# Characterization of the Amyloid Fibril as a Cross-$\beta$ Protein* (34110)

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The major component of amyloid is a fibrous protein (1) with a unique organization as seen by high resolution electron microscopy (2). A second component, a pentagonal rod, has been isolated from amyloid deposits and demonstrated to be identical with a circulating $\alpha$ globulin (P-component) (3). This component is distinct from the fibril in its ultrastructure, chemistry, and immunological interactions and constitutes only a minute part of amyloid deposits.

In 1960 we obtained an X-ray diffraction powder pattern of an amyloid-rich tissue. In subsequent years improved methods of isolation of amyloid were devised and X-ray diffraction patterns of these more purified preparations were obtained. Results were presented[1] that indicated the presence of a cross-$\beta$ pattern. The present study was carried out on amyloid fibrils isolated by various techniques to determine whether this molecular structure was consistently present in all preparations, including those freed from the pentagonal unit.

*Materials and Methods.* Amyloid fibrils were obtained from the spleens of patients with primary, secondary, and myeloma-associated amyloidosis and prepared by the following methods: (1) homogenization of whole amyloid-laden spleen, centrifugation and recovery of a top-layer rich in amyloid fibrils (4); (2) sucrose gradient separation of the top-layer specimen to obtain a purer preparation (5); (3) reprecipitation of amyloid fibrils after they had been solubilized in dilute alkali at pH 11.5 (6); (4) a "water-soluble" amyloid prepared after purifying amyloid-laden spleen by exhaustive saline washes followed by homogenization in water (7).

In addition, several of the above preparations were further purified by delipidization using a chloroform-methanol (2:1) mixture for lipid extraction 8). As a control normal human spleen tissue was prepared and examined in a similar manner.

Aliquots of the above preparations were suspended in dilute ammonia (pH 11.5) or water by stirring gently overnight. The suspensions were then centrifuged at 50,000 rpm (159,000$g$) in a Spinco model L65 ultracentrifuge, with a fixed-angle rotor. Virtually all of the protein was spun down into a compact pellet by this technique. The pellet was carefully removed and allowed to dry in air at room temperature on a siliconized microscope slide. A sample of 0.5 $\times$ 2 mm was cut from the resulting thin film. One end of this sample was attached with glue to the end of a thin glass fiber, and mounted so that the surface of the film was parallel to the X-ray beam, with the X-ray beam passing through the 0.5-mm dimension. Either the centrifugation or the drying, or a combination of both, developed sufficient orientation in the sample to produce clearly oriented X-ray diffraction patterns.

X-ray diffraction patterns were obtained with nickel-filtered copper radiation. Wide-angle diffraction patterns were obtained with a Norelco microcamera, with a 100$\mu$ glass-

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[1] Boston University Seminars in Molecular Biology, Apr. 12, 1968 and Boston University School of Medicine, April 6, 1967.

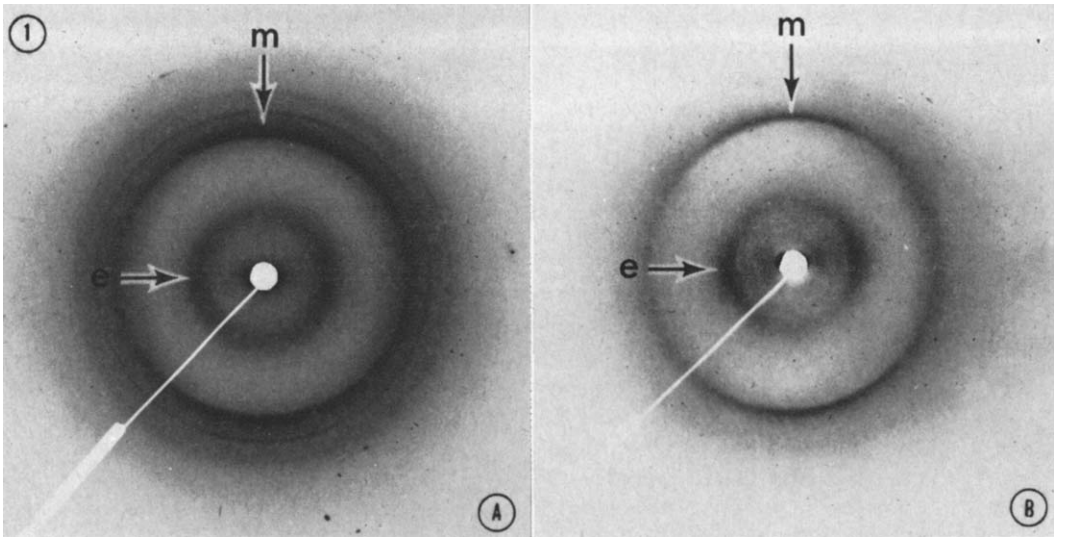


FIG. 1. X-ray diffraction patterns of amyloid (1A) and delipidized amyloid (1B) isolated from a patient with primary amyloidosis. The 4.68 A meridional arc (m), and 9.8 A equatorial arc (e) are indicated. Crystalline diffraction spots from an unidentified contaminant which were occasionally seen are evident in Fig. 1A.

