Cholesterol and Phosphatidylethanolamine Lipids Exert Opposite Effects on Membrane Modulations Caused by the M2 Amphipathic Helix

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Introduction

Membrane curvature remodeling induced by amphipathic helices (AHs) is essential in many biological processes. We used a model amphipathic peptide, M2AH, derived from influenza A M2 protein as a template to study how AHs may promote membrane curvature by altering membrane physical properties. M2 of influenza A virus is a versatile protein composed of an extracellular domain, a transmembrane helix, and a cytoplasmic domain. M2 forms a low-pH activated proton channel. The channel activity of M2 is essential for virus unpacking. The cytoplasmic domain of M2 contains a membrane-associated AH called M2AH that spans residues 47–62. Here, we used M2AH as a template to assess how AHs may compensate the curvature-associated energy penalty. In particular, we used electron paramagnetic resonance (EPR) spectroscopy to explore changes in membrane material properties.

Experimental

EPR spectra were collected using a Bruker E680 spectrometer and a HiPER spectrometer at the NHMFL. Lipid bicelles and lipid vesicles with different lipid compositions, with 1% mol of 5-doxyl stearic acid (5-SASL), were prepared in buffers with defined pH values. M2AH was added to the lipids at increasing peptide/lipid (P/L) ratios. Lipid and peptide mixtures were loaded into glass capillary tubes before EPR measurements.

Results and Discussion

We used EPR spectroscopy to explore changes in lipid chain mobility and chain orientational order. To determine the role of different lipid species in mediating M2AH-membrane interactions, we selectively studied model lipid membranes containing phosphatidylcholine (POPC), cholesterol (Chol), anionic phosphatidylglycerol (POPG), and phosphatidylethanolamine (POPE) lipids. Our EPR spectroscopy showed that M2AH reduced lipid chain mobility of lipid vesicles (**Fig.1**) and had a minimal effect on lipid chain orientational order defined using lipid bicelles (data not shown). The EPR data are consistent with the surface-bound state of M2AH that acts as a chain mobility inhibitor. By comparing results from different lipid bilayers, we found that cholesterol enhanced the activity of M2AH in inducing bilayer pits and altering lipid chain mobility. The results were explained by considering specific M2AH-cholesterol recognition and/or cholesterol-induced expansion of interlipid distance. Moreover, lipid chain mobility measurements revealed a modest effect of anionic lipids. This highlights that membrane interaction of M2AH is mainly driven by hydrophobic forces. Lastly, we found that phosphatidylethanolamine lipids inhibited the activity of M2AH.

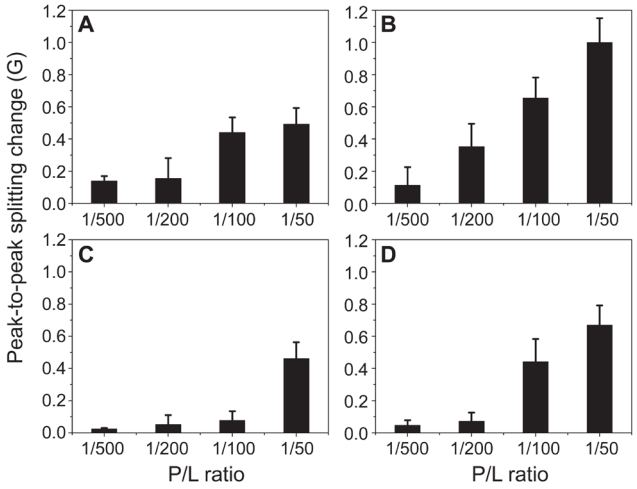

Fig.1. EPR spectroscopy revealed M2AH-induced changes of lipid chain mobility for liposomes composed of (A) POPC, (B) POPC+30%Chol, (C) POPC+40%POPE, and (D) POPC+20%POPG. For each lipid composition, four P/L ratios were examined. Positive changes of the peak-to-peak splitting ($2A_{//}$) reflect lower lipid chain mobility.

Conclusions

The obtained biophysical data highlight that M2AH may facilitate membrane curvature reorganization by altering membrane material properties. By determining the impacts of M2AH on physical properties of lipid bilayers containing different lipid species, our study provides useful insights into the role of various lipids in regulating M2AH-mediated membrane restructuring.

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References

[1] Pan, J., *et al.*, BBA Biomembranes, **1861**, 201-209 (2019).