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Amyloid of Medullary Carcinoma of the Thyroid; Partial Characterization

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ABSTRACT

Amyloid was isolated from lymph node metastases of a medullary thyroid carcinoma. SDS electrophoresis and gel filtration revealed a major subunit protein of MW less than 10 000. This subunit was capable of forming fibrils when dialysed in a solution against water. The amino acid composition of the subunit differed unequivocally from that of calcitonin. The amyloid also differed from systemic amyloids, since it did not form a top layer when homogenized and, further, did not seem to contain significant amounts of tryptophane. Since the amyloid of medullary carcinoma of the thyroid showed definite similarities to islet amyloid it is concluded that these two amyloids form a special class.

INTRODUCTION

Medullary carcinoma of the thyroid gland (MCT) is an entity which has only been described fairly recently. The tumour has its origin in the parafollicular or C cells. Its most characteristic feature is the presence of amyloid. This amyloid has the typical properties of all amyloids, i.e. an affinity for Congo red with green birefringence when studied in a polarization microscope, and an ultrastructure of criss-crossing fine fibrils.

The nature of the amyloid of MCT has been a matter of dispute (cf. 5). Pearse et al. (7), applying histochemical methods, were unable to demonstrate tyrosine and tryptophane in the amyloid as in amyloids of some other tumours of "APUD" origin and suggested that these amyloids form a special class ("APUD" amyloid). No detailed analysis of isolated amyloid of MCT appears to have been made, however. It was the aim of this study to isolate and characterize this amyloid and to compare it with some other amyloids.

MATERIAL AND METHODS

Tumour tissue was obtained from a 38-year-old man who had a medullary carcinoma of the thyroid with metastases in the lymph nodes of the neck. A radical cervical dissection was performed and the lymph nodes were found to be almost completely replaced by amyloid rich tumour tissue.

Isolation of amyloid

Lymph nodes (about 5 g) were dissected free of connective tissue. The subsequent procedure was the same as for isolation of amyloid of the islets of Langerhans (11, 12), which is a modification of the method described by Pras et al. (9). Briefly, the tissue was repeatedly homogenized in normal saline followed by distilled water. The sediment was frozen and the bottom layer, which contained most of the amyloid, was separated from the top, which was discarded. After a few further homogenizations this procedure was repeated. The final material, which was seen by light microscopy to be almost pure amyloid, was then lyophilized. A small sample for use in the amino acid analysis (see below) was digested by collagenase, as described by Cohen & Calkins (1).

For comparison, isolated islet amyloid (12) and amyloid fibrils from patients with different forms of systemic amyloidosis, isolated by the method of Pras et al. (9), were studied.

Electron microscopy

A diluted suspension of native fibrils or precipitated protein is distilled water was mixed with an equal amount of 2% ammonium molybdate in distilled water. A drop of the mixture was placed on formvar coated copper grids, the excess was blotted off and the grids were air-dried. The preparations were then studied in a Zeiss electron microscope (EM 9).

Spectrophotometry

1.6 mg of the lyophilized material was dissolved in 3 ml 0.1 M sodium hydroxide. After centrifugation the clear supernatants were read in a spectrophotometer between 220 and 340 nm.

Electrophoresis

SDS electrophoresis was performed as described by Harada et al. (4) after denaturation of the fibrils in 6 M guanidine HCl. Cytochrome C, insulin and glucagon were used as molecular weight markers.

Gel filtration

Amyloid was dissolved in 6 M guanidine HCl–0.1 M tris buffer with 0.1 M dithiothreitol (4) and applied to a