capillary collimator and a specimen-to-film distance of 15 mm or with a flat-film camera with 57.6 mm specimen-to-film distance and a beam-size of about 0.5 mm at the film. Low angle diffraction photographs were obtained with a Rigaku-Denki Model 2 low-angle camera-diffractometer.

*Results.* Figure 1A shows a typical wide-angle X-ray diffraction pattern of amyloid. A sharp meridional arc at 4.68 A and a more diffuse equatorial arc at 9.8 A are clearly evident. These characteristic reflections indicate that amyloid, prepared as above, has a cross-$\beta$ configuration. All amyloid fibril specimens (primary, secondary, myeloma-associated) showed these patterns.

In addition to the cross-$\beta$ reflections at 9.8 A and 4.68 A, some amyloid diffraction patterns also show unoriented reflections at 4.13 A, 5.17 A, and 5.76 A and equatorial reflections at 46.5 A, 34.3 A, 17.0 A, and 9.3 A. These non-cross-$\beta$ reflections did not appear with any consistency, and when present had variable intensities relative to the cross-$\beta$ reflections, and to one another. They were greatly reduced in intensity (but did not disappear altogether) on patterns obtained from delipidized amyloid (Fig. 1B) and many of the spacings were suggestive of lipid material. This evidence strongly suggests that the non-cross-$\beta$ lines observed were due to a lipid contaminant, and were not a characteristic of the amyloid material itself.

X-ray powder diffraction patterns of lyophilized amyloid or of amyloid-rich tissue showed a diffuse halo at approximately 9.8 A, and a relatively sharp ring at 4.68 A, superimposed on a more diffuse halo centered at about 4.5 A. The radial sharpness of this ring, corresponding to the 4.68 A meridional reflection observed with oriented cross-$\beta$ specimens, distinguishes the amyloid powder pattern from powder patterns of another $\alpha$ or $\beta$ protein, although these materials have diffraction maxima at the same spacings. [This same distinguishing sharpness of the 4.68 A ring has previously been noted in frog epidermis prekeratin (9)]. The powder patterns also frequently show a moderate to strong lipid ring at 4.13 A. Normal spleen tissue prepared as described above invariably yielded collagen X-ray diffraction patterns.

*Discussion.* Originally the cross-$\beta$ configuration in proteins was thought to occur only in denatured forms of $\alpha$ or parallel $\beta$ structures. More recently, this configuration has

been found in a variety of proteins prepared by very mild methods, including insect silk (10), embryonic dental enamel (11), and prekeratin. The present report indicates that amyloid should be added to the list of naturally occurring cross-$\beta$ proteins.

Ashkenazi *et al.*, have reported X-ray diffraction studies on amyloid (12). They did not interpret their results as suggesting a cross-$\beta$ structure, although their published pattern resembles those described here. They interpret the equatorial reflections at 46.5 A, 34.3 A, 17.0 A, as indicating the presence of parallel arrays of scattering elements separated by a mean distance of 47 A. Our studies strongly suggest these reflections are due to a lipid-contaminant.

The cross-$\beta$ configuration structure is generally assumed to be closely related to the pleated-sheet configurations proposed by Pauling and Corey (13) with the polypeptide chain running transverse to the fiber axis of the specimen. This suggests, in view of the ultrastructural fibrillar appearance of amyloid noted by Shirahama and Cohen (2), that the width of the pleated-sheets (in the direction of the polypeptide chain length) must be small, perhaps as small as 10–15 A, or that a fibrous modification of the pleated-sheet exists (14).

Finally, the fact that all types of amyloid specimens, prepared in several fashions, give a cross-$\beta$ diffraction pattern (as do lyophilized preparations of amyloid or amyloid-rich tissue) strongly suggests that the cross-$\beta$ configuration exists in the intact protein, and is not produced by denaturation.

*Addendum.* Since the preparation of this report, Eanes, E.D. and Glenner, G.G., [J. Histochem. Cytochem. **16** 673 (1968)] have published patterns essentially identical with those of the delipidized amyloid reported here.

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