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Accumulation of polystyrene oligomers alters lipid membrane phase order and miscibility

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Introduction

The cell membrane is the first barrier encountered by any foreign object entering an organism, and therefore is the primary candidate of investigation in assessing possible toxicity of plastic nano fragments. In particular, the membrane phase behavior plays a crucial role in maintaining cell functionality, and even minute changes in membrane phase properties can result in a potential threat for the organism. Several coarse-grained molecular simulation studies suggest that polystyrene (PS) may alter the phase behavior and properties of lipid membranes. Rossi *et al.* [1] described that the presence of PS oligomer and polymers changes the bending rigidity of POPC membranes (16:0-18:1-phosphocholine) and moreover affects the phase coexistence when cholesterol is present in the bilayer. Bochicchio *et al.* [2] showed that PS stabilizes phase coexistence in model bilayers. These results indeed point out to a potential hazard since protein functionality is partially controlled by the membrane organization. In this work we investigate the effects of polystyrene oligomers (Mn = 500 Da) on the phase transition of lipids bilayers composed of saturated, or unsaturated, or a mixture of both, lipids. We investigate the changes in phase transition using differential scanning calorimetry, and Laurdan fluorescence spectra, extracting information on the structure and the thermodynamics. Moreover, we directly visualize the changes on the membranes at the micrometric scale using epifluorescence microscopy.

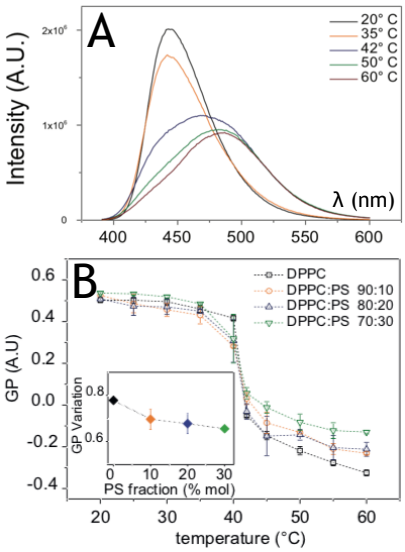
Results

Small angle neutron scattering (SANS) was used both to confirm the presence of polystyrene within the lipid membrane and to obtain information about the changes on the bilayer structure induced by the oligomers. Liposomes of DPPC were compared to liposomes of DPPC:PS (70:30), at 25°C, *i.e.* in the gel, L_0 phase, and at 50°C, *i.e.* in the fluid, L_α phase. Though SANS curves do not allow to quantitatively determine the lipid:polymer ratio, results clearly show that PS is successfully incorporated in the membrane, and that liposomes do not aggregate upon PS incorporation (results not shown).

General Polarisation (GP) of Laurdan was calculated from Laurdan emission spectra (Fig. A) as:

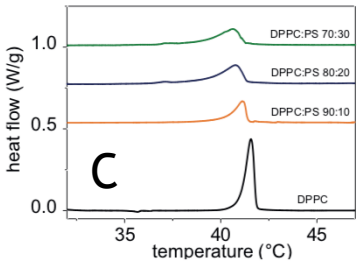
$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}}$$

GP was used to highlight changes in the membrane order upon incorporation of the oligomers (see reference paper [3]). Laurdan spectral properties reflect the hydration level in the headgroup region of the lipids. Figure B illustrates how GP evolves with temperature and PS fraction in DPPC:PS liposomes. The strong increase in GP with PS fraction in the fluid phase (high temperature), can be attributed to a lower number of water molecules due to the presence of polystyrene, hinting a higher packing order of the lipid molecules induced by PS.

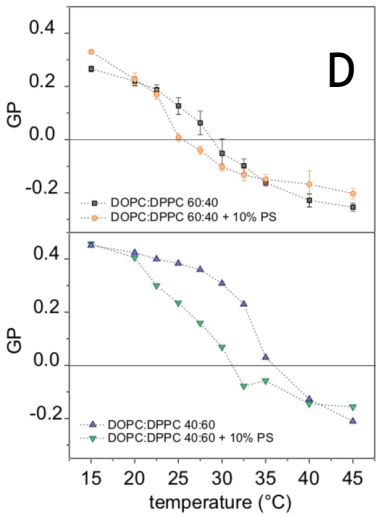


Combined with SANS data, these results indicate a different localisation of the polymer within the bilayer in the gel (L_0) and fluid (L_α) phases.

Gel-to-fluid transition is inhibited by PS oligomers. Differential scanning calorimetry (DSC) thermographs (Fig. C) show that, with increasing amount of polystyrene incorporated in the membrane, the main transition temperature T_m slightly decreases, while the peak significantly decreases in intensity and broadens, suggesting a loss in enthalpy and cooperativity. That results are consistent with the effects reported for hydrophobe/lipid bilayer [4,5] or pheromones/lipid bilayer [6] interactions.



Polystyrene increases miscibility between So and Lα phase. Figure D shows the effect on GP of the presence of PS in two binary membrane compositions, *i.e.* DOPC:DPPC 40:60 and 60:40. Both mixtures exhibit S_0/L_α (gel/fluid) phase coexistence at low temperature, and a homogeneous, L_α phase at 45°C. The presence of PS (10 mol%) decreases the transition temperature T_m (obtained as the intercept at GP=0), all the more so when DPPC fraction increases. These results are confirmed by image analysis of fluorescently labelled Giant Vesicles under a microscope (not shown). A thermodynamic model was developed, that considers the gel-to-liquid transition in mean-field Ising model. Using the values obtained from DSC on DPPC:PS bilayers (Fig. C), the model reproduces the data presented in Fig. D, as well as other results taken from literature.



Conclusions

Our results indicate that low molecular weight polystyrene incorporated within the hydrophobic region of lipid bilayers not only distributes differently between the S_0 (gel) and L_α (fluid) bilayer phases, but also perturbs significantly the phase behavior, in particular by decreasing the transition temperature between S_0 and L_α phases in binary, saturated/ unsaturated lipid mixtures.

References

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