Virchows Arch. A Path. Anat. and Histol. 373, 161 – 166 (1977)

Virchows Archiv A

Amyloid of Human Islets of Langerhans

II. Electron Microscopic Analysis of Isolated Amyloid

Per Westermark

Institute of Pathology, University of Uppsala, Box 553, S-751 22 Uppsala, Sweden

Summary. Isolated amyloid from the islets of Langerhans of patients with maturity onset diabetes mellitus was compared with amyloid fibrils from patients with different types of systemic amyloidosis. It was found that systemic amyloids had in common rigid and non-branching filaments with a width of about 75 Å and that these filaments sometimes were attached laterally, forming thicker fibrils. Similar filaments could also be extracted from islet amyloid but the main part of this amyloid was built up by large aggregates of very thin and often very wavy units. This structure, which has not been previously described in human amyloid, probably explains some properties of isolated islet amyloid.

Key words: Amyloid fibrils – Islets of Langerhans.

Introduction

Since the first description of amyloid as an ultrastructurally fibrous protein (Cohen and Calkins, 1959), a large number of studies have shown that amyloid fibrils isolated from patients with systemic amyloidoses are approximately 100 Å wide, non-branching and rigid (Emeson et al., 1966; Shirahama and Cohen, 1967; Cohen, 1968; Gueft et al., 1968; Hirschl, 1969; Cohen and Shirahama, 1973), and that these fibrils are built up by finer subunits. Attempts to find differences between fibrils from different types of systemic amyloids have failed (Cohen and Shirahama, 1973) and even some laboratory-made pure protein fibrils can share at least some of the amyloid ultrastructural patterns (Glenner et al., 1974; Westermark, 1974a). The studies have hitherto been made on amyloid fibrils isolated from patients or animals with systemic amyloidosis, but amyloid also occurs in non-systemic forms. A well-known example is amyloidosis

Supported by the Swedish Medical Research Council (Project No. 102) and Nordisk Insulinfond. Thanks are due to Anni Bedy and Cristina Bittkowski for skilled technical assistance

of the islets of Langerhans. This amyloid is probably a product of the B-cells (Westermark, 1973) and is chemically distinctly different from systemic amyloids (Westermark, 1975b and 1976). The present paper is an ultrastructural study where isolated islet amyloid has been compared with amyloid fibrils from patients with systemic amyloidosis. A structure is described which hitherto is unique to islet amyloid.

Material and Methods

The pancreas of two patients with maturity onset diabetes mellitus and marked amyloidosis of the islets of Langerhans were used. The pancreases were treated by the method of Pras et al. (1968) and the water extractable material (Pras et al., 1968) as well as the bottom layer (Westermark, 1975b) were used for electron microscopy. As controls, amyloid fibrils from five patients with systemic amyloidoses of various types were isolated by extraction with distilled water (Pras et al., 1968). Insulin fibrils were created as described by Burke and Rougvie (1972). Dilute suspensions of fibrils in water were put on formvar coated copper grids and air-dried after that the excess was blotted off with a filter paper. The specimens were then negatively stained with 2% ammonium molybdate or 2% phosphotungstic acid at pH 5.3. Pieces of tissue were fixed in glutaraldehyde, post-fixed in osmium tetroxide, embedded in Epon and ultrathin sections were cut on an LKB Ultrotome. The sections were contrasted with uranyl acetate and lead citrate. The specimens were studied in a Zeiss EM 9 electron microscope at 60 kV or in a Jeol 100 C electron microscope at 80 kV.

Observations

Tissue Sections

In systemic amyloidosis, the amyloid appeared as a rather loose arrangement of criss-crossing, straight, non-branching fibrils of usually about 100 Å thickness. Sections of islet amyloid in part closely resembled this appearance. However, in many areas it was difficult to find any fibrillar substructure, but the amyloid had a rather homogeneous structure.

Isolated Amyloid

Systemic Amyloid. The ultrastructure of amyloid fibrils from cases of systemic amyloidosis has been described in several papers and is only briefly described here. The fibril morphology differed from case to case but all preparations had in common a filament[1] of a width of about 75 Å (Figs. 1–3). These filaments were sometimes separated, especially in two cases of secondary amyloidosis (Fig. 2) but were often attached laterally, forming thicker fibrils. Sometimes the subunits (protofibrils) of the filaments were evident, especially in a case of primary amyloidosis (Fig. 1). These protofibrils were about 40 Å in width. The fibrils were straight and non-branching and their length varied greatly.

