Student Travel Award Recipient

Structural and Functional Insights into EchAMP, a Unique Monotreme Antimicrobial Protein Expressed During Lactation

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Monotremes, the egg-laying mammals with the ability to lactate are the only living representatives of ancient oviparous mammals with the primitive form of lactation. During evolution, they split from the therian lineage (present days placental mammals) about 166-210 mya and so displays a unique combination of reptilian and mammalian reproductive features. They lay parchment-shelled eggs which upon hatching give birth to altricial young ones developmentally equivalent to a 40-day old human embryo1. The mother's milk secreted from the nipple-less mammary patches is the only source of nourishment and protection for these altricial youngs against pathogens present in their environment. Monotreme milk thus is rich in bioactives and can be a novel source of antimicrobial molecules. Echidna AntiMicrobial Protein (EchAMP) is one such protein expressed in the echidna milk2. It is the tenth most abundant transcript with conserved secretory signal sequence and multiple putative sites for post-translational modifications2.

The detailed functional characterization of this protein was hindered because of the exclusivity of echidna as a geographically confined species and poorer protein yields with several eukaryotic expression systems. However, in the present study, we successfully purified the EchAMP protein in optimum quantities using a bacterial expression system. Unlike its eukaryotic counterpart, the recombinant protein from bacteria lacks post-translational modification but shows activity in our antimicrobial assays. Structurally the protein is intrinsically disordered as suggested by tryptophan fluorescence, circular dichroism, and NMR spectroscopy and like most of the IDPs upon thermal melting exhibit propensity to form transient a-helices. The in vitro antimicrobial assays with the purified EchAMP protein showed activity against both the Gram-positive (Bacillussubtilis, Staphylococcus aureus) and the Gram-negative micro-organisms (Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Enterococcus faecalis). This broad-spectrum antimicrobial activity is species specifically dose-dependent and mostly bacteriolytic as confirmed by live-dead staining and scanning electron microscopy. Moreover, the observed bacteriolytic activity was relatively higher for bacillus compared to other species used in the assay.

Echidna is a terrestrial animal that lives in burrows and so essentially needs protection against spore-forming soil microbes often known to be associated with infections in the lactating glands. Our studies using tryptophan fluorescence and circular-dichroism spectroscopy with amphipathic molecules and bacterial membrane mimics such as SDS, lipopolysaccharide (LPS) and liposomes (SUVs) respectively suggested that like other intrinsically disordered AMPs

(Cecropin A and Magainin 2)3 EchAMP too exhibits a propensity to fold into helices upon interaction with the bacterial envelope. Thereby gaining functionally active conformation and killing cells by compromising their membrane integrity and causing cytoplasm
leaking.

To summarize, EchAMP in light of its evolutionary conserved association only with monotremes and its broad-spectrum antimicrobial activity can be a promising candidate of choice against multiple infections associated with the lactating mammary gland in humans and livestock.