Structure of the Fiber Core of Orb2A, A Functional Amyloid, Revealed by Micro-electron Diffraction

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Amyloid protein aggregation is typically associated with neurodegenerative diseases such as Alzheimer's disease. However, not all amyloid proteins are pathogenic in their aggregated state. The neuronal cytoplasmic polyadenylation element binding (CPEB) protein has been shown to form "functional" amyloid aggregates in several model organisms. CPEB is a regulator of synaptic mRNA translation, and CPEB aggregation has been shown to be an important step in the formation of long-term memory. Our work is focused on the Drosophila CPEB homolog, termed Orb2A. The first 9 N-terminal amino acid residues of Orb2A are necessary for its aggregation, and this segment has been suggested to form the amyloid fiber core. Intriguingly, a single point mutation of the phenylalanine residue in the 5th position to tyrosine (F5Y) decreases Orb2A aggregation and impairs long-term memory formation in Drosophila. To understand the structural basis for Orb2A aggregation, we characterized this critical 9-residue segment of Orb2A, which we call M9I-WT. Using micro-electron diffraction, we determined the crystal structure of M9I-WT at a resolution of 1.0 Å. The segment forms an array of parallel in-register β-sheets, which are held together tightly by inter-strand aromatic and hydrophobic side chain interactions. We also compared the structural properties of the wild-type protein with interface-blocking mutants, and observed mutant segment fibrils are shorter and exhibit poorer ordering. Our model provides an explanation for the decreased aggregation observed for the F5Y mutant, and offers a hypothesis for how the addition of a single atom (the tyrosyl oxygen) can affect memory. Ultimately we aim to understand the differences between functional and pathological amyloids, and thus further our understanding of amyloid disease mechanisms, and improve therapeutic strategies.