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Mini-Review

Self Assembly of Short Aromatic Peptides into Amyloid Fibrils and Related Nanostructures

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ABSTRACT

The formation of amyloid fibrils is the hallmark of more than twenty human disorders of unrelated etiology. In all these cases, ordered fibrillar protein assemblies with a diameter of 7–10 nm are being observed. In spite of the great clinical important of amyloid-associated diseases, the molecular recognition and self-assembly processes that lead to the formation of the fibrils are not fully understood. One direction to decipher the mechanism of amyloid formation is the use of short peptides fragments as model systems. Short peptide fragments, as short as pentapeptides, were shown to form typical amyloid assemblies in vitro that have ultrastructural, biophysical, and cytotoxic properties, as those of assemblies that are being formed by full length polypeptides. When we analyzed such short fragments, we identified the central role of aromatic moieties in the ability to aggregate into ordered nano-fibrillar structures. This notion allowed us to discover additional very short amyloidogenic peptides as well as other aromatic peptide motifs, which can form various assemblies at the nano-scale (including nanotubes, nanospheres, and macroscopic hydrogels with nano-scale order). Other practical utilization of this concept, together with novel β breakage methods, is their use for the development of novel classes of amyloid formation inhibitors.

THE ULTRASTRUCTURAL AND PHYSIOLOGICAL PROPERTIES OF AMYLOID FIBRILS

The formation of amyloid protein deposition is associated with major human diseases. A partial list includes Alzheimer's disease, Parkinson's disease, Type II diabetes, Prion disorders and many more.[1-3] There are more than twenty human disorders that are associated with the formation of amyloid fibrils. In all these cases, fibrillar assemblies that have a diameter of 7–10 nm are being observed by electron microscopy (EM) or atomic force microscopy (AFM).[1-3] The amyloid fibrils are well-ordered assemblies that have a typical 4.6–4.8 Å X-ray fiber diffraction reflection on the meridian. This reflection is consistent with high degree of order along the long-axis of the fibrils. The order of the fibrils is also reflected in their typical green-gold birefringence when examined between cross-polarizers upon staining with the Congo Red dye. The process of formation of well-ordered amyloid fibrils was depicted as "one dimensional crystallization" by Peter Lansbury and coworkers.[4]

Amyloid assemblies also show a predominant β sheet structure as shown by circular dichroism (CD) and Fourier-transformed infrared (FTIR) spectroscopy. This secondary structure nicely correlates with the well-ordered X-ray fiber diffraction pattern as the stacking of β strand (as observed in other elongated biological assemblies such as silk and β helix protein) is consistent with those parameters. This secondary structure is also a key feature that is being used to inhibit amyloid formation by the use of β breaker elements as will be further described here. A very interesting point is that fibrils of different origins (e.g., from the brain of Alzheimer's disease patients and the pancreas of individuals affected with Type II diabetes) show remarkable biophysical and ultrastructural properties as described above. However, despite the remarkable similarity, no simple homology between the amyloid-forming proteins is apparent.

DO AMYLOID FIBRILS REPRESENT THE TRUE PATHOLOGICAL ASSEMBLIES?

Although fibrillar amyloid assemblies are identified in various amyloid diseases, it is not clear whether these are the genuine pathological species in those disorders. Recent studies suggested that actually early soluble oligomeric assemblies, rather than mature fibrils, may represent the pathological agents in various amyloid disorders (Fig. 1).[5-18] The most studied

system is that of Alzheimer's disease β amyloid polypeptide in which small soluble assemblies appeared to be correlated with memory impairment in cellular and rodent models.[5,15,17-18] The in vivo detection of soluble oligomeric assemblies occurs well before the detection of amyloid deposits and it is correlated with impairment. Indeed soluble assemblies, such as the dodecameric Aβ*56 (Fig. 1), were found to directly affect the process of long-term potentiation (LTP), a synaptic activity that is associated with memory and learning.[17-18]

