Vol. 183, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1992 Pages 227-231

CREATION OF AMYLOID FIBRILS FROM MUTANT ASN187 GELSOLIN PEPTIDES

C.P.J.Maury and E-L.Nurmiaho-Lassila

Fourth Department of Medicine and Department of Electron-microscopy, University of Helsinki,SF-00170 Helsinki Finland

Received January 20, 1992

Summary:The amyloid protein in familial amyloidosis,Finnish type, is a 71 amino acid long fragment of the inner region of mutant Asp_{187}→Asn gelsolin.The mechanism of gelsolin amyloid formation was tested with synthetic 11 and 30 residue peptides corresponding to the normal and mutant sequence of gelsolin.Fibrils meeting the morphologic criteria of amyloid were formed from the mutant Asn_{187} peptides.Substitution of the normal Asp_{187} residue with the mutant Asn residue resulted in a 9-fold increase in fibrillogenicity as determined by quantitative fluorometry.The present study demonstrates the first successful in vitro creation of amyloid-like fibrils from Asn_{187} gelsolin peptides and provides evidence that amyloid formation in Finnish amyloidosis is a direct consequence of the Asp_{187}→Asn substitution in gelsolin.

Familial amyloidosis ,Finnish type (Finnish hereditary amyloidosis, Meretoja amyloidosis) is an autosomal dominant form of systemic amyloidosis clinically characterized by corneal lattice dystrophy(type II),progressive cranial neuropathy,skin changes, as well as renal and cardiac manifestations (1).The amyloid subunit protein accumulating in the tissues of patients with Finnish amyloidosis is different from all other amyloid proteins described, showing amino acid sequence homology with variant gelsolin(2-4). The complete primary structure of the amyloid protein has been elucidated, demonstrating that it is a 71 amino acid long polypeptide derived from the inner,actin-modulating domain of mutant Asp_{187}→Asn gelsolin by limited proteolysis(5).Expression of Asn_{187} gelsolin in Finnish amyloidosis is the result of a point mutation, G→A, at nucleotide 654 in the gelsolin gene(6,7).

The mechanism of amyloid formation in Finnish amyloidosis has not been defined.A crucial question is whether or not the Asp_{187}→Asn substitution is directly involved in amyloidogenesis.To address this issue,we studied the in vitro amyloid fibril forming capacity of synthetic peptides corresponding to the mutant and normal sequences of gelsolin.The results indicate highly increased fibrillogenic properties of the peptides containing the mutant sequence.

0006-291X/92 $1.50

Materials and Methods

Peptides.Synthetic peptides >95% pure (Multiple Peptide Systems, San Diego, CA) corresponding to normal or mutant gelsolin were used (Table1).

Fibril formation.The synthetic peptides were dissolved in 10% acetic acid(8) or in water at a concentration of 5mg/ml.Samples were analyzed by light microscopy(Congo-red staining, polarized light) and electron microscopy(negative staining).

Quantitation of fibril formation.For quantitative estimation of amyloid fibrils, a fluorometric assay was used(9).The method is based on the bright fluoresence of thioflavine T in the presence of amyloid fibrils at the excitation and emission maxima at 450 and 482nm, respectively.

Electron microscopy.Samples were placed on copper grids and stained with 1% phosphotungstic acid and pH was adjusted to 6.5 with potassium hydroxide.The grids were examined in a JEOL JEM 100CX electronmicroscope operated at 60kV.

Results

Fig.1. shows the relationship between the synthetic peptides, gelsolin and the amyloid protein accumulating in the tissues in a fibrillar form in Finnish type familial amyloidosis.Peptide $P_{1-30/N}$ corresponds to the amino terminal portion of the amyloid protein and peptide $P_{10-20/N-A}$ to the sequence around the substitution site. After dissolution in acetic acid or water the mutant peptides $P_{1-30/N}$ and $P_{10-20/N-A}$, and to a lesser degree the normal peptides $P_{1-30/D}$ and $P_{10-20/D}$, formed material that exhibited congofilia and green birefringence when examined in polarized light. Ultrastructurally the fibrils created from the mutant peptides were amyloid-like(Fig.2.)The fibril forming capactity of the mutant $P_{1-30/N}$ and normal $P_{1-30/D}$ peptides was compared in a quantitative assay

