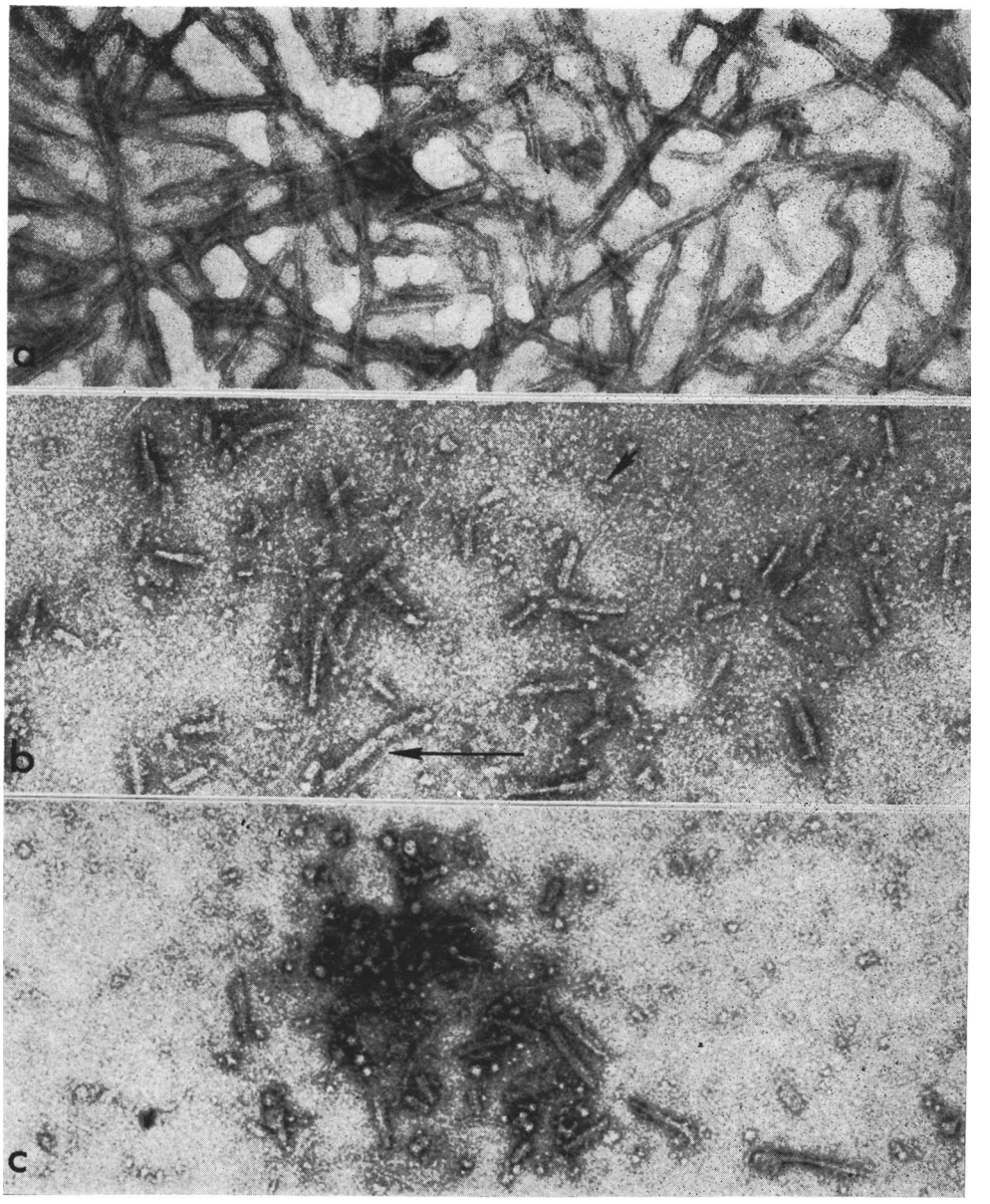

FIG. 1a. Native amyloid fibrils of indeterminate length show lateral aggregation of 50 Å filaments. Negative contrast with 2% phosphotungstic acid. (b) Fragments of amyloid digested with sodium hydroxide, NaOH, for 1 hr (DAM). Short arrow points to short stubby fragment measuring only ± 200 Å in length whereas long arrow points to much longer fibril. Lateral aggregation of filaments is discernible in almost all fragments. (c) DAM following 3 hr digestion of native amyloid fibrils. The number of shorter fragments is increased. Magnification a–c: ×90,000.

little if any cross reactivity was noted between the respective native proteins (13, 14).

The apparent dissociation of antibody production to the conformational determinants and determinants associated with the primary amino acid sequence may well prove of practical value in studying amyloid. Since antibodies to the fibrillar subunit react only with

amyloid derivatives having the characteristic tertiary structure and not with the ubiquitous immunoglobulins which appear to be the basic subunit, they should be useful in detecting amyloid in tissues diagnostically and in studying some of the similarities and differences in the fibrillar structure of different types of amyloid. In contrast antibodies to the basic subunit will prove of value in comparing the primary structure of different types of amyloid.

Summary. Antisera reactive specifically with DAM (degraded amyloid) and amyloid and unreactive with any serum proteins appear to be directed to conformational antigenic determinants since they no longer react with DAM after complete reduction and alkylation.

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