Will the discovery of cross-α amyloid-like fibrils herald a new definition of amyloid?

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In microorganisms, functional amyloid is often involved in virulence mechanisms. *Staphylococcus aureus* secretes a 22-residue phenol-soluble modulin α3 (PSMα3) peptide, capable of forming elongated fibrils, with the appearance and staining properties typical of amyloid. Researchers working to solve the 3D structure of PSMα3 fibrils by X-ray crystallography were in for a surprise. As reported by Tayeb-Fligelman *et al* in their recent publication in *Science*, fibrils of PSMα3 revealed a distinctive "cross-α" amyloid-like architecture instead of the classical cross-β spine, hitherto considered the hallmark of amyloid.

BACKGROUND

Amyloid is the term used to describe smaller soluble aggregates or larger insoluble elongated fibrillar structures formed under certain conditions by many proteins, which otherwise fold into one globular structure, the native state. In contrast to the folding of globular proteins, a single protein sequence can aggregate into several distinct amyloid structures, termed polymorphs, and a given polymorph can propagate itself by seeding. Amyloid fibrils can be self-assembled *in vitro* from short peptides, polypeptides and proteins, many, but by no means all of which, are implicated in disease.

The association of amyloid fibrils with devastating diseases such as Alzheimer's, Parkinson's and bovine spongiform encepalophathy (BSE), commonly known as mad cow disease, has somewhat deflected attention from the many instances of amyloid production which is beneficial, or even essential, to the producing organism's survival. The latter kind of amyloid has been dubbed 'functional amyloid' to distinguish it from the pathogenic, disease-associated variety. In bacteria, fungi, as well as insects, amyloid fibres are exploited for their unique mechanical and biological properties (Fowler, 2007). Chorion proteins in insects and fish, spidroin in the spider, and the Pmel17 fibres essential for mammalian melanosome biogenesis are examples of functional amyloid. More than 30 different peptide hormones have been found to form amyloids *in vitro*, indicating their contribution to normal cell and tissue physiology (Maji *et al*, 2009; Badtke *et al*, 2010).

In microorganisms, functional amyloid is often involved in virulence mechanisms and pathogenicity. Many bacteria are capable of producing functional amyloids that serve as a biofilm matrix component. Biofilm development in *Staphylococcus aureus* is mediated by the coordinated production of the biofilm matrix, which can be composed of polysaccharides, extracellular DNA and proteins including amyloid fibres (Schwartz *et al*, 2016).

The amyloid fibers produced by *S. aureus* are composed of small peptides called phenol soluble modulins (PSMs), which in some circumstances act as toxins, affecting neutrophil chemotaxis and cytolysis. However, factors influencing the transition of PSMs from soluble toxin to inert fibril in the biofilm environment are not well understood. The 22-residue peptide PSMa3 is the most cytotoxic and lytic member of the PSM family (Tayeb-Fligelman *et al*, 2017).

Although amyloidogenic proteins show a variety of amino acid sequences and biological functions and adopt different secondary structures, they also form amyloid fibrils whose spines share common features. Amyloid fibrils have several distinct biochemical properties, such as causing birefringence of the dye Congo red (CR) and a spectral shift of the dye thioflavin T (ThT). The commonly accepted biophysical definition of amyloid fibrils until now has been their cross–β X-ray diffraction pattern, resulting from folding of the polypeptide chain into β-sheets arranged parallel to the longitudinal fibril axis, with constituent β-strands arranged perpendicular to this axis (Nelson *et al*, 2005; Sawaya *et al*, 2007) (Figure 1). The implication of this is that α-helical amyloidogenic sequences in proteins presumably have to unfold and refold into β-strands during amyloid formation.

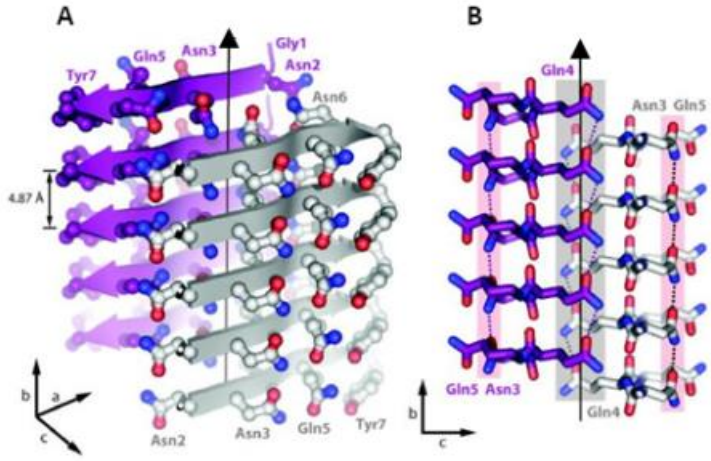

Figure 1. Structure of GNNQQNY, the amyloid fibril-forming peptide from yeast protein Sup35. (A)The pair-of-sheets structure of GNNQQNY, showing the backbone of each α-strand as an arrow, with ball and stick sidechains protruding. Sidechains protruding from the two sheets form a dry, tightly self-complementing steric zipper, bonding the sheets. Within each sheet, every segment is bound to its two neighbouring segments via stacks of both backbone and sidechain hydrogen bonds. (B)The steric zipper viewed edge on (down the **a** axis). The vertical shift of one sheet relative to the other, allows interdigitation of the sidechains emanating from each sheet. The amide stacks of the dry interface are shaded in grey at the centre, and those of the wet interface are shaded in pale red on either side (Nelson et al, 2005). Reprinted by permission from Macmillan Publishers Ltd:Nature (Nelson et al, 2005), copyright (2005).

