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THE N-TERMINAL SEGMENT OF PROTEIN AA DETERMINES ITS FIBRILLOGENIC PROPERTY

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SUMMARY: The amyloid fibril protein AA consists of a varying long N-terminal part of the precursor protein serum AA. By using synthetic peptides corresponding to human and murine protein AA segments and cyanogen bromide fragments of human protein AA, we show evidence that the amyloidogenic part of the molecule is the first 10-15 amino acid long segment. Amino acid substitutions in this part of the molecule may explain why only one of the two mouse SAA isoforms is amyloidogenic.

Amyloid fibrils are formed by aggregation of certain small proteins in cross β-pleated sheet conformation (1). In human, 14 different proteins are known to give rise to amyloid fibrils todate (2). Why certain proteins tend to adopt this structure in vivo is not completely known but several of them have been shown to form amyloid-like fibrils in vitro.

Serum amyloid A (SAA) is an acute phase apolipoprotein produced by the liver and the plasma concentration of SAA can rise thousand-fold in association with different inflammatory or malignant diseases (3-5). SAA belongs to a family of proteins, in human coded by at least three different genes. Five different cDNA forms of SAA have been detected in one single individual (6). The SAA molecules can become partially C-terminally degraded and the remaining N-terminal part (protein AA) form fibrils that are deposited as amyloid extracellularly in the tissue.

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The present study was undertaken to study the in vitro formation of amyloid-like fibrils from peptides corresponding to segments of human and mouse SAA. Specific questions were if the predicted hairpin segment or the N-terminal part had amyloidogenic property and in the latter case, if there was any difference between mouse SAA_1 and SAA_2.

MATERIAL AND METHODS

Synthetic peptides

Peptides corresponding to positions 2-12, 13-23, 24-34 and 35-45 of human SAA (6-8) (Table 1) were synthesized by automatic solid-phase synthesis on a model 430A peptide synthesizer (Applied Biosystems). Peptides corresponding to positions 1-11 of the murine SAA_1 and SAA_2 molecules (9, 10) were synthesized in the same way. The peptides were C-terminally amidated. The compositions of the synthesized peptides were analyzed by mass spectrometry that showed the expected molecular masses.

Protein AA peptides

Amyloid fibrils were extracted from the kidney of a patient with AA-amyloidosis and protein AA was purified as described (11). The purified protein, known to contain two methionine residues, was subjected to cyanogen bromide (CNBr) cleavage (12), dissolved in 5 M guanidine HCl and gelfiltered through a 0.9x120 cm Sephacryl S200 (Pharmacia, Uppsala, Sweden) column, equilibrated with the same solution. The resulting three peak materials were applied to a Brownlee 4.6x30 mm C_4 reversed phase column, eluted with a linear gradient of 0-70% acetonitrile-0.1 % trifluoroacetic acid and dried in vacuum. The three split products were identified by amino acid analyses which gave the expected compositions.

Fibril formation in vitro

The fibrillogenic properties of the synthetic peptides and the peptides obtained by CNBr cleavage of protein AA, were studied as described with minor modification (13, 14). Aliquots of the peptides were suspended in 1.75 M acetic acid at a concentration of 10 mg/ml and left in room temperature for 24 hours. After that samples had been taken for light and electron microscopy (see below), neutralization of the solutions was performed with concentrated NH_4OH and again left at room temperature for 24 hours after which new samples were taken. For light microscopy, drops were dried on glass slides and stained with Congo red and viewed in polarized light for green birefringence, typical of amyloid.

Electron microscopy

After dilution (1:20 in distilled water), the peptide solutions were adsorbed to carbonized formvar coated nickel grids (200 mesh/inch).

Negative staining was performed with 2% uranyl acetate at pH 4.5. The samples were examined in a Jeol 2000 electron microscope at an acceleration voltage of 100 kV.

