Carbohydrate Composition of Amyloid Components[1] (35536)

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Amyloid, a pathological deposit in the tissues of patients with amyloidosis, contains one major protein component, the fibril, and one minor component, the pentagonal structure (1, 2). The characterization of these two proteins remains incomplete. The amino acid composition of the fibril, determined on a limited number of specimens, and the amino acid composition of the pentagonal structure have recently been presented (2, 3). Amyloid also contains a small amount of carbohydrate, but studies of the individual component sugars of the fibril and the pentagonal structure have not been reported. The results of such a study are presented below.

Materials and Methods. Fibrils were obtained from the spleens of two patients with primary amyloidosis by a modification of the procedure of Pras *et al.*(4). Seven to 10 g of spleen were repeatedly homogenized in 0.15 *M* NaCl, and centrifuged each time at 12, 000*g* for 20 min. The supernatants of the first two extractions were discarded; and the remaining six supernatants were pooled and saved for the isolation of the pentagonal structure. The insoluble precipitate remaining after saline extraction was homogenized at high speed in deionized and deaerated water, centrifuged, and the first supernatant was discarded. The water extraction was repeated until the absorbance at 280 mμ of the supernatant became minimal. All protein-containing extracts were pooled, and the proteins were reprecipitated by addition of 2 *M* NaCl to a final concentration of 0.15 *M*. This was followed by a second series of extractions with water to obtain "purified fibrils."

Pentagonal structures were prepared from one of the spleens mentioned above and from the kidney of a patient dying with secondary amyloidosis. The pentagonal structures in the original combined saline supernatants were purified by repeated cycles of precipitation in 0.1 *M* acetate buffer at pH 4.5 and solubilization in glycinate buffer at pH 9.5 (5). Further purification was achieved by filtration through Bio-Gel P-300 in a column equipped with an upward-flow adaptor.

Preparations of fibrils and pentagonal structures were applied to formvar coated grids, and were stained negatively with phosphotungstic acid for examination in the electron microscope.

Starch gel electrophoresis of the preparations was performed in 0.1 *M* glycinate buffer, pH 9.0, and borate buffer, pH 8.5. The gels were stained with amido black.

Cellulose acetate electrophoresis was carried out in a Beckman microzone apparatus using 0.075 *M* barbital buffer, pH 8.6.

Sedimentation velocity studies were performed in a Spinco Model E ultracentrifuge.

Amino acid analyses of the fibril and the pentagonal structures were carried out on a Spinco amino acid analyzer using the accelerated system of Spackman *et al.* (6) after hydrolysis of the preparations for 20 hr at 105° with 6 *N* HCl.

Amino sugars were analyzed on the short column of the amino acid analyzer after hydrolysis of the preparations in 4 *N* HCl for 4 hr at 105° and by the method of Good and Bessman (7).

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Neutral sugars in the two preparations were determined by gas liquid chromatography of their alditol acetates (8). Sialic acid was measured by the thiobarbituric acid method of Warren (9). Hexuronic acid was measured by the carbazole method according to the modification of Bitter and Muir (10).

Results and Discussion. The best recovery of fibrils in the aqueous extract was 40% of the dry weight of the spleen; whereas the recovery of pentagonal structures was only 2% of the dry weight of the tissue. Thus it is evident that the fibrils constitute the major component of the amyloid. This is consistent with the observations of previous investigators (1).

Electron microscopy of the fibril preparations revealed only the presence of the typical double-stranded fibrils 80–100 Å in diameter. Similarly, only pentagonal structures, as described by Bladen *et al.* (11), were found upon examination of these preparations in the electron microscope. The pentagonal structures migrated as a single band in starch gel electrophoresis. The fibril preparations remained at the origin and did not migrate into the gel. Microzone electrophoresis of the pentagonal structures revealed a single zone with a mobility close to that of serum albumin. Ultracentrifugation of the pentagonal structure preparations revealed a single sharp peak sedimenting at approximately 11S; whereas the aqueous fibril extract sedimented above 40S. The pentagonal structures readily dissolved in 0.1 *M* glycinate buffer, pH 9.5; whereas the fibril preparations were poorly soluble, even after repeated sonication in this buffer. Thus the fibrils and the pentagonal structures are different with respect to morphology, molecular size, and solubility.

Amino acid analyses of both preparations revealed the absence of hydroxyproline and hydroxylysine, indicating the absence of collagen. The amino acid analyses generally agreed with the results reported in the literature (3, 4, 12) and are thus not reported here.

Carbohydrate analyses (Table I) revealed the presence of sialic acid, glucosamine, glucose, galactose, and mannose, and small amounts of fucose in both preparations. The pentagonal structure preparations contained approximately 4 and 5% carbohydrate and the fibril, half that amount. Furthermore, the molar ratios of the individual sugars in the two preparations were different. The carbohydrate moiety of the pentagonal structure was richer, with respect to glucosamine and sialic acid, than the fibril preparations. Galactose was consistently the major neutral sugar in the fibrils; whereas the molar ratio of galactose to mannose in the pentagonal structure was close to unity.

TABLE I. Carbohydrate Composition of Amyloid (μmole/mg).

	Fibril		Pentagonal structure	
	Spleen	Spleen	Spleen	Kidney
Fucose	0.0021	0.003	0.005	0.001
Mannose	0.0189	0.013	0.053	0.028
Galactose	0.0234	0.022	0.052	0.031
Glucose	0.0118	0.008	0.016	0.011
Hexosamine	0.034	0.039	0.098	0.075
Sialic acid	0.017	0.018	0.036	0.04
Total % CHO	2.14	2.08	5.13	3.85

The application of the carbazole test for hexuronic acid produced a yellow rather than the typical pink color usually seen with hexuronic acids. This color was more typical of neutral sugars than of hexuronic acid and it was concluded that hexuronic acid was not a component of either preparation. This is in contrast with the results of other investigators who have reported the presence of hexuronic acid in fibril preparations (2–4). This may reflect a greater purification of the preparations in the present study in view of the observation of Dalferes *et al.* (13) that amyloid-laden tissues contain considerable acid mucopolysaccharide, mainly heparitin sulfate.

These data indicate that amyloid fibrils and pentagonal structures from the same specimen differ with respect to their carbohydrate composition, as well as in their physical properties. This reinforces the general conclusion reached by others (2, 5) that the two proteins are chemically quite distinct. The availability of more material and further analyses will be required to solve the question of the identity of amyloid occurring in the primary and secondary forms of the disease.

Summary. The two ultrastructural components of amyloid, the fibril and the pentagonal structure, have been isolated and their carbohydrate composition was determined. The data add to the evidence that these structures are different glycoproteins and are distinct from other fibrous proteins.

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