ATOMIC STRUCTURE OF PSMα3 FIBRILS RESEMBLES CANONICAL AMYLOID

Previous structure determination by solution NMR had revealed that PSMα3 forms amphipathic helices in its native state, but the helical structure itself is apparently not sufficient for PSMα3 molecules to enact their biological activities (Cheung et al, 2014). Tayeb-Fligelman et al found that PSMα3 formed elongated and unbranched fibrils (Figure 2), which bound ThT, generating a characteristic amyloid-fibrillation curve. Unlike previously characterized amyloid proteins which convert into β-pleated structures during fibril formation (Eisenberg & Jucker, 2012), they found that PSMa3 maintained its α-helical conformation, both in solution and in the fibrils.

The X-ray diffraction pattern of PSMa3 indicated that the fibrils were built from the stacking of α helices, rather than the β-sheets of canonical cross-β amyloid. The crystal structure of full-length PSMα3, solved *de novo* at 1.45 angstrom resolution, revealed a distinctive "cross-α" amyloid-like architecture, in which amphipathic α-helices stacked perpendicular to the fibril axis such that hydrophobic faces of the helices interact, holding the α-helical sheets tightly together, similar to the steric zipper structure observed for cross-β amyloid fibrils (Figure 2).

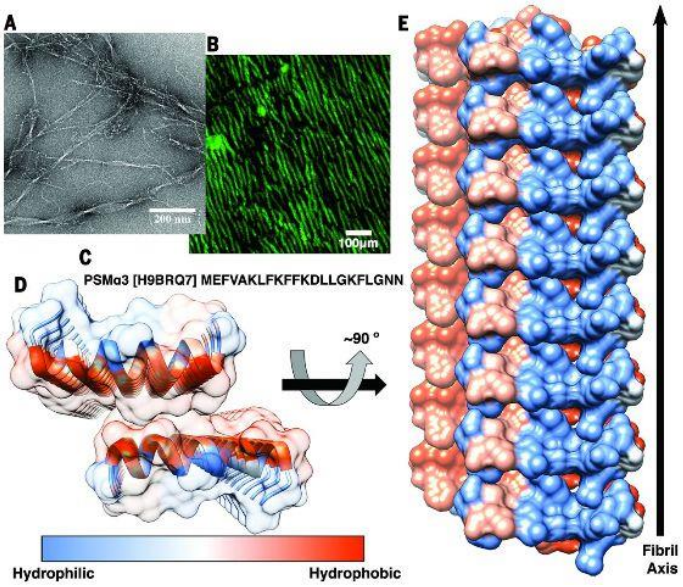

Figure 2. (A) An electron micrograph of *S. aureus* PSMα3 fibrils. (B) Fluorescence microscopy images of ThT–stained PSMα3 fibrils. (C) Amino acid sequence of *S. aureus* PSMα3 (UniProt accession number in brackets). (D&E) The crystal structure of PSMα3 at 1.45-Å resolution, coloured according to hydrophobicity (see scale bar). (D) A view down the fibril axis. PSMα3 forms parallel α-helical stacks, rendered as ribbons with semitransparent surface. Facing helical sheets are oriented head to tail. (E) Perpendicular view of fibril axis. The helices, shown in surface representation, run horizontally. Eight layers of α-helices forming the cross-α structure are depicted. The α-helical sheets interact via their hydrophobic face, creating a tight interface. From Tayeb-Fligelman et al, 2017. Reprinted with permission from AAAS.

PSMα3 Fibril Formation and Toxicity

Tayeb-Fligelman et al also investigated the role played by fibril formation in PSMα3 cytotoxicity by creating mutant peptide sequences to discover which residues are crucial for fibril formation. They found that non-fibril-forming mutants were less cytotoxic, although one such mutant still formed helices, leading to the conclusion that helical formation alone is not sufficient for cytotoxicity. Cytotoxicity was reduced in the presence of surfactant that inhibits fibril formation. The authors conclude that formation of cross-α amyloid-like fibrils by PSMα3 is required for cytotoxicity and may play a key role in the pathogenicity of *S. aureus*.

Summing Up

This is the first report of a cross-α amyloid-like fibril and perhaps more examples will follow. Should this be the case, the definition of amyloid may have to expand to include α-helices, not just β-strands.

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