Table 1. Designation and amino acid sequences of the synthetic peptides

Peptide	Residue number	Sequence	Position in gelsolin	
			Normal	Mutant
$P_{1-30/D}$	30	ATEVPVSWESFNNGDCFILDLGNNIHQWCG	173-202	
$P_{1-30/N}$	30	ATEVPVSWESFNNGNCFILDLGNNIHQWCG		173-202
$P_{10-20/D}$	11	SFNNGDCFILD	182-192	
$P_{10-20/N-A}$	11	SFNNGNCFILD-amide		182-192

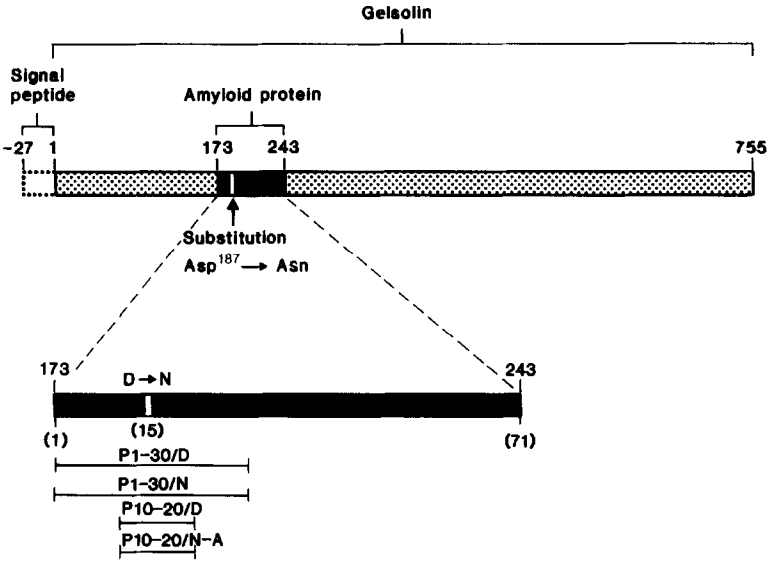

Fig.1. Relationship between the synthetic peptides, mature gelsolin and the amyloid protein accumulating in the tissues of patients with Finnish type familial amyloidosis

utilizing thioflavine T.The mutant peptide showed on an average a 9-fold higher tendency to form fibrils than the corresponding normal peptide (Table 2).

Discussion

The present study clarifies the molecular basis of amyloid formation in gelsolin-related amyloidosis.It is shown for the first time that gelsolin peptides containing the Asn_{187}sequence are amyloidogenic,i.e.produce fibrils that meet the criteria of amyloid (Congofilia, green birefringence in polarized light,ultrastructure).As shown by quantitative fluorometry, substitution of the normal Asp_{187} residue with the mutant Asn_{187}residue in the 30residue peptide,corresponding to the aminoterminal portion of the amyloid protein, caused a highly increased fibrillogenic tendency in vitro.These findings provide strong evidence that the Asp_{187}→Asn substitution in gelsolin in Finnish amyloidosis is directly involved in pathogenesis by rendering the mutant gelsolin molecule a high amyloidogenic potential.Interestingly, all Finnish families so far studied(10), as well as two unrelated American families (10,11) with Finnish type gelsolin-related amyloidosis have the same Asn_{187} mutation in gelsolin, suggesting that the Asp_{187}→Asn substitution in gelsolin may be particularly amyloidogenic.Also the shorter, 11 residue gelsolin peptide spanning over the mutation site and containing the Asn_{187} residue, formed amyloid-like fibrils in vitro.This is in accordance with previous studies

