Interactions between Model Membranes and Influenza Virus Protein M2-Derived Peptides Studied by EPR Techniques

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Introduction

Influenza A is responsible for seasonal outbreaks of the flu including extremely deadly outbreaks such as the Spanish Flu. Continuous research into many aspects of the virus mechanism of action is critical for human health. M2ah is a peptide extracted from the M2 protein, which is a protein expressed by the influenza A virus that has been linked to viral invasion and release. A crucial step for an effective viral infection is the budding and fission of host cell plasma membranes. Here, the perturbation effects of M2ah on membrane-mimicking systems were examined using Electron Paramagnetic Resonance (EPR) spectroscopy.

Experimental

EPR experiments were conducted using a Bruker E680 spectrometer in X-band (9.5 GHz) and a HiPER spectrometer in W-band (95 GHz) 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. M2ah 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 308 K for lipid bicelles.

Results and Discussion

Lipid fluidity changes are reflected in differences in the spectral peak-to-peak splitting ($\Delta A_{//}$), as shown in **Fig.1**. M2ah has a moderate influence on the mobility of lipid POPC vesicles, where the $\Delta A_{//}$ change at the highest P/L ratio has a value of ~0.5 Gauss, indicating that the lipid mobility is slightly reduced. Cholesterol has a significant influence on the mobility induced by M2ah, displaying a ~1 Gauss increase at the same P/L ratio. This result suggests that M2ah interacts more strongly with membranes containing high percentages of cholesterol, which is characteristic of Influenza A lipid viral envelope.

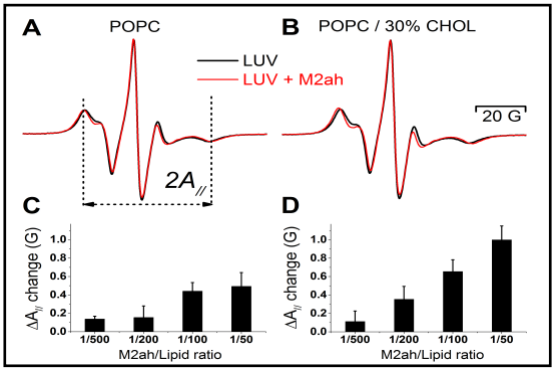

Fig.1 Upper panel: EPR spectra of liposomes labeled with 5-SASL for (A) POPC and (B) POPC/30% cholesterol recorded at 295 K at P/L =1/50. EPR spectra of bare liposomes (black) are overlaid with the spectra in the presence of M2ah (red). Lower panel: Fluidity changes upon peptide binding are represented by $2A_{//}$ changes ($\Delta A_{//}$) at various P/L ratios for (C) POPC and (D) POPC/30% cholesterol

The overall lipid disordering induced by M2ah has been tested with DMPC/DHPC bicelles with increasing quantity of M2ah peptide. Data have been simulated to obtain the lipid order parameter S_{20}. **Fig.2** shows the lipid order parameter S_{20} calculated for M2ah at increasing P/L ratios compared to that of model peptides Alamethicin and Magainin II. M2ah seems to have little to no influence on lipid disordering, a result that opens question on the details of M2ah interacting with zwitterionic membranes in these specific experimental conditions.

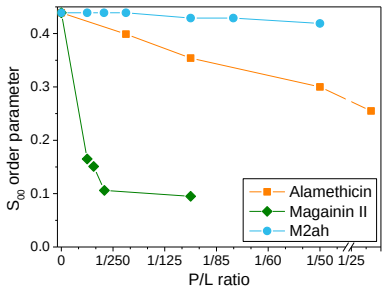

Fig.2 Plot of the orientational order parameter S_{20} as a function of the P/L ratio. For comparison, the reported values for Alamethicin and Magainin 2 are also displayed.

Conclusions

Data on M2ah show that it has a low propensity to interact with POPC liposomes, while it has a preference for cholesterol-containing membranes, which is a feature shared by mammalian membranes and Influenza viral envelope. The lipid disordering profile shows a very low to absent ability of M2ah to perturb and disorder zwitterionic bilayers; further experiments will clarify whether M2ah forms very ordered pores or doesn't cause membrane permeability; we will also verify if M2ah is able to perturb cholesterol-containing bicelles.

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