[1] The nomenclature of Shirahama and Cohen (1967) is used here

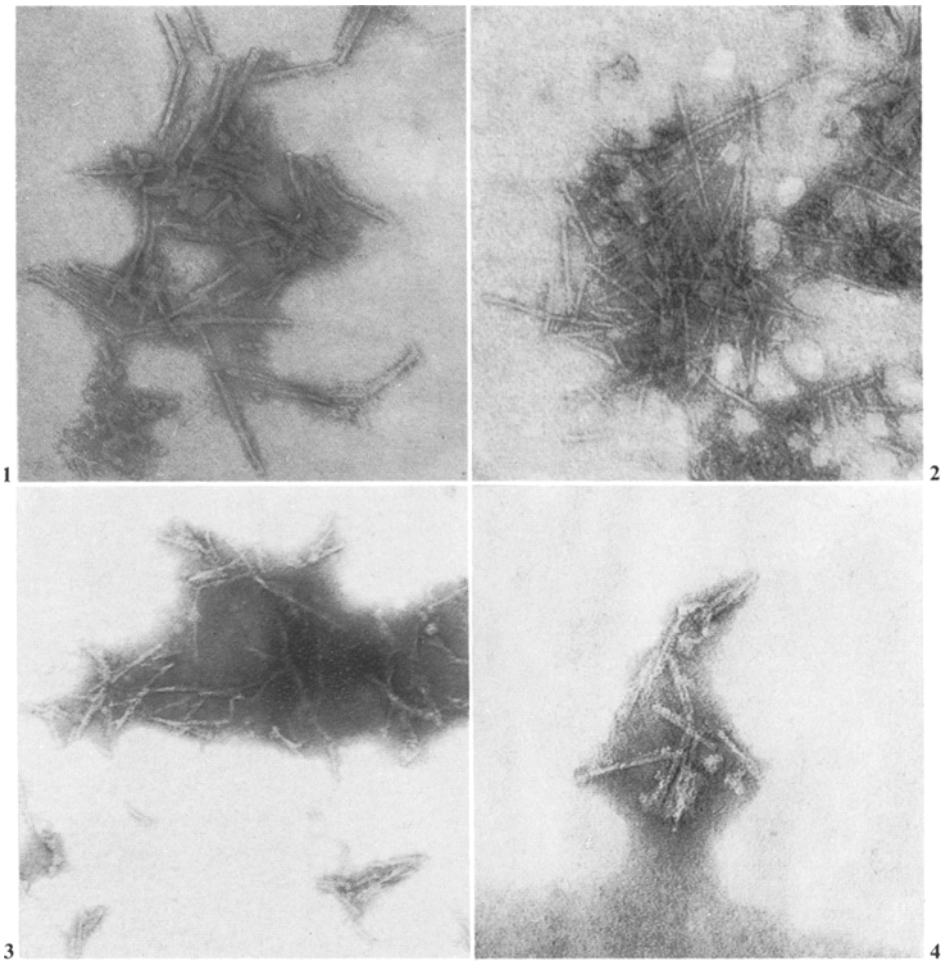

Fig. 1. Isolated amyloid fibrils from a patient with primary amyloidosis. Besides filaments of the same dimensions as in Figure 2 multiple protofibrils of about 40 Å in width are found. Negatively stained with ammonium molybdate. × 100,000

Fig. 2. Isolated amyloid fibrils from a patient with secondary amyloidosis. Filaments are about 75 Å in width. Negatively stained with phosphotungstic acid. × 100,000

Fig. 3. Isolated amyloid fibrils from a patient with senile heart amyloidosis. Filaments of about 75 Å in width are dominating. Negatively stained with ammonium molybdate. × 100,000

Fig. 4. Isolated amyloid fibrils from the islets of Langerhans (water extractable material). The fibrils are morphologically comparable to some fibrils seen in Figures 1–3. Negatively stained with ammonium molybdate. × 130,000

Islet Amyloid

Water Extractable Material. This material contained mainly cell membranes and other unrecognisable particles. However, rather frequently areas with filaments which were about 75 Å in width, rigid, and non-branching, were seen

