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Alzheimer's disease: Structure of aggregates revealed

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Abstract

A technical feat achieved by two independent groups has enabled resolution of the molecular structure of a form of the amyloid- β protein that plays a major role in Alzheimer's disease.

The aggregation of short amyloid- β ($A\beta$) proteins in brain tissue leads to Alzheimer's disease. Most aggregated $A\beta$ exists in filamentous assemblies called fibrils, which are 5 to 10 nanometres in diameter, and can be many micrometres in length. The structural details of $A\beta$ fibrils have proved tough to define, because the assemblies' insolubility and noncrystallinity prevent structure determination by standard methods. Now two independent groups (Wälti *et al.*¹ writing in the *Proceedings of the National Academy of Sciences* and Colvin *et al.*² writing in the *Journal of the American Chemical Society*) report full molecular structures for fibrils formed by the version of $A\beta$ comprised of 42 amino-acid residues ($A\beta_{42}$), which is widely believed to be of primary importance in most cases of Alzheimer's disease³. Their findings have broad implications for our understanding of this disease and for the development of drugs and diagnostic imaging agents.

The new $A\beta_{42}$ fibril structures are derived primarily from solid-state nuclear magnetic resonance (NMR) data. As the name suggests, solid-state NMR is aimed at samples that are not liquids or solutions. Similar to the solution NMR techniques more familiar to most chemists and biologists, solid-state NMR provides valuable information about the molecular structure, molecular motions and other properties of samples under study, but does not require solubility or crystallinity. Application of solid-state NMR to assemblies of proteins or short amino-acid chains called peptides is a rapidly growing area of research, with amyloid fibril structures being one of the main targets. From the standpoint of methodology alone, the new structures represent a major triumph.

One big problem in structural studies of amyloid fibrils is that they are typically polymorphic — they can adopt multiple distinct molecular structures, often depending on subtle variations in fibril growth conditions^{4,5}. This polymorphism, together with the fact that roughly half of $A\beta_{42}$ is hydrophobic, makes it hard to prepare the milligram-scale, structurally homogeneous $A\beta_{42}$ fibril samples required for solid-state NMR.

To overcome this problem, the two groups grew their $A\beta_{42}$ fibrils in aqueous solutions with different combinations of pH, temperature, and ionic strength. They also adopted an approach developed⁴ to prepare fibrils of the 40-residue version of the protein ($A\beta_{40}$), in which fibrils were grown in several successive rounds, seeding each from the last, to purify a single predominant structure from an initial mixture of polymorphs. Despite differences in

sample preparation conditions, the A β ₄₂ fibril structures determined by the two groups are nearly identical. Apparently, then, this A β ₄₂ structure is thermodynamically stable and forms efficiently over a range of conditions.

So what is the structure of A β ₄₂ fibrils? Both groups find that their A β ₄₂ molecules adopt a roughly S-shaped conformation, comprised of short β -strand segments that are linked by bends. Molecules stack directly on top of one another along the direction in which the fibrils grow, forming in-register stacks of parallel cross- β subunits, where the term “cross- β ” refers to a structure in which β -strands of neighboring molecules are linked by hydrogen bonds along the growth direction⁶. Each fibril contains two such subunits, arranged with two-fold symmetry about the growth axis (Fig. 1a).

The cross- β subunit structures determined by Colvin *et al.* and Wälti *et al.* agree well with a previous study⁷. However, this earlier work did not show how subunits interact to produce two-fold symmetry and did not uniquely define the conformation of amino-acid residues 34–36. The excellent overall agreement among these three independent studies, which used different strategies for labelling their samples, different combinations of solid-state NMR measurements and different molecular modelling approaches, makes the results highly reliable.

A β ₄₀ is more abundant than A β ₄₂ in the healthy human brain, but is less hydrophobic and hence less aggregation-prone. Similar to A β ₄₂, A β ₄₀ fibrils have been shown^{4,8,9} by solid-state NMR to contain in-register, parallel cross- β subunits, arranged with either two-fold or three-fold symmetry. But many details of the molecular conformations and the interactions within and between molecules in A β ₄₀ and A β ₄₂ fibrils are quite different (Fig. 1b). It seems that these structural differences, especially interactions that involve residues 41 and 42 (which are absent from A β ₄₀), would make it impossible for A β ₄₀ to form stable fibrils that are more similar to the newly determined A β ₄₂ structure.

Additionally, whereas the C-terminal segment of the protein is exposed on the surface in A β ₄₂ fibrils, this segment is buried in the core of A β ₄₀ fibrils. Differences in surface composition and structure may affect the neurotoxicity of A β ₄₀ and A β ₄₂ fibrils, conferring different interactions with neuronal membranes, membrane-bound receptor proteins or the cerebral vasculature, different abilities to stimulate inflammation, and different antibody recognition properties.

These structural properties provide a likely explanation for the observation that A β ₄₂ fibril fragments cannot be extended by A β ₄₀ molecules⁷ — a process called cross-seeding. An absence of cross-seeding might affect how A β ₄₀ and A β ₄₂ fibrils propagate within brain tissue in Alzheimer's disease, determining whether the two species act independently or in concert.

Specific structural features revealed by the new solid-state NMR studies may also allow the design of new chemical compounds that either inhibit fibril formation or bind tightly to fibrils, with enhanced abilities to distinguish between A β ₄₀ and A β ₄₂. Such compounds are of potential interest for prevention or treatment of Alzheimer's disease, for diagnostic imaging, and for research into the roles of the two peptides in the disease.

Finally, both groups prepared the A β ₄₂ fibrils for their experiments *in vitro*. Does the same structure develop naturally in human brain tissue, and do other A β ₄₂ fibril polymorphs also develop? Wälti *et al.* show that their fibrils are bound by the same panel of antibodies that recognize A β deposits in brain tissue, providing evidence for their biomedical relevance. Solid-state NMR measurements of A β ₄₂ fibrils derived from brain tissue, as has been reported for A β ₄₀ fibrils⁹, are a sensible direction for future research.

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Figure 1. Molecular structures of Alzheimer's-disease-associated amyloid fibrils

(a) Colvin *et al.*² and Wälti *et al.*¹ used solid-state nuclear magnetic resonance (NMR) to define very similar structures of fibrils formed by the 42-amino-acid version of amyloid- β protein ($A\beta_{42}$). Here, a cartoon representation of the structure from Colvin *et al.* (Protein Data Bank file 5KK3) is viewed down the fibril growth axis, with arrows indicating β -strand segments. (b) One $A\beta_{42}$ molecule from Colvin *et al.*, in orange, is compared with one molecule from a previously determined structure⁹ of fibrils formed by the 40-amino-acid version ($A\beta_{40}$, Protein Data Bank file 2M4J), in blue. Ellipses indicate examples of differences in amino-acid sidechain interactions, for phenylalanine-19 (F19) and lysine-28 (K28). Residues 1-13 are omitted.