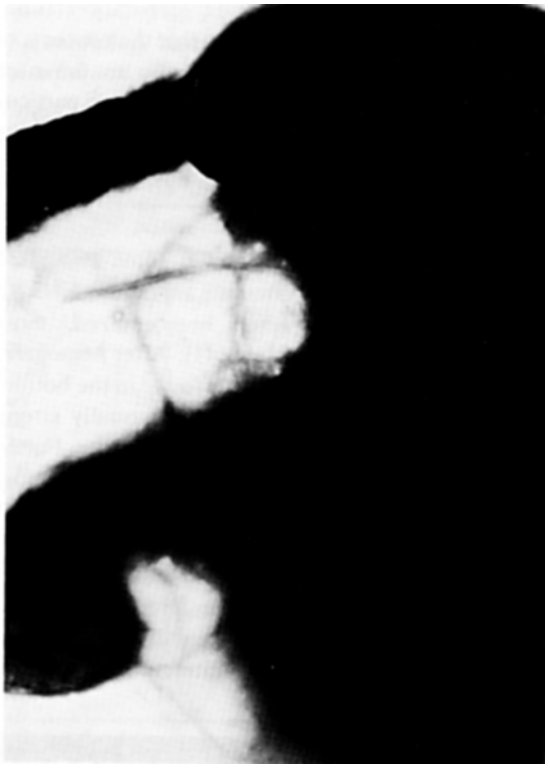

Fig. 1. Typical electron microscopic appearance of amyloid of MCT: large, electron dense particles from which fine slender fibrils sometimes extend. Negatively contrasted by ammonium molybdate. ×25 000.

1.6×100 cm Sephadex G 50 column equilibrated with 5 M guanidine HCl in distilled water. Suitable fractions were dialysed against water in tubings with reduced porosity.

Amino acid analysis[1]

About 1 mg of material was hydrolyzed in 6 M HCl at 110°C for 24 hours. Half cystine was measured as cysteic acid after oxidation. The analyses were performed in an automatic amino acid analyser.

RESULTS

When homogenized and centrifuged most of the amyloid of MCT appeared in the bottom layer as particles of varying size. Only a small amount was found in the top layer. The amyloid was rather difficult to dissolve in 0.1 M NaOH or in 6 M guanidine HCl and differed from the systemic forms in this way. However, it was not as difficult to dissolve as islet amyloid (12).

Electron microscopy

The amyloid fibrils occurred as irregular clumps of varying size. These clumps often appeared compact and electron dense, but a more loose arrangement was sometimes seen and occasionally fine, rather long and wavy fibrils extended from electron dense particles (Fig. 1). However, single fibrils were rarely found. The electron microscopic picture resembled that of islet amyloid (12), but differed from that of systemic amyloids where the fibrils were more separated from each other.

Spectrophotometry

The ultraviolet absorption spectrum of the amyloid showed no peaks in the range 230–340 nm (Fig. 2). Only slight irregularities occurred between 280 and 290 nm, which might have been due to a trace of tryptophane. The absorption spectrum was very similar to that of islet amyloid but differed unequivocally from that of systemic amyloids, all of which showed typical tryptophane peaks.

Electrophoresis

SDS electrophoresis revealed one major band and several very weak minor bands. The major band moved faster than cytochrome C (MW 12 400) and only a little slower than insulin (MW 5 800) (Fig. 3).

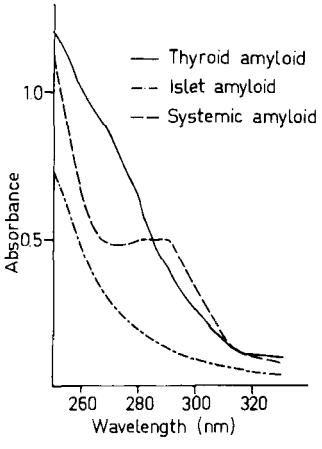

Fig. 2. Ultraviolet absorption spectra of amyloid of MCT, islet amyloid and a primary systemic amyloid. The islet amyloid and the amyloid of MCT show no peaks, while the systemic amyloid presents the typical tryptophane peaks at 281 and 289 nm.

[1] Performed by the Central Amino Acid Analysis Laboratory, Institute of Biochemistry, University of Uppsala.