RESULTS

The ability of the synthetic peptides to give rise to fibrils is shown in Table 1. The synthetic peptides corresponding to human SAA(13-23), SAA(24-34) and SAA(35-45) were easily soluble in both solvents. Neutralization of the acidic solution did not give rise to any gel and no green birefringence after staining with Congo red was seen at polarization microscopy. No fibrils were found in the electron microscope. The synthetic peptides corresponding to human SAA(2-12) and to mouse SAA_1(1-11) and SAA_2 (1-11) were virtually insoluble at neutral pH. While the human peptide was only partly dissolved by 10% acetic acid, both the murine peptides dissolved readily. After 24 hours, the solutions containing human SAA(2-12) and murine SAA_2 (1-11) had given rise to gels, which had affinity for Congo red and showed green birefringence. In both cases, an abundance of fibrils of similar appearance were found electron microscopically. The fibrils were long, slender and slightly wavy (Fig. 1). They were flattened and composed of very thin, parallely attached filaments which were twisted with a periodicity varying between 16 and 75 nm. The widest parts of the fibrils measured 10-16 nm.

The three protein fragments, obtained by cleavage of purified human protein AA in methionine residues, were 16, 6 and approximately 40 (the exact length of the studied protein AA was not known) amino

Table 1. Amyloidogenic properties in vitro of synthetic peptides corresponding to parts of human and mouse SAA

Synthetic Peptide	Sequence	Fibril formation
Human SAA (2-12)	SFFSFLGEAFD	Yes
Human SAA(13-23)	GARDMWRAYSD	No
Human SAA(24-34)	MREANYIGSDK	No
Human SAA(35-45)	YFHARGNYDAA	No
Mouse SAA_1(1-11)	GFFSFVHEAFQ	No
Mouse SAA_2(1-11)	GFFSFIGEAFQ	Yes