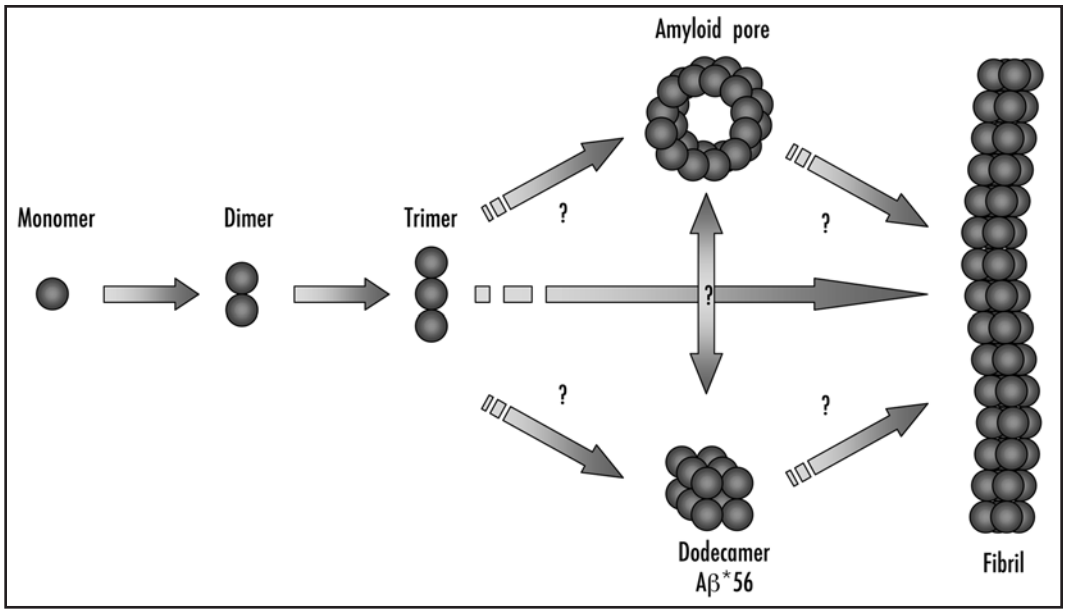

Figure 1. The molecular cascade that leads to the formation of amyloid fibrils. The formation of amyloid fibrils is a sequential process that proceeds from monomeric species into well-ordered amyloid fibrils. It is still unclear whether the formation of soluble oligomers such as the Aβ*56[17] and the annular amyloid pores,[8] is an on-pathway or an off-pathway process.

The new appreciation for the role of early oligomers in amyloid-associated disease pathology clearly suggests that very early events of amyloid formation should be inhibited. Therefore the early molecular recognition and self-assembly processes should be marked as the key target for the development of therapeutic agents that could control the formation of the pathological species. The genuine understanding of the molecular interfaces that mediate recognition and association is consequently critical for future pharmacological developments.

THE USE OF PEPTIDE MODELS TO STUDY THE MECHANISM OF AMYLOID FORMATION

While amyloid fibrils are being formed in most cases by polypeptides of 30–40 amino acids or even longer, other studies had demonstrated that peptide fragments, as short as hexapeptides, form amyloid fibrils of similar physical and ultrastructural properties as described above.[19-23] The first hexapeptide system that was studied is the NFGAIL amyloid-forming peptide fragment from the islet amyloid polypeptide (IAPP).[19] This peptide was found to assemble into typical amyloid fibrils that show the ultrastructure, molecular conformation, and cytotoxicity that are similar to that of amyloid deposits that are formed by the full-length polypeptide.[19]

To decipher the mechanism of amyloid formation, we systematically analyzed short peptide fragments to pinpoint residues that play a role in the molecular recognition and self-assembly process. In the first experimental system, a short amyloid forming motif from the diabetes-related IAPP was studied.[24] Using a systematic alanine scan, we identified the key role of phenylalanine in the NFGAILSS motif (Fig. 2).[24] It was revealed that any amino-acid, but the phenylalanine, could be substituted to an alanine and a significant level of formation of amyloid fibrils would still be detected (Fig. 2).[24] Only when the phenylalanine was change to an alanine, no amyloid formation could be observed under the experimental conditions. When vertical scan of this peptide fragment was made, it was found that modification of the phenylalanine into aliphatic amino acids significantly reduced the ability of the peptide to form typical amyloid fibrils.[25] On the other hand modification of the phenylalanine to tryptophan (that is less hydrophobic than phenylalanine) allows the formation

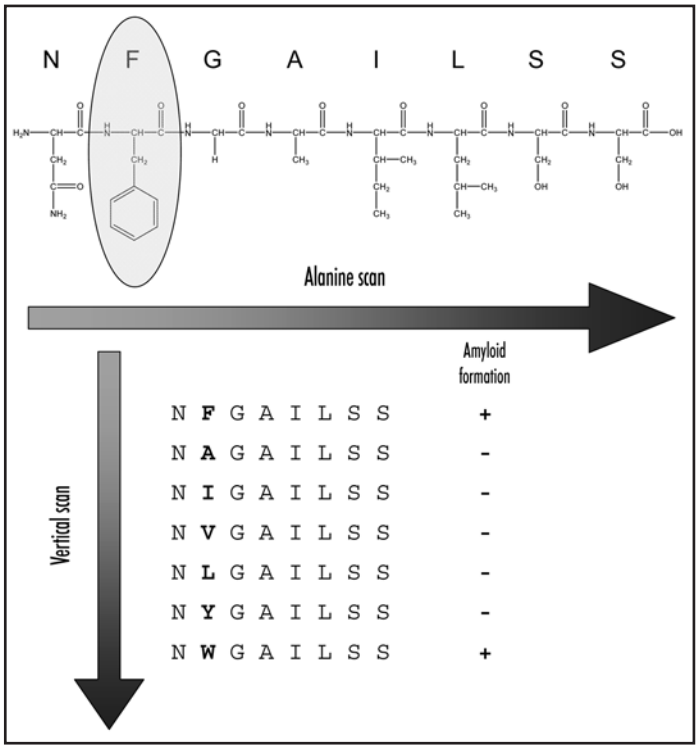

Figure 2. Systematic study of amyloid formation. Alanine scan was first used to identify the phenylalanine as the most important residue in amyloid formation by this fragment. This was followed by vertical scan in which the role of aromatic as compared to aliphatic residues was revealed.

of amyloid-like structure (Fig. 2).[26] In a later study by Dobson and coworkers, in which the amyloidogenic propensity of all naturally occurring amino acids was compared, aromatic amino acids were determined to have the highest amyloid forming propensity.[27] The amyloidogenic potential of the aromatic amino acid is significantly higher than that of the aliphatic ones.

