Perturbation of model membranes by two antimicrobial peptides with different hydrophobicity

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Antimicrobial peptides (AMPs) are promising candidates for the development of new drugs to face bacterial resistance to traditional antibiotics. Indeed, these peptides do not act on a specific cellular target but they interact with bacterial membranes, perturbing their permeability and causing bacterial death. The mechanism of pore formation is still debated; however, it is clear that AMPs cause a stress in the membrane by binding to the outer leaflet and inserting themselves below the polar headgroups. The antimicrobial activity and selectivity are determined by the interplay between electrostatic and hydrophobic driving forces: electrostatic interactions drive selective association of AMP to bacterial (anionic) membranes, whereas the hydrophobic effect defines the depth of insertion. Recently, the addition of an aromatic end tag to the sequence of AMPs was proposed as a general method to increase AMP activity, without reducing their selectivity [1, 2].

Here, we used spectroscopic methods to characterize differences in activity and selectivity between two peptides: GKH17 (Gly-Lys-His-Lys-Asn-Lys-Gly-Lys-Lys-Asn-Gly-Lys-His-Asn-Gly-Trp-Lys) and its analogue GKH17-3W (Gly-Lys-His-Lys-Asn-Lys-Gly-Lys-Lys-Asn-Gly-Lys-His-Asn-Gly-Trp-Lys-Trp-Trp-Trp), modified by the addition of three Trp residues at the C-terminus.

Peptide-membrane binding studies indicated that GKH17-3W has a higher affinity for anionic membranes than GKH17, also under physiological ionic-strength conditions. At the same time, its association to neutral membranes remains minimal, as required for a good selectivity. Quenching experiments confirmed that GKH17-3W inserts into membranes, locating under the polar headgroups. Peptide-induced leakage experiments highlighted again the higher activity of GKH17-3W, compared to GKH17. Furthermore, both peptides caused liposome aggregation and fusion; these effects could contribute to their antimicrobial action.

In agreement with biological assays [1, 2], GKH17 resulted to have a very low-lipid bilayer perturbing activity on model membranes: its strong hydrophilicity reduces its affinity for membranes and does not allow it to insert at the correct depth inside the bilayer. By contrast, the Trp end tag provided the correct hydrophobicity required for activity, without increasing toxicity significantly. Our data also suggest that the antimicrobial activity of GKH17 e GKH17-3W is not based on pore formation only: these peptides could induce the agglutination of bacteria, and thus prevent the diffusion of infection [3]..

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