Cholesterol and Unsaturations Induce Resistance to Magainin H2 Pore Formation through Opposing Effects on the Modulation of Lipid Packing

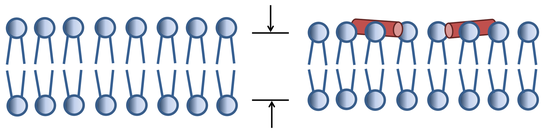
Nathaly Marín-Medina, Steve Trier, Rafael Clavijo, Chad Leidy, Manu Forero-Shelton

Department of Physics, Universidad de los Andes, Bogotá, Colombia

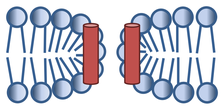
INTRODUCTION

Antimicrobial peptides (AMPs) found in nature use different bacterial killing mechanisms. The interest of understanding these mechanisms at a molecular level has increased, considering their potential to treat antibiotic-resistant bacterial infections.

Short cationic AMPs, like Magainin H2 (MagH2), permeabilize bacterial membranes by inducing transmembrane pores. These AMPs initially bind in an orientation parallel to the membrane as an α-helix, embedded into the lipid headgroup region inducing membrane thinning [1].



Beyond a threshold peptide-to-lipid ratio, AMPs bind perpendicular to the membrane and form pores [1].



Increased headgroup spacing has been proposed to stabilize peptides in their parallel configuration, inhibiting peptide insertion and pore formation [2]. We aim to determine if the headgroup spacing (as reported by Laurdan GP) is a direct predictor of MagH2 activity on model membranes, given that unsaturations and cholesterol have opposite effects on this spacing.

REFERENCES

[1] Huang HW (2000) *Action of antimicrobial peptides: two-state model*. Biochemistry (Mosc) 39: 8347–8352.

[2] Strandberg E et al. (2012) *Lipid shape is a key factor for membrane interactions of amphipathic helical peptides.* Biochim Biophys Acta Bba-Biomembr 1818:1764-1776.

ACKNOWLEDGEMENTS

Funding for this project was obtained from:

- a Colciencias scholarship (Convocatoria N° 567 - 2012, NM)
- an internal research grant from the Faculty of Sciences (Convocatoria 2013-2, NM) at Universidad de los Andes
- a research grant from Colciencias (123048925268, CL)
- Whitaker International Program (ST)

Travel expenses were covered by a grant from the Faculty of Sciences at Universidad de los Andes.

We wish to thank the Biophysics Research Group at Universidad de los Andes for their support in the lab work and helpful discussions.

RESULTS

How does headgroup spacing (as reported by Laurdan GP), which is modified by unsaturations and cholesterol in opposite ways, affect MagH2 activity on model membranes?

Headgroup spacing increases when comparing monounsaturated (POPC) and diunsaturated (DOPC) lipids, and decreases when cholesterol is added.

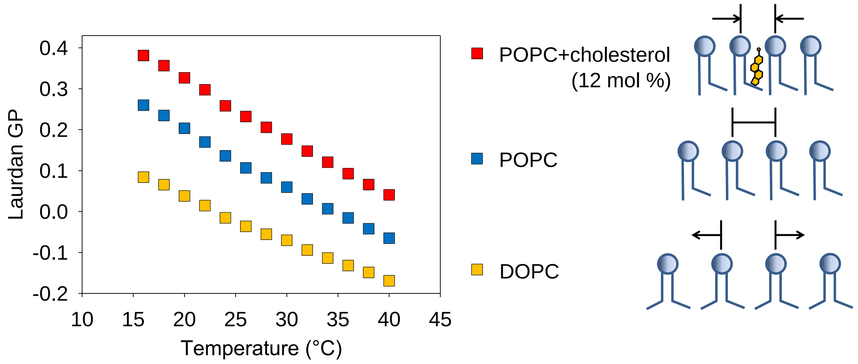


Fig. 1 Laurdan reports the extent of water penetration into the bilayer interfacial region, which is correlated with headgroup spacing. Higher values of Laurdan generalized polarization (GP) correspond to a reduced level of headgroup spacing.

GP is calculated as $GP = (I_{440} - I_{490})/(I_{440} + I_{490})$ where $I_{440/490}$ is the emission intensity at 440 or 490 nm.