Based on the mechanistic insights, we identified novel fragments, as short as tetrapeptides, which could form amyloid-like nanostructures. The two notable systems are the human calcitonin that forms amyloid assemblies in the case of thyroid carcinoma[22] and the ubiquitous Medin deposits.[23] In the first case, the known information was the pH-dependence of the process of amyloid formation by the full length Calcitonin polypeptide.[22] This suggested that charged amino-acids, that can change their ionization-state in various pH conditions, may have a role in the process of amyloid formation. These led to the exploration of short charged-aromatic DFNKF pentapeptide and DFNK tetrapeptide fragments that can form ordered fibrillar structures.[22] While the pentapeptide formed typical amyloid fibrils, the fibrils formed by the tetrapeptide showed somewhat thicker diameter. In the other study the amyloidogenic potential of a hexapeptide fragment of human medin, a protein that forms amyloid deposits that is observed practically in all individuals above the age of 60. The aromatic hexapeptide, NFGSVQ, formed typical amyloid structures with the ultrastructural and biophysical properties as described above.[23] Also in the case of the calcitonin and medin fragments, the change of the phenylalanine reside to aliphatic residues significantly reduced the level of amyloid formation.[22-23]

While the amyloidogenic potential of the aromatic moieties is well appreciated there is still a debate regarding the molecular mechanism of their action. We previously suggested that stacking interactions of aromatic moieties may be the root for their ability to efficiently mediate the formation of amyloid fibrils.[28] The order and directionally of π-π stacking interactions was suggested as a driving force for the efficient formation of ordered amyloid assemblies.[28] Indeed, in several structural studies of amyloid assemblies, typical aromatic interactions were being observed at high resolution using NMR, X-ray, and electron diffraction.[29-32] In addition also theoretical studies, including parameter-free models and molecular dynamics, support this notion.[33-37] Yet, other studies had indicated that properties of the aromatic moieties, other than their stacking interactions, may be the root for their activity at least in some systems.[38] We hope that further studies will help to give better answer to this important scientific quest.

THE SELF-ASSEMBLY OF SHORTER AROMATIC PEPTIDES

The calcitonin study was the first demonstration that a peptide, as short as a tetrapeptide, can form ordered fibrillar structures. Similar to the calcitonin work, we identified very short amyloidogenic motifs in many other amyloid-forming proteins and polypeptides. In our path to search for the smallest amyloidogenic structural motif, we studied the core recognition motif of the Alzheimer's β amyloid peptide. We revealed that the diphenylalanine peptide forms discrete and well-ordered tubular nanostructures.[39] The formed nanotubes may share some structural properties with the amyloid fibrils as they have similar vibrational spectrum and birefringence as compared to the fibrils. The even simpler diphenylglycine peptide forms nano-spherical assemblies which are similar to the tubular ones.[40] This indicates that the very simple dipeptide aromatic motifs contain all the molecular information needed to form well-ordered supramolecular structures at the nano-scale.

Later studies from our group reveled that other aromatic homodipeptides could form various structures at the nano-scale. The structures included, besides nanotubes and nanospheres, also fibrillar assemblies, nano-plates and hydrogels with nano-scale order.[41-43] These structures are currently being explored for their utilization in various nanotechnological applications.[44-45]

INHIBITION OF AMYLOID FORMATION BY PEPTIDE FRAGMENTS

We explored various directions towards the use of aromatic amino acids in the process of self-assembly at the nano-scale level, and particularly amyloid formation, to develop novel amyloid formation inhibitors. The aromatic moieties represent recognition interfaces that mediate the very early stage of amyloid formation. We utilize both peptide-based[26] and small-molecule-based[46-47] approaches to develop new inhibitors for the process of amyloid formation at its very early stage. When peptide inhibitors are being used, we utilize the novel inhibitory strategy that is based on the use of the α-aminoisobutyric acid as a β breaker of exceptional potency.[48]

SUMMARY AND FUTURE PROSPECTS

Amyloid nanofibers are naturally occurring self-assembled biological structures. Aromatic moieties play a central role in amyloid fibrils self-assembly by extremely short peptides. We speculate that the stacking interactions rather than mere hydrophobicity may provide energetic contribution as well as order and directionality in the self-assembly of amyloid structures. The hypothesis suggests a new approach to understand the self-assembly mechanism, enables the identification of novel motifs, and indicates new ways to control this process.

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