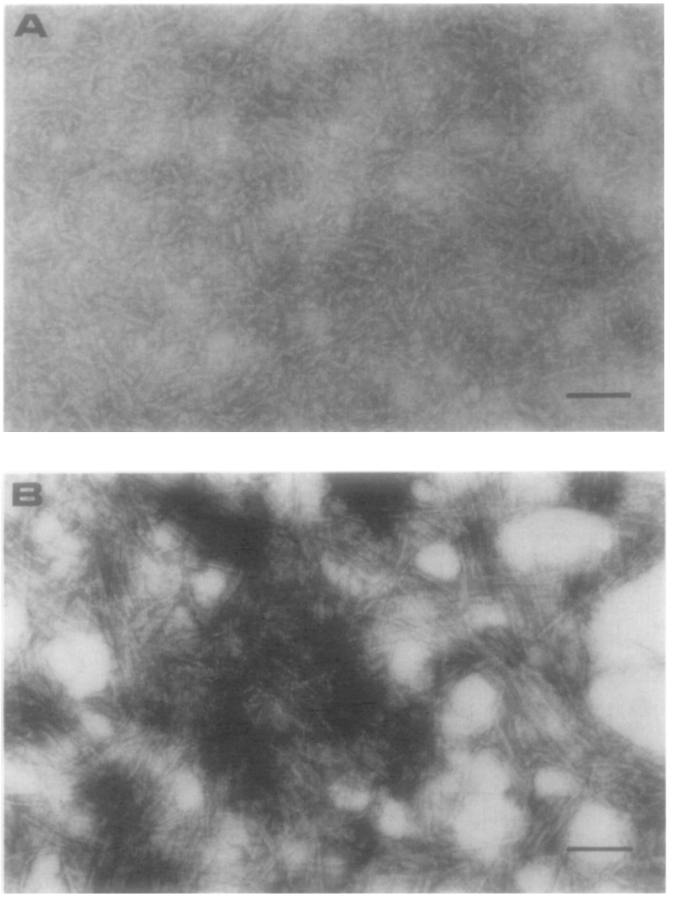

Fig.2. Electron micrographs of fibrils formed from normal, $P_{1-30/D}$ (A) or mutant, $P_{1-30/N}$ (B) gelsolin peptides. The fibrils created from the mutant peptide are amyloid-like. Magnification x 85000. Bar, 100nm.

demonstrating amyloid-like fibril formation of short synthetic peptides related to other types of amyloid proteins, including islet amyloid polypeptide(12), Alzheimer beta-protein(13) and transthyretin (14).

Table 2. Comparison of the fibril forming capacity of normal and mutant gelsolin peptides by quantitative fluorometry using thioflavine T.

		Fluorescence at 482 nm %, relative	
Peptide	n	Median	Range
$P_{1-30/D}$ (normal)	5	10	4-18
$P_{1-30/N}$ (mutant)	5	86	38-100

Acknowledgments

This study was supported by the Sigrid Juselius Foundation and the Academy of Finland.

References

1. Meretoja,J.(1969) Ann.Clin.Res. 1,314-324.
2. Maury,C.P.J., Alli,K., and Baumann,M.(1990) FEBS Lett. 260,85-87.
3. Maury,C.P.J.(1990) Biochim.Biophys.Acta 1096,84-86.
4. Ghiso,J. Haltia,M.,Prelli,F.,Novello,J.,and Frangione,B.(1990) Biochem.J. 272,827-830.
5. Maury,C.P.J. (1991) J.Clin.Invest. 87,1195-1199.
6. Maury,C.P.J.,Kere,J.,Tolvanen,R.,and de la Chapelle,A. (1990) FEBS Lett.276,75-77.
7. Levy,E.,Haltia,M.,Fernandez-Madrid,I,Koivunen,O.,Ghiso,J., Prelli,F.,and Frangione,B.(1990) J.Exp.Med. 172,1865-1867.
8. Westermark,P.,Engström,U.,Johnson,K.H.,Westermark,G.T.,and Betsholz,C.(1990) Proc.Natl.Acad.Sci.USA 87,5036-5040.
9. Naiki,H.,Higuchi,K.,Hosokawa,M., and Takeda,T.(1989) Anal.Biochem 177,244-249.
10. de la Chapelle,A.,Kere,J.,Sack,G.H.Jr.,Tolvanen, R., and Maury,C.P.J. (1992)Submitted.
11. Gorevic,P.D.,Munoz,P.C.,Gorgone,G.,Purcell,J.J.Jr.,Rodrigues,M., Ghiso,J.,Levy,E.,Haltia,M.,and Frangione,B.(1991)N.Engl.J.Med. 325,1780-1785.
12. Glenner,G.G.,Eanes,D.,and Wiley,C.A.(1988) Biochem.Biophys.Res. Commun. 155,608-614.
13. Gorevic,P.D.,Castano,E.M.,Sarma,K.and Frangione,B. (1987) Biochem.Biophys.Res.Commun. 147,854-862.
14. Gustavssaon,A.,Engström,U., and Westermark,P. (1991) In Amyloid and Amyloidosis1990 (J.B.Natvig et al. Eds.)Kluwer Academic Publishers ,Dordrecht.