Synergistic interactions between antimicrobial peptides and non-specific lateral crowding in membranes revealed by solid-state ^{19}F-NMR

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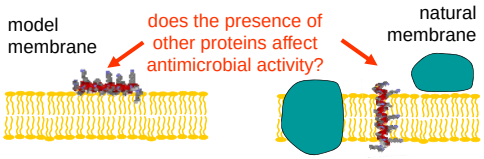
Summary

The activity of antimicrobial peptides relies on the interaction with the target membrane, where the interplay with the lipids, but also with proteinaceous components could be important. E.g. other antimicrobial peptides can modulate or synergisti-cally enhance activity [1]. To gain insight in the influence of lateral crowding and specific peptide interactions on the antimicrobial mechanism, we analyzed the behaviour of PGLa in the presence of other peptides mimicking a protein-rich membrane. We found a pronounced change of the PGLa activity in the presence of Magainin-2, and confirmed the synergism of these two peptides [1]. The other peptides did not induce equivalent changes in the PGLa behaviour, indicating a specific interaction of PGLa and Magainin-2.

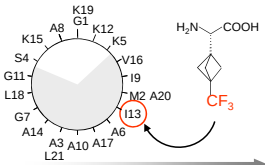


The peptide PGLa

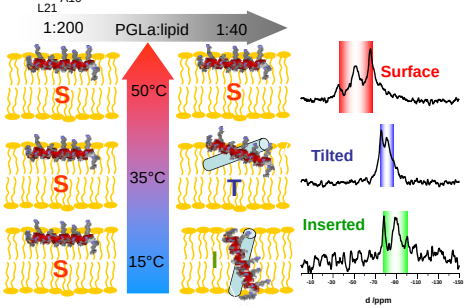
We tested the effect of proteins on the activity of antimicrobial peptides using PGLa as an example. This a-helical peptide, derived from frog skin, forms a polar side with 4 lysines, opposed by an alanine-rich hydrophobic side.

Different states of insertion into DMPC membranes were found recently for PGLa [2,3]. At low protein:lipid ratios, PGLa lies flat on the bilayer surface (S-state). At high concentrations PGLa is surface aligned only at high temperatures. At temperatures just above the lipid phase transition, the peptide tilts into the membrane (T-state). Below the phase transition, PGLa inserts completely in a nearly upright position (I-state). The PGLa activity might be related to dimers formed in the T and I states.

^{19}F-NMR to probe the structure



The insertion state of PGLa was monitored using ^{19}F-solid state NMR on oriented samples. To this aim we labelled PGLa with CF_3-bicyclopentylglycine, which links the CF_3-label to the peptide backbone in a rigid way [4].



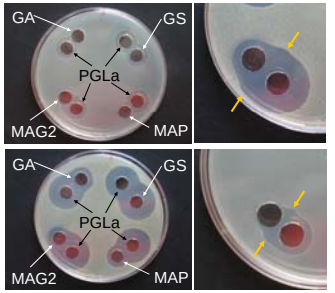
In oriented samples, solid state NMR resonances become orientation dependent. Thus, position and splittings of the CF_3-triplet signal reflect the orientation of PGLa. This way, all three states result in distinct ^{19}F-NMR spectra.

Results: Antimicrobial activity

As a first step we evaluated which influence the peptides, used in this NMR study to mimick crowding, have on the antimicrobial activity of PGLa. PGLa combined with Magainin-2 (MAG2) leads to growth inhibition zones typical for a synergistic behaviour (see yellow arrows), whereas all other peptides do not change PGLa activity.

Escherichia coli DH5a (gram-)

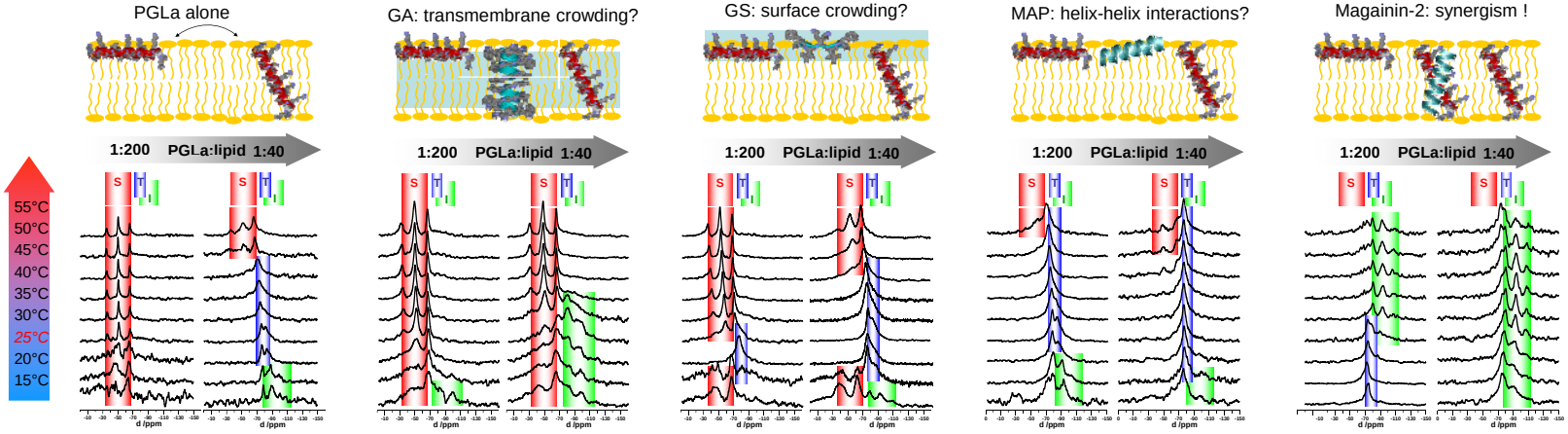
Micrococcus luteus ATCC 4698 (gram+)



Results: ^{19}F-NMR

The insertion state of PGLa was followed by ^{19}F-NMR in the presence of a second peptide. We used gramicidin-A (GA), gramicidin-S (GS), a model-amphipathic peptide (MAP) and magainin-2 (MAG) to mimick crowding in different regions of the bilayer, and to probe specific interactions.

Samples were prepared with two PGLa concentrations: at low PGLa:DMPC (1:200), where only the S-state states occurred in the absence of a second peptide, and at high PGLa:DMPC (1:40), where the inserted T and I states were found. The total peptide:lipid ratio was 1:20.



Conclusions

The behaviour of PGLa changes only marginally in the presence of gramicidin-A, gramicidin-S or MAP. Magainin-2 on the other hand turns PGLa into the inserted I-state even at low PGLa concentrations. These results indicate that the presence of membrane proteins as such ("crowding") has only little effect on the activity of antimicrobial peptides. Only when paired with a particular partner, such as magainin-2, the activity changes profoundly. The basis for the synergistic activity enhancement between PGLa and magainin-2 thus seems to be a specific interaction between these peptides.

Acknowledgements

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Literature

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