



Interaction Between Model Membranes and a Lipopeptide Studied Using EPR

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Introduction

As an effort to oppose the ever-growing antibiotic resistance, antimicrobial peptides (AMPs) are being extensively studied to develop more effective antibacterial reagents. The interest in AMPs is raised by their abilities to exert their antibacterial activities through nonspecific interactions with bacterial membranes, which lead to direct membrane disruption avoiding the development of resistance. Many AMPs are rich in cationic residues, so they are capable of selectively interacting with bacterial membranes, which are rich in negatively charged lipids, versus mammalian cell membranes, which are zwitterionic and contain cholesterol. However, natural AMPs are often susceptible to proteolytic degradation. The synthesis and study of new synthetic AMPs is of great importance to develop new antibiotic drugs with no protease vulnerability.

Experimental

EPR experiments were conducted using a Bruker E680 EPR spectrometer. Lipid bicelles and lipid vesicles with different lipid compositions, with 1% mol of 5-doxy stearic acid (5-SASL) or 5-doxy methyl stearate (5-MeSL) spin labels, were prepared in buffers with varied pH values. A lipopeptide, MSA652, was added to the lipids at increasing peptide/lipid (P/L) ratios. Lipid/Peptide mixtures were loaded into glass capillary tubes. Spectra were collected at room temperature for lipid vesicles and 308K for lipid bicelles.

Results and Discussion

MSA652 has a moderate influence on the mobility of lipid vesicles of different compositions, as shown in Fig.1. With 5-SASL, the highest fluidity changes occur in POPC liposomes; MSA652 has a significantly reduced effect on liposomes with cholesterol, while negatively charged liposomes (PC/PG) have an in-between profile. Interestingly, with 5-MeSL as a spin label, the interaction of MSA652 with PC/PG liposomes is unaltered, while the interaction with zwitterionic liposomes is greatly reduced.

The overall lipid disordering induced by MSA652 has been tested with DMPC/DHPC bicelles with increasing quantity of the MSA652 peptide. The data have been simulated to obtain the lipid order parameter S_{20} . Fig.2 shows the lipid order parameter S_{20} calculated for MSA652 at increasing P/L ratios compared to that of

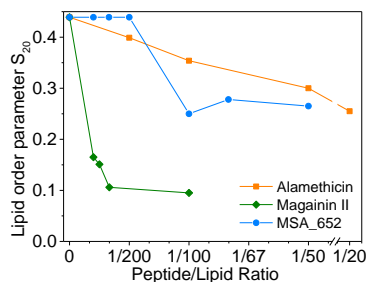


Fig.2 Plot of the orientational order parameter S_{20} as a function of the P/L ratio. For comparison, the reported values for Alamethicin and Magainin II are also displayed.

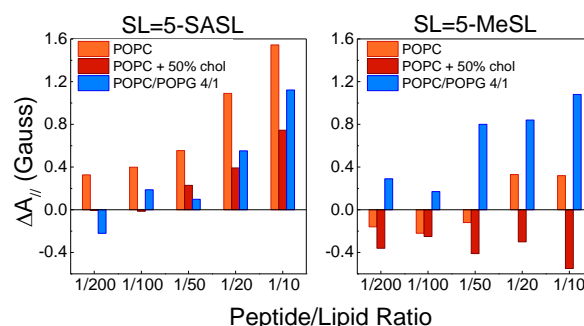


Fig.1 Membrane fluidity changes in the presence of MSA652. The motional parameter ΔA_{ij} , i.e. the peak to peak splitting, quantifies the difference in mobility between liposomes with and without peptides.

model peptides Alamethicin and Magainin II. MSA652 order parameter decreases slowly, resembling Alamethicin profile. Next, we plan to determine peptide-induced disordering on negatively charged bicelles and to assess peptide-induced pore formation with leakage experiments.

Conclusions

Data on MSA652 lipopeptide show that it has a scarce propensity to interact with cholesterol-containing membranes, while it has a preference for negatively charged lipids. The difference observed for POPC liposomes is attributed to the negative charge of 5-SASL: once this is removed, MSA652 effect on those liposomes is also reduced. The lipid disordering profile shows a low ability of MSA652 to perturb and disorder zwitterionic bilayers: further experiments will clarify whether MSA652 forms ordered pores or doesn't cause membrane permeability.

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