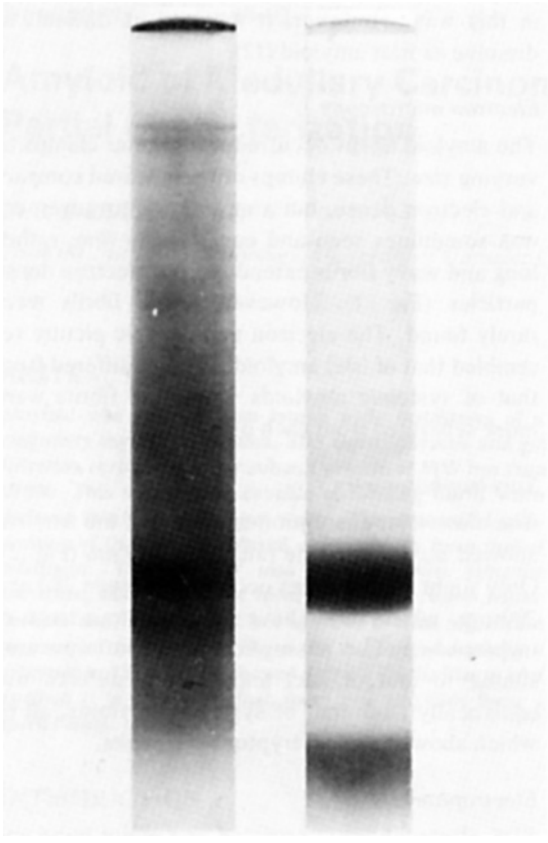

Fig. 3. SDS electrophoresis of amyloid of MCT (left) and cytochrome C (MW 12400), insulin (MW 5800) and glucagon (MW 3485) (right). The major band of the amyloid moves slightly slower than insulin.

Gel filtration

Gel filtration resulted in one major V_0 peak and a smaller second peak. SDS electrophoresis showed that this second peak corresponded to the band of MW 6000 of the unpurified fibrillar material. When this peak was dialysed against distilled water a precipitate appeared, which on electron microscopy displayed fine, rather long and wavy fibrils (Fig. 4).

Amino acid analysis

The amino acid composition is given in Table I. The native amyloid of MCT had rather high contents of aspartic acid, serine, glutamic acid and glycine. Arginine and half cystine were present in fairly large amounts. The tyrosine value was rather low. The subunit material did not differ appreciably from the native amyloid in its amino acid composition, except for the content of glycine. The amino acid composition of one islet amyloid preparation (Is 62) is also given in Table I; it is seen that the contents of most amino acids are fairly similar to those of amyloid of MCT, with a few exceptions, in particular glutamic acid and alanine.

DISCUSSION

The amyloid of MCT differs in some important respects from the systemic amyloids hitherto characterized. Thus, when homogenized, most amyloids form a soft top layer (1). After homogenization the amyloid of MCT was found in the bottom layer and this is probably due to unusually strong binding of the amyloid fibrils to one another. Further, tryptophane, a very typical amino acid of most amyloids (2, 4), was not present in significant amounts in the amyloid of MCT.

In some respects the amyloid of MCT closely resembles islet amyloid. The latter also forms aggregates of fibrils when homogenized (12). Further, as is shown in Fig. 2, the ultraviolet absorption

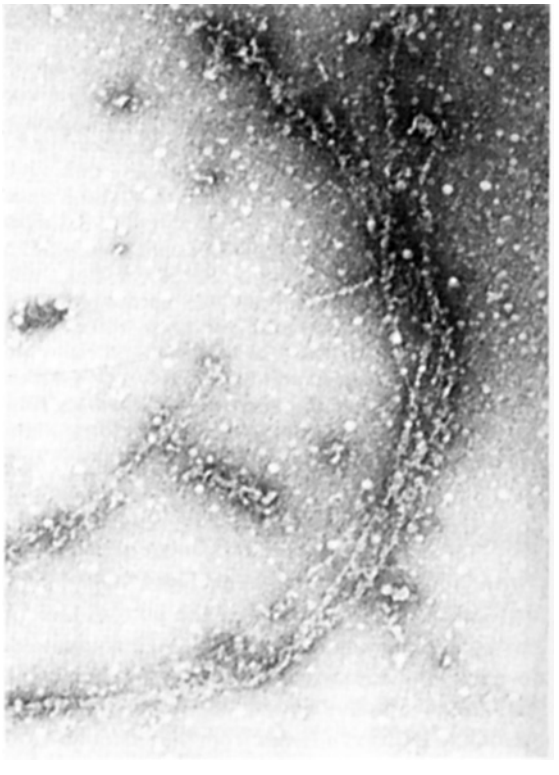

Fig. 4. Precipitate of the major subunit material of amyloid of MCT. The protein forms long wavy fibrils. Negatively contrasted by ammonium molybdate. ×85000.