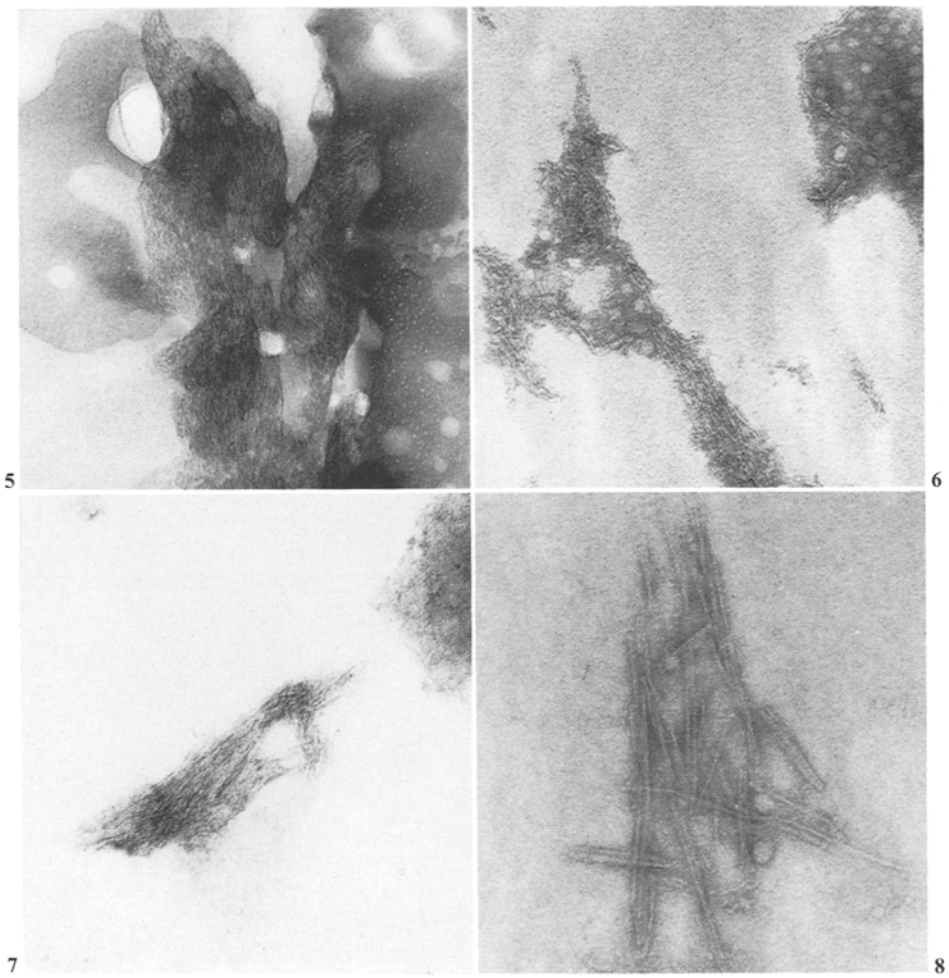

Fig. 5. Isolated amyloid from the islets of Langerhans (bottom layer). This particle consists of several hundreds of fine, 30–40 Å thick, wavy filaments, closely attached laterally. No fibrils are seen. Negatively stained with ammonium molybdate. × 100,000

Fig. 6. Isolated amyloid from the islets of Langerhans. The fine filaments are in this picture more separated from each other, but do not form fibrils. Negatively stained with ammonium molybdate. × 130,000

Fig. 7. Isolated amyloid from the islets of Langerhans. A smaller particle. Negatively stained with ammonium molybdate. × 130,000

Fig. 8. Insulin fibrils. Rather long filaments of about 40–50 Å in width are seen, sometimes attached laterally to fibrils. Negatively stained with phosphotungstic acid. × 100,000

(Fig. 4). These filaments were similar to those found in systemic amyloid. Sometimes broader fibrils were also found.

Bottom Layer. This material showed a picture, which differed from the water extractable material. In contrast to systemic amyloid, fibrils composed of fila-

ments were rare. Instead, a common finding was large aggregates of more or less parallelly attached thin fibrillar structures with a width of 30–40 Å (Fig. 5). These structures thus could correspond to the protofibrils of systemic amyloid (Shirahama and Cohen, 1967). The "protofibrils" were most often wavy and not as straight as amyloid filaments. It was also very common to find separated wavy "protofibrils", which occasionally formed small networks (Figs. 6 and 7). Rarely, fibrils of the same appearance as in the water extractable material were found.

Insulin Fibrils

Insulin fibrils were composed of parallelly attached, often very long subunits of about 40 Å in width (Fig. 8). These subunits were straight or slightly wavy but never showed the pattern of islet "protofibrils".

Discussion

Morphologically, amyloid seems to be composed of two different structures, the P-component and the fibril. The P-component is a pentagonally shaped protein (Skinner et al., 1974) which is soluble in normal saline and which easily can be extracted from amyloid-laden organs after homogenization in that solution. All human amyloids studied until now, including those of the islets of Langerhans (Westermark et al., 1975) and of medullary carcinoma of the thyroid (Sletten et al., 1976) contain P-component. The other component, the fibril, which exhibits the staining properties of amyloid substance, can usually be extracted from amyloid-infiltrated organs with distilled water and it is possible in this way, to separate fibrils from other insoluble tissue components (Pras et al., 1968). Islet amyloid and amyloid of medullary carcinoma of the thyroid are exceptions from this rule since most of these amyloid materials are not extractable with distilled water (Westermark, 1975a and b).