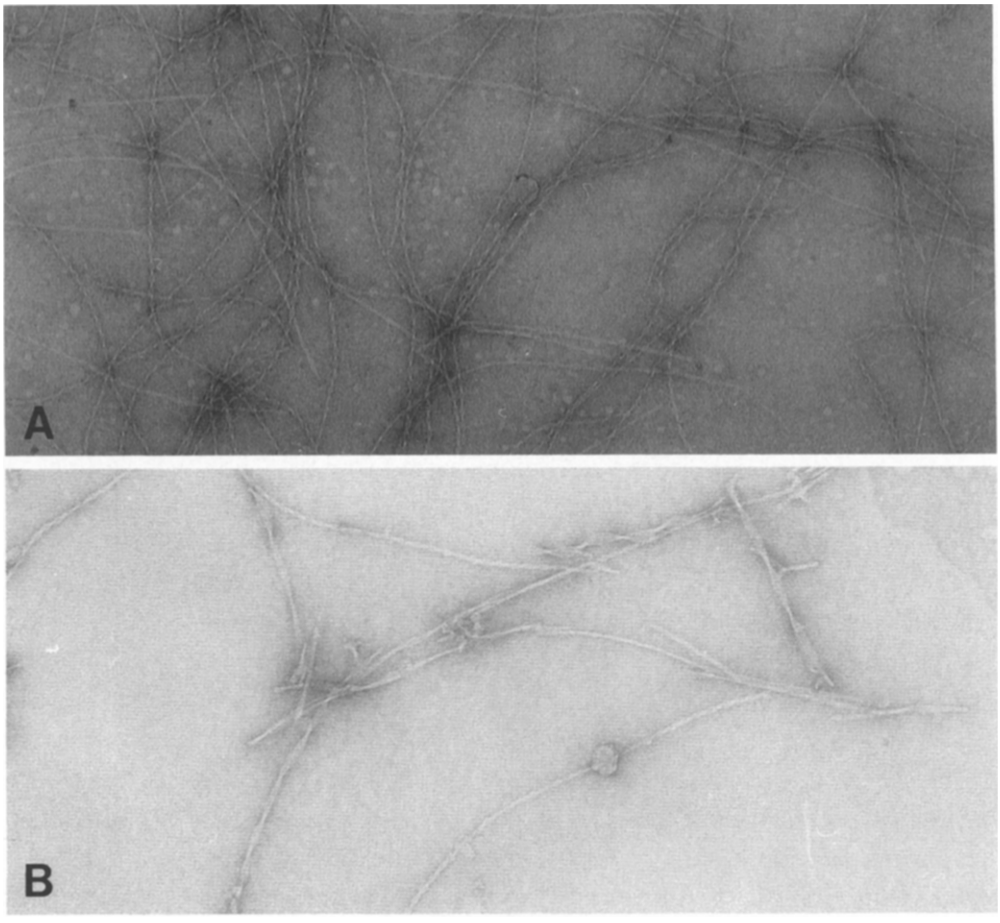

Fig. 1. Fibrils formed from synthetic peptides, corresponding to positions 2-12 of human SAA (A) and to positions 1-11 of murine SAA_2 (B). A, X 109,000 and B, X 82,000.

acid residues long. Of these three, only the 16 amino acid N-terminal peptide gave rise to amyloid-like fibrils in vitro.

The synthetic peptide corresponding to mouse SAA_1(1-11) did not give rise to fibrils neither in acidic nor in neutral condition. Neutralization of the acidic solution of this peptide resulted in a non-fibrillar precipitate.

DISCUSSION

Protein AA is formed by cleavage and removal of a C-terminal part of the larger SAA protein. The N-terminal segment of SAA is always intact in protein AA except for one or two N-terminal amino acid

residues. In humans, AA-proteins varying from 94 to 43 residues (position 2-44) have been characterized (11, 15) which indicates that an amyloidogenic region is present within the N-terminal part of the molecule. In studies with synthetic peptides corresponding to segments of other amyloid-forming proteins, we have found that known or predicted β-strands have the property to form amyloid-like fibrils in vitro (13, 14). For example, two different transthyretin β-strands gave rise to fibrils while a third TTR-fragment with little β–structure did not (13). We therefore expected that the 35-45 segment of human SAA, which contains the only predicted β-strands of short protein AAs (16) should be amyloidogenic. However, the negative result with this experiment indicated that the amyloidogenic property of SAA is not dependent of this segment. Negative results were also obtained with SAA(13-23) and SAA(24-34) while an undecapeptide, corresponding to the desArg N-terminal part of human SAA turned out to be strongly fibrillogenic in vitro. The relevance of the findings with synthetic fibrils was verified with CNBr cleaved protein AA fragments of which only the N-terminal 16 amino acid residue long peptide gave rise to amyloid-like fibrils in vitro. This hydrophobic segment of SAA contains a predicted lipid-binding part of the molecule (16) and is probably normally buried in the lipid particles of HDL. Therefore, the segment is not normally exposed in the plasma. Experiments in the mouse have shown that in prolonged inflammation, degradation of SAA in the liver creates SAA-fragments of the size of protein AA and these are delivered to the plasma unbound to lipid (17). It has been shown that HDL can bind not only SAA but also protein AA. The latter binding is weaker, however, and is more easily displaced by other apolipoproteins (18). Therefore, we postulate that protein AA is cleaved in one site, transported by the plasma bound to lipid particles and delivered from these at the site of deposition. Such a mechanism could explain why protein AA can vary in size between individuals but is more or less uniform in a single individual (11, 15).

In the mouse, three different genes coding for SAA (SAA_{1-3}) and one pseudogene, all located to chromosome 7, have been found (9). While SAA_3 is not found in plasma, SAA_1 and SAA_2 are found to circulate at equal levels. However, only SAA_2 is deposited as amyloid (10, 19). This selective withdrawal and amyloid deposition of one SAA isoform does not seem to occur in human. In the first 25 amino acid residues, the two murine SAA isoforms differ only in positions 6 and 7 where SAA_1 has Val-His and SAA_2 Ile-Gly (9, 10). Very interestingly, the synthetic peptide corresponding to the N-terminal 11 amino-acid-residue-

segment of SAA_2 gave rise to amyloid-like fibrils in vitro while the corresponding segment of mouse SAA_1 did not. This finding indicates that differences in the N-terminal part of SAA determines the amyloidogenicity of SAA. No N-terminal variation is seen between the different human SAA isoforms and this might explain why especially amyloidogenic human SAA variants have not been found.

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