Biophysical studies of cell penetrating peptides (CPP) on 3D membrane model systems

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Aim

The main goal of this project is using cell-penetrating peptides (CPPs) as the tool for membrane lipid intervention, enabling them to induce apoptotic signalling and act as chemosensitizers. The strategy will consist of constructing CPPs on the basis of the structure of a peptide ($S4_{13}PV$), which was shown in previous studies to display low level of cytotoxicity, high ability to transpose membranes and competence as a nucleic acid delivery system [1]. In order to uncover the mechanisms underlying the membrane translocation of a scrambled (scr) and a wild type (wt) $S4_{13}PV$ and their ability for nucleic acid delivery, biophysical studies were performed to characterize peptide-membrane interactions using membrane model systems.

Main achievements

The structural modifications of 3D model systems made of lipids representative of zwitterionic membranes (DPPC), negatively charged membranes (DPPG) and membranes containing non-bilayer lipids (DOPE:DOPG (7:3)) induced by increasing concentration of CPPs were studied by small-angle (SAXS) and wide-angle x-ray (WAXS) scattering. SAXS experiments showed that $S4_{13}PV$ peptide interacts preferentially with anionic rather than zwitterionic membrane lipids, promoting alterations of the lipid packing and inducing lateral phase separation. From the SAXS patterns of the $L_{\beta'}$ phase of the negatively charged membrane it is possible to conclude that the addition of peptides led to a lipid phase separation. Indeed, a non-influenced DPPG $L_{\beta'}$ phase occurs ($d \approx 5.0$ nm not relevantly changed comparing with pure DPPG) together with an interdigitated phase (much smaller d values ≈ 3.0 nm characteristic of $L_{\beta I}$). This additional interdigitated phase, visible in the SAXS regime, can be confirmed in the corresponding WAXS patterns (Figure 1).

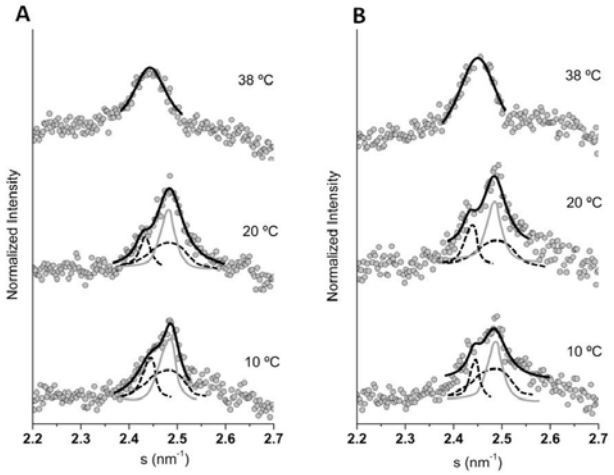

Figure 1: WAXS patterns of (A): $S4_{13}PV_{scr}$ (50:1) and (B) DPPG: $S4_{13}PV_{wt}$ (50:1) at the temperatures of 10, 20 and 38 °C. Solid lines give the best fit of the Lorentzian's analysis model to the scattered intensities. At temperatures of 10 and 20 °C the fitting can be described as the superimposition of three peaks corresponding to the orthorhombically packed acyl chains (dashed black lines) and interdigitated acyl chains (gray line).

In agreement with our results, it was reported that peptides, depending on their structure and concentration might tip the balance between headgroup and hydrocarbon chain interactions and induce interdigitated phases in PG [2]. These interdigitated phases are of great interest since although being observed only in the gel phase; their effects may propagate into physiologically fluid phase in terms of membrane thinning and membrane perturbation.

Furthermore, SAXS data showed that the CPPs studied induce the generation of non-lamellar phases in model membrane systems containing non-bilayer lipids. SAXS measurements were performed at two different temperatures: 20 and 50 °C, where only lamellar phases (L_α) are expected. At the temperatures tested, several small angle diffraction peaks were detected in the DOPG:DOPE mixture. The observed reflections in the absence of peptides are consistent with the coexistence of two lamellar phases: a remaining L_β, corresponding to the peaks positioned at lower lattice spacings and a L_α phase corresponding to the peaks positioned at higher lattice spacings. The first and second order Bragg peaks of each of these lipid phases presented an indexed ratio of 1:2, which indicates the lamellar nature of these phases. Concentration dependent effects occurred upon interaction of either of the two CPPs with the lipid mixture. Both peptides promoted the formation of non-lamellar phases at lower temperatures (50 ºC) than the expected for this lipid mixture, which according to the literature occurs at about 65 ºC [2]. In the presence of $S4_{13}$-PV_{scr}, several small angle reflections were observed at 50 ºC. The first order reflection at 5.06 ± 0.05 nm and the second order reflection at 2.63 ± 0.05 nm were assigned to the lamellar phase (indexed ratio of 1:2). Whereas the first order reflections at 4.65 ± 0.05 and 5.98 ± 0.05 nm and the second order reflections at 2.63 ± 0.05 nm and 2.97 ± 0.05 nm were assigned to an inverted Hexagonal (H_{II}) phase (indexed ratio of 1:√3:2). $S4_{13}PV_{wt}$ peptide also proved to be able to promote the formation of non-lamellar phases, in this case consistent with the formation of a cubic phase and a small amount of H_{II} phase. Thus, at this temperature, the first order reflections at 4.65 ± 0.05, 5.42 ± 0.05 and 6.55 ± 0.05 nm and the second order reflections at 2.73 ± 0.05 nm and 2.98 ± 0.05 nm were assigned to the cubic phase (indexed ratio of 1:√3:√4:√5) and a H_{II} phase (indexed ratio of 1:√3:2).

Conclusions

Consistent with our results, upon interaction with the CPPs, ionic bonds are expected to form between the positive charges of the peptide and the anionic phospholipids. Subsequently, lateral phase separation occurs with the appearance of defects at the boundaries of domains with different lipid composition. From the CPPs embedded in the membrane, two consequences might be predicted depending on the disorder level of the lipid domains. In the case of highly ordered domains (< Tm, Figure 2), the hydrophobic mismatch will promote fatty acid chain interdigitation with an additional increase of order. However, in the case of liquid-disordered domains (> Tm, Figure 2), the insertion of the peptide may afford a higher separation and mobility of the hydrocarbon chains, leading to an increase of the curvature stress with destabilization of the bilayer and eventual formation of inverted micelles, which could create conditions for the peptide translocation across the membrane thickness.

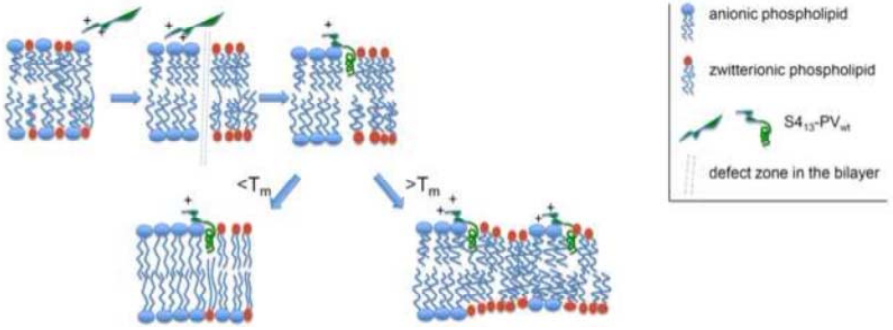

Figure 2: Schematic prediction of $S4_{13}$-PV_{wt} with membranes.

References

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