Table 1. *Amino acid composition of native amyloid of a medullary carcinoma of thyroid, of its subunit material and of an islet amyloid preparation (Is 62)*

Values are expressed as residues per 100 residues found. N.D.=not determined

	Medullary carcinoma amyloid		Native islet amyloid
	Native	Subunit	
Asp	9.7	10.4	12.0
Thr	7.3	5.8	8.5
Ser	8.9	10.3	10.0
Glu	12.2	15.1	7.5
Pro	6.0	5.8	3.6
Gly	10.2	6.8	10.5
Ala	5.7	7.7	9.4
Cys/2[a]	3.7	2.1	4.1
Val	3.9	4.2	5.6
Met	1.3	2.9	1.0
Ile	2.8	1.5	3.6
Leu	7.3	8.6	7.7
Tyr	2.2	2.0	2.8
Phe	4.5	3.0	4.6
His	2.3	2.8	2.0
Lys	5.7	4.8	3.1
Arg	6.5	6.4	4.1
Hypo	N.D.	N.D.	Trace
Trp	N.D.	N.D.	N.D.

[a] Determined as cysteic acid.

spectra of the two amyloids are almost identical, with no peaks at 280 or 289 nm. As is seen in the table, the amino acid compositions are similar with a few exceptions, especially the content of glutamic acid, where the difference is 4.7 residual per cent. In view of this difference, and the difference in molecular weight of the major proteins (12), it can be stated that the major proteins of islet amyloid and of amyloid of MCT are not identical but are probably closely related. Thus it seems fully justifiable to place these amyloids in a special class, as has been suggested by Pearse et al. (7).

SDS electrophoresis revealed that amyloid of MCT contained a major subunit protein (protein AMCT[1]) which had a molecular weight of nearly 6000 daltons. This major subunit protein was capable of forming fibrils when dialysed in a solution against water. This capacity supports the view that the low molecular protein is an important part of the amyloid fibril of MCT, since both the light chain proteins of amyloid (3, 10) and amyloid protein AA (8, 13) have this property. It has been proposed that calcitonin or a precursor of calcitonin (5) may be the origin of protein AMCT. This seems very doubtful, however. The calcitonin monomer is a much smaller molecule than protein AMCT and, furthermore calcitonin contains no arginine (6), an amino acid present in such a large quantity in the native amyloid of MCT and in the small molecular weight material that it is unlikely to be due to contamination.

[1] According to the nomenclature accepted at the International Symposium on Amyloidosis, Helsinki, Aug. 1974.

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REFERENCES

1. Cohen, A. S. & Calkins, E.: The isolation of amyloid fibrils and a study of the effect of collagenase and hyaluronidase. J Cell Biol *21:* 481, 1964.
2. Cooper, J. H.: An evaluation of current methods for the diagnostic histochemistry of amyloid. J Clin Pathol *22:* 410, 1969.
3. Glenner, G. G., Ein, D., Eanes, E. D., Bladen, H. A., Terry, W. & Page, D.: The creation of "amyloid" fibrils from Bence Jones proteins in vitro. Science *174:* 712, 1971.
4. Harada, M., Isersky, C., Cuatrecasas, P., Page, D., Bladen, H. A., Eanes, E. D. Keiser, H. R. & Glenner, G. G.: Human amyloid protein; chemical variability and homogeneity. J Histochem Cytochem *19:* 1, 1971.
5. Ljungberg, O.: On medullary carcinoma of the thyroid. Acta Pathol Microbiol Scand, Sect. A., Suppl. *231,* 1972.
6. Neher, R., Riniker, B., Rittel, W. & Zuber, H.: Menschliches Calcitonin. III. Struktur von Calcitonin M und D. Helvet Chim Acta *51:* 1900, 1968.
7. Pearse, A. G. E., Ewen, S. W. B. & Polak, J. M.: The genesis of apudamyloid in endocrine polypeptide tumours: histochemical distinction from immunamyloid. Virchows Arch Abt B *10:* 93, 1972.
8. Pras, M. & Reshef, T.: The acid-soluble fraction of amyloid—a fibril forming protein. Biochim Biophys Acta *271:* 193, 1972.
9. 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.
10. Shirahama, T., Benson, M. D., Cohen, A. S. & Tanaka, A.: Fibrillar assemblage of variable segments of immunoglobulin light chains: an electron microscopic study. J Immunol *110:* 21, 1973.
11. Westermark, P.: On the nature of the amyloid in human islets of Langerhans. Histochemistry *38:* 27, 1974.
12. Westermark, P.: Amyloid of human islets of Lang-

erhans. I. Isolation and some characteristics. In preparation 1975.
13. Zuckerberg, A., Gazith, J., Rimon, A., Reshef, T. & Gafni, J.: The structural subunit of amyloid. Isolation and characterization of a polypeptide capable of fibril formation. Eur J Biochem *28:* 161, 1972.

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