

Structure and ionization state of DPPG monolayers

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Electrostatic interactions play an important role in the functioning of biological membranes. Membranes often bear a negative charge, mainly due to a presence of acidic phospholipids as well as membrane proteins and gangliosides. Therefore, it is important to study the phase behavior and structure of acidic phospholipids in dependence on pH, ionic strength and the nature of counterions (size, charge, hydrophobicity). Modern experimental techniques as grazing incidence X-ray diffraction (GIXD), X-ray reflectivity (XR), surface potential measurements and Infrared Reflection Absorption Spectroscopy (IRRAS) allow the determination of the ionization state in a Langmuir monolayers at the air/water interface.

Phosphatidylglycerols (PGs) are components of many biological membranes, especially bacterial cell membranes, and they are also one of the important components of pulmonary surfactants. Area/pressure isotherms and monolayer structures of PGs are strongly dependent on subphase composition. At 20 °C on pure water, dipalmitoyl-phosphatidylglycerol (DPPG) exhibits a condensed phase already at zero surface pressure. A second-order transition between two condensed phases appears at 32 mN/m. The same behavior is observed at low pH and in the presence of divalent cations. In contrast, in the presence of alkali cations a phase transition from LE (liquid-expanded) to LC (condensed) was observed. IRRAS data demonstrate that on pure water the phosphate group of DPPG is almost not charged (approximately 10% charged groups).

The structure of DPPG on different subphases was elucidated by GIXD using the liquid surface diffractometer at the undulator beamline BW1 at HASYLAB, DESY. Figure 1 shows selected contour plots for DPPG monolayers at 25 mN/m on different subphases. At pH 2, the DPPG chains are less tilted than at pH 7. Additionally, the DPPG monolayer structure at pH 2 is affected by the presence of cesium ions. The type of the cation on the DPPG structure has a much smaller influence than the pH value. Although the tilt angles of DPPG to the surface normal are very similar on CsCl and LiCl, the monolayer structures differ slightly. The DPPG chains on CsCl are arranged in an orthorhombic lattice and tilted in the nearest neighbor direction (NN). In contrast, an oblique structure with chains tilted into an intermediate direction between NN and NNN (next nearest neighbor) directions is observed on LiCl (Fig. 1).

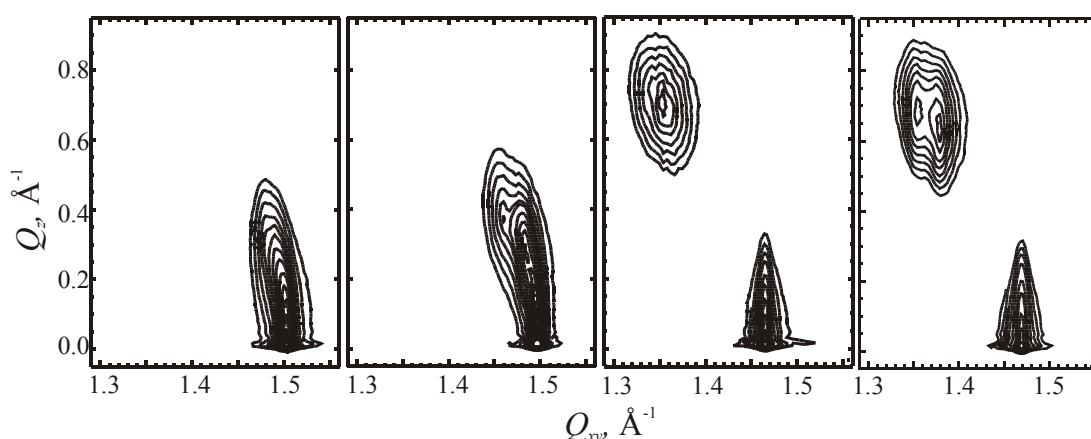


Figