Spot the Difference: Function versus Toxicity in Amyloid Fibrils

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Keywords

Amyloid, cryo electron microscopy, Orb2, CPEB, Alzheimer, memory

Abstract

In a recent study, **Hervas *et al*.** extracted Orb2 fibrils, which are involved in long-term memory formation from *Drosophila*-brains, characterised their function, and determined their structure using cryo-EM. The fibrils show a remarkable resemblance to Aβ fibrils associated with Alzheimer's disease, highlighting the subtle difference between functional and dysfunctional amyloid.

Main text

Amyloid, first observed in 1639, typically shows a cross β-sheet fibrillar structure and was initially associated with tissue damage and human disease [1]. The best known examples of amyloid diseases are neurodegenerative disorders such as Alzheimer's (Amyloid-β (Aβ)/Tau), Parkinson's (α-synuclein), and Huntington's (huntingtin with polyQ expansion) diseases [1]. However, some amyloid fibrils are involved in biological functions [2-4]. In 2000, Wösten and de Vocht pioneered the term "functional amyloid" based on their work on hydrophobins, a family of small proteins in fungi that self-assemble at hydrophobic/hydrophilic surfaces and are important for growth and development [3]. Since then, functional amyloids have been identified in bacteria (e.g. curli (biofilm formation)), fungi (e.g. HET-s (programmed cell death)), yeast (e.g. Ure2p (nitrogen catabolism)), plants (e.g. luminidependens (regulating flowering)), arachnids (e.g. spidroin (spider silk)), and mammals (e.g. pmel17 (melanin formation)), highlighting the wide range of their functional roles [2-4]. Pmel17, the first functional amyloid protein identified in humans, was described only 15 years ago. Today, functionally important amyloids in humans are known to be involved in several functions, including hormone storage (peptide hormones), regulating necrosis (receptor interacting protein1/RRIP3), and removing damaged sperm (semenogelin proteins 1/SEM2) (reviewed in [4]).

One family of functional amyloids include RNA-binding proteins of the cytoplasmic polyadenylation element binding protein (CPEB) family [5]. In a number of organisms, including humans, CPEB is located in germ cells and the central nervous system, and is involved in controlling polyadenylation and translation, crucial for cell division during development, but also in the formation and storage of long-term memories [5]. For its function in memory, CPEB has been shown to be required to self-

assemble into prion-like self-propagating fibrillar aggregates [6]. These fibrils bind mRNAs and control translation of synaptic proteins, changing the protein composition within the neuron and, by stabilizing enhanced synaptic activity, allowing long-term memory formation and storage. Focusing on the activity and structure of fibrils of the CPEB orthologue, Orb2, from the brains of the fruit fly *Drosophila melanogaster*, Hervas *et al*. [7] started to answer the fascinating, and key, question of how 'molecular memory' is made.

Whilst Orb2 is monomeric in *Drosophila* early embryos, Hervas *et al*. [7] extracted Orb2 fibrils (as well as oligomeric and monomeric Orb2) from the brains of adult flies. The authors found that while extracted monomeric Orb2 supresses translation of a specific mRNA by binding to a protein GC13928 *in vitro,* binding of oligomeric or fibrillar Orb2 to a different protein, CG4612, activates translation. For example, the oligomers and fibrils of Orb2 positively regulate translation of mRNA coding for tequila, a protein required for long-term memory formation, while the monomer supresses tequila production, explaining how translation regulation is brought about by this CPEB protein. Hence, the function of Orb2 depends critically on its state of assembly and the repertoire of mRNAs being translated.

Hervas *et al*. [7] also showed that Orb2 fibrils have the biophysical properties of amyloid - the ability to seed (catalyse) fibril growth of monomers, to bind the amyloid-specific fluorescent dye Thioflavin-T and amyloid-specific antibody OC, and to be resistant to proteases, heat, and SDS treatment. The authors then exploited the powers of cryo-electron microscopy (EM) to solve a high-resolution structure (2.6 Å) of Orb2 fibrils isolated from the fly brains. Their beautiful atomic structure revealed that Orb2 fibrils are comprised of three identical protofilaments related by C3 symmetry that form a triangular cross section **(Figure 1)**. In the fibril core, each protofilament adopts a hoop-like conformation with a wide turn, linking two β-strands that stack into a cross-β structure typical of amyloid. Only 31 residues (176-206) of the 704 residue protein form the amyloid core, with less well-defined protein density likely belonging to the protein interaction domain and RNA-recognition motif, which would allow these functional regions to bind other molecules to fulfil their physiological function. Interestingly, the fibril core is hydrophilic due to the 20 glutamines and 7 histidines within the structure. The inter-protofilament polar interface is built of three glutamines and two histidines forming multiple hydrogen-bonds, stabilising the fibril structure.