Both unsaturations and cholesterol increase resistance against MagH2 activity despite having opposite effects on headgroup spacing.

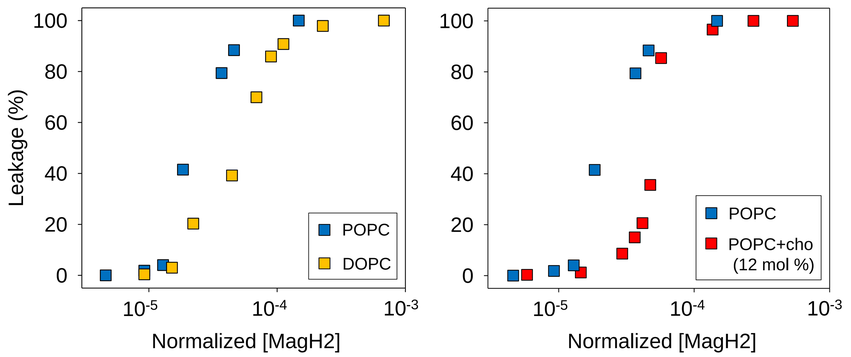
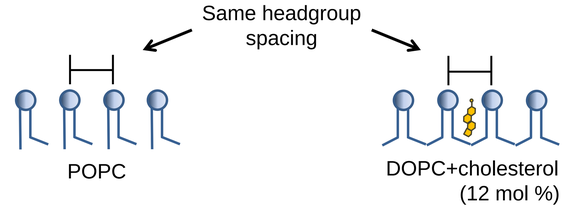


Fig. 2 MagH2 activity is determined by monitoring the leakage of entrapped calcein from large unilamellar vesicles (LUVs) of 100 nm nominal diameter, due to transmembrane pore formation.

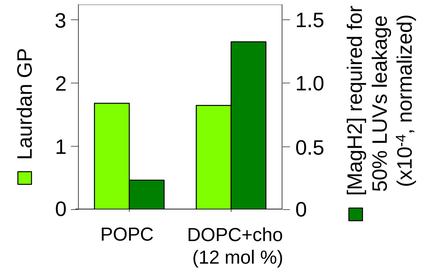
Leakage at higher normalized concentrations of MagH2 corresponds to higher resistance against the peptide, where we have normalized the peptide concentration to the total vesicle content in the sample.

How do two lipid compositions with the same spacing compare in terms of their resistance to MagH2?

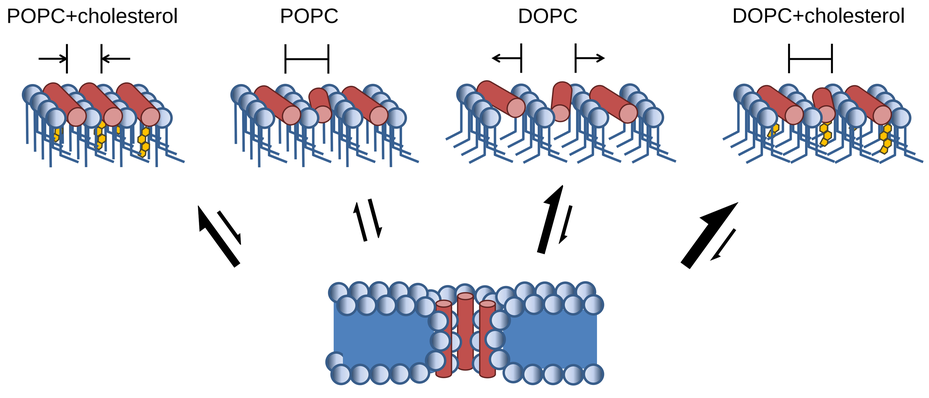


DOPC+cholesterol vesicles show a higher resistance against Mag-H2 than POPC vesicles with the same headgroup spacing (as reported by Laurdan).

Fig. 3



CONCLUSIONS AND PERSPECTIVES



- Headgroup spacing alone does not directly predict MagH2 activity. Our results indicate that cholesterol has an additional inhibiting effect on MagH2-induced pore formation not related to the regulation of headgroup spacing.
- In order to avoid alternate mechanisms by which cholesterol could affect pore formation or Laurdan measurements, we propose to match the headgroup spacing of POPC and DOPC by changing temperature.