One reason why systemic amyloid fibrils are extractable with distilled water seems to be the fact that 1–3 filaments are held together in units (fibrils) but that these units easily can be separated from each other. In contrast, as is shown in the present paper, a large part of the islet amyloid is built up by large aggregates of very thin fibrils (protofibrils) and these particles are probably not water soluble. These large aggregates probably correspond to the rather homogeneous areas seen in positively stained sections where the very fine fibrils probably are too small to be visualized with this technique (cf. Shirahama and Cohen, 1967). Besides the aggregates, the islet amyloid after homogenization revealed fibrils indistinguishable from other amyloid fibrils and which could be extracted with distilled water.

The insulin fibrils resembled the fibrils of systemic amyloids and the relatively rare fibrils of islet amyloid rather than the aggregates of very thin fibrils reported here. Material with immuno-reactivity of insulin can be extracted from islet amyloid (Westermark, 1974a and b) but insulin does not seem to constitute any major part of it (Westermark, 1976b). However, regarding the similarity

of islet amyloid to amyloid of medullary carcinoma of the thyroid (Pearse et al., 1972; Westermark, 1975a) and the hormone nature of the latter (Sletten et al., 1976), it seems very probable that an insulin-related protein forms a major part of islet amyloid.

Thus, two rather different structures of islet amyloid were found. Whether these two structures are chemically identical or not is unknown at present.

References

Burke, M.J., Rougvie, M.A.: Cross-β protein structures. I. Insulin fibrils. Biochemistry **11**, 2435 (1972)

Cohen, A.S.: High-resolution ultrastructure, immunology and biochemistry of amyloid: In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J.H. and Cohen, A.S., eds.), p. 149. Amsterdam: Excerpta Medica Foundation 1968

Cohen, A.S., Calkins, E.: Electron microscopic observation on a fibrous component in amyloid of diverse origins. Nature (Lond.) **183**, 1202 (1959)

Cohen, A.S., Shirahama, T.: Electron microscopic analysis of isolated amyloid fibrils from patients with primary, secondary and myeloma-associated disease. Israel J. med. Sci. **9**, 849 (1973)

Emeson, E.E., Kikkawa, Y., Gueft, B.: New features of amyloid found after digestion with trypsin. J. Cell Biol. **28**, 570 (1966)

Glenner, G.G., Eanes, E.D., Bladen, H.A., Linke, R.P., Termine, J.D.: β-pleated sheet fibrils. A comparison of native amyloid with synthetic protein fibrils. J. Histochem. Cytochem. **22**, 1141 (1974)

Gueft, B., Kikkawa, Y., Hirschl, S.: An electron microscopic study of amyloidosis from different species. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J.H. and Cohen, A.S., eds.), p. 172. Amsterdam: Excerpta Medica Foundation 1968

Hirschl, S.: Electron microscopic analysis of human amyloid. J. Ultrastruct. Res. **29**, 281 (1969)

Pearse, A.G.E., Ewen, S.W.B., Polak, J.M.: The genesis of apud-amyloid in endocrine polypeptide tumours: histochemical distinction from immunamyloid. Virchows Arch. Abt. B Zellpath. **10**, 93 (1972)

Pras, M., Schubert, M., Zucker-Franklin, D., Rimon, A., Franklin, E.C.: The characterization of soluble amyloid prepared in water. J. clin. Invest. **47**, 924 (1968)

Shirahama, T., Cohen, A.S.: High-resolution electron microscopic analysis of the amyloid fibril. J. Cell Biol. **33**, 679 (1967)

Skinner, M., Cohen, A.S., Shirahama, T., Cathcart, E.S.: P-component (pentagonal unit) of amyloid: Isolation, characterization and sequence analysis. J. Lab. clin. Med. **84**, 604 (1974)

Sletten, K., Westermark, P., Natvig, J.B.: Characterization of amyloid fibril proteins from medullary carcinoma of the thyroid. J. exp. Med. **143**, 993 (1976)

Westermark, P.: Fine structure of islets of Langerhans in insular amyloidosis. Virchows Arch. Abt. A Path. Anat. **359**, 1 (1973)

Westermark, P.: On the nature of the amyloid in human islets of Langerhans. Histochemistry **38**, 27 (1974a)

Westermark, P.: Islet amyloid—a fibrillar from of insulin? Acta endocr. (Kbh.) **77**, Suppl. 190, 35 (1974b) (Abstr.)

Westermark, P.: Amyloid of medullary carcinoma of the thyroid: Partial characterization. Upsala J. med. Sci. **80**, 88 (1975a)

Westermark, P.: Amyloid of human islets of Langerhans. I. Isolation and some characteristics. Acta path. microbiol. scand., Sect. C **83**, 439 (1975b)

Westermark, P.: The nature of amyloid in islets of Langerhans in old age. In: Amyloidosis (Wegelius, O. and Pasternak, A., eds.). New York-London: Academic Press 1976 (in press)

Westermark, P., Skinner, M., Cohen, A.S.: The P-component of amyloid of human islets of Langerhans. Scand. J. Immunol. **4**, 95 (1975)

Received November 4, 1976