Comparing the fibril architecture of this work with previously published structures of functional and disease-related amyloid fibrils demonstrates the peculiarity of this three protofilament amyloid structure. The literature is dominated by amyloid structures built of two protofilaments twisted around each other [8]. Two other examples of a C3 symmetric fibril with three protofilaments have been reported to date, formed from the presynaptic Amyloid beta peptide ($A\beta_{40}$) *in vitro* [9] and by elongation of seeds from a patient with Alzheimer's disease with $A\beta_{40}$ [10] **(Figure 1)**. Interestingly, whilst Orb2 aggregates help to form memory, Aβ is involved in Alzheimer's disease, a type of dementia that destroys memory and thinking processes [11]. Can one explain these contrary activities for such similar fibril architectures based on their molecular structure? Focusing on the differences reveals characteristics possibly explaining their opposing effects: 1. Whilst Orb2 fibrils have a hydrophilic core (formed from glutamine and histidine residues), the core of $A\beta_{40}$ fibrils is hydrophobic (formed mainly of valine, methionine, glycine, isoleucine). Changes in pH would destabilise the functional amyloid core, allowing fibril depolymerisation and reformation, enabling the dynamics required for memory formation. By contrast, the uncharged core of toxic amyloid would be less affected by pH differences. 2. Both fibril structures show ~30 residues within the inflexible, structured fibril core, hence in $A\beta_{40}$ only the first ~4-10 residues are unstructured, while in Orb2B (the longest Orb2 isoform primarily involved in fibril formation and memory), more than 650 residues are dynamically disordered, enabling interactions with mRNAs and proteins and controlling

translational processes of synaptic proteins. 3. Orb2 fibril extraction from ~3 million flies resulted in one structure. By contrast, Aβ amyloid isolated from Alzheimer's brains revealed variations between patients and dependent on disease phenotype [11]. Hence, to fulfil a physiological function, fibrils might need to adopt a uniquely defined structure across individuals. It should also be remembered that function and toxicity are not black and white: for example, $A\beta_{40}$ fibrils have been reported to protect against microbial infection suggesting they also have a functional role [4].

In summary, the report by Hervas *et al*. highlights the commonality in architecture of both functional and pathogenic amyloid and reveals how the details of the fibril structure are crucial in spotting the differences between fibrils of physiological function with pathogenic ones.

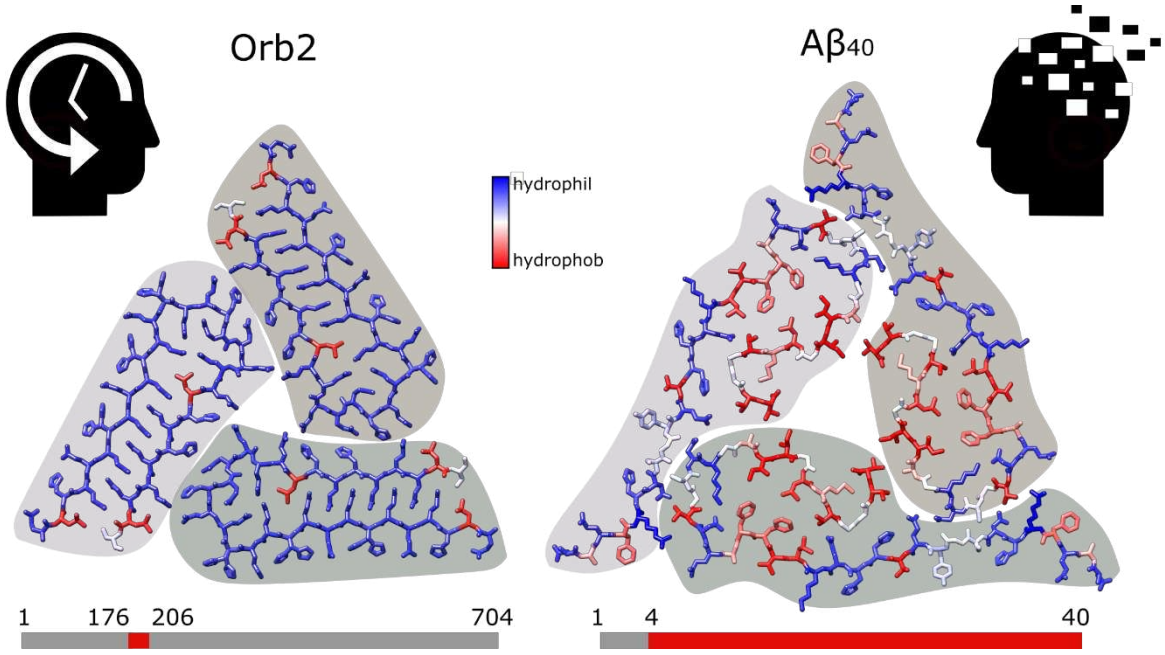

Figure 1: Comparison between the functional amyloid fold of Orb2 (PDB: 6VPS) (left) versus the toxic fibril architecture of Aβ40 (PDB: 2M4J) (right)*. Whilst Orb2 is involved in long term memory formation, Aβ40 is involved in the neurodegenerative disorder Alzheimer's disease. Both structures (one from Drosophila brain and one from human brain) have C3 symmetry with three protofilaments (highlighted in yellow, green and pink) forming the mature fibril. While the core of Orb2 is mainly hydrophile (blue), the Aβ40 core is hydrophobic (red). Also, for Aβ40 nearly the whole protein is structured in the fibril core (red bar), while for Orb2 the majority of the protein sequence is dynamically disordered (grey bar) allowing functionally important interactions with other molecules.*

Acknowledgements

We acknowledge, with thanks, funding from the Wellcome Trust (SMU (215062/Z/18/Z) and SER (204963)).

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