Supramolecular structure of an antimicrobial peptide

Antimicrobial activity via self-assembly into stable helical fibrils of a human-derived peptide

Antimicrobial peptides (AMPs) are a diverse group of molecules that evolved in most living organisms over 2.6 billion years, and can provide new therapeutic venues to combat severe infections, killing cancerous cells and for immunomodulation. Importantly, AMPs are thought to induce less microbial resistance compared to conventional antibiotics. However, their relatively low efficacy and bioavailability, as well as their lack of chemical stability, discouraged development into therapeutic agents. Self-assembly of AMPs is functionally relevant and can enhance antimicrobial activity [1]. Moreover, the formation of supramolecular ordered structures of AMPs can provide immense stability against heat, shear force and chemical and proteolytic degradation.

The human LL-37 antimicrobial peptide (AMP) cleaved from cathelicidin is expressed by various mammalian cells and is considered to play an important role in the first line of defence against pathogens. LL-37 undergoes further proteolytic cleavage by host proteins, microbiome and invading pathogens, to yield numerous derivatives with a diverse array of selectivity against microbial strains and additional functions within the immune system [2]. The LL-3717-29 fragment (amino-acid residue 17-29, 13 residues long), although not detected directly in vivo, was suggested to serve as the active core of LL-37, showing a different spectrum of antibacterial activity compared to the full-length LL-37 and other fragments [3]. It generates an amphipathic helix with a large hydrophobic moment of 0.85 and a net charge of +4 (compared to 0.52 and +6, respectively, of the entire LL-37).

While crystal structures of short helical AMPs are rather rare, we determined the crystal structure of LL-3717-29, at 1.35 Å resolution using X-ray diffraction data collected at EMBL micro-focus beamline P14 at the PETRA III synchrotron (PDB ID 6S6M). Crystals could be only formed under one specific condition among 1440 tested solutions. The crystals diffracted well only when 2-methyl-2,4-pentanediol (MPD) was added before flash freezing. Data processing and refinement were highly challenging but nevertheless rewarding. The structure revealed a novel fibril structure (Fig. 1), composed of self-assembled amphipathic helices into a densely packed and elongated hexameric structure. This fibrillar assembly is comprised of four-helix bundles stabilised by a hydrophobic core, while a network of polar interactions stabilises interactions between bundles. Overall, the fibrillar assembly creates alternating hydrophobic and polar (positively charged) patches on its surface (Fig. 2), suggesting interactions and disruption of negatively charged lipid bilayers such as bacterial membranes.

In accordance with the fibrillar crystal structure, LL-3717-29 formed wide ribbon-like fibrils, which interacted with M. luteus bacterial cells (Fig. 2). Structure-guided mutagenesis confirmed the importance of self-assembly to the antibacterial activity. For example, the substitution of the amino-acid residue isoleucine 24, deeply buried within the four-helix

Figure 1
Novel fibrils of the human LL-3717-29 formed around bacterial cells. A transmission-electron micrograph of human LL-3717-29 showing wide fibrils formed around M. luteus cells. High-resolution structure of LL-3717-29 illustrated in a salmon-coloured ribbon representation along the fibril.
bundle, abolished antibacterial activity and the formation of fibrils that interact with the bacterial cells (Fig. 3), despite maintaining the amphipathic nature and total charge. In contrast, a mutation in a relatively exposed position (with minimal inter-helical contacts), despite change in amphipathicity, did not affect activity and showed the formation of nanofibrils and aggregation around the bacteria.

Certain AMPs were suggested to assemble into well-ordered fibrils that resemble amyloids [4], which are proteins associated with neurodegenerative and systemic diseases known to form ultra-stable cross-β fibrils composed of tightly mated β-sheets [5]. Yet LL-37(17–29) lacks the amyloid continuous sheets and also differs from helical fibrils such as collagen, actin and fibrinogen. Nevertheless, the helical LL-37(17–29) shares fibril thermostability often observed for β-rich amyloids and remained stable up to 80°C. Overall, LL-37(17–29) presents a type of self-assembly which is distinct from other protein fibrils, with a role in direct killing of bacterial cells still to be fully determined.

The findings expose a scaffold for wide-ranging applications in bio and nanotechnology, regenerative medicine and bioengineering, with the invaluable advantage of an inherent antibacterial activity. Links between fibril formation and antimicrobial activity are accumulating [6], and LL-37(17–29) provides atomic-level insight for such example. Further elucidation of the interplay between antimicrobial activity and fibril formation and morphology will aid the design of AMPs with enhanced potency, selectivity, stability, bioavailability and shelf-life. Successful design of such functional nanostructures with tuneable self-assembly might provide novel antibacterial therapeutics or coating of medical devices, and may target other roles of AMPs in immunomodulation and in killing cancerous cells.

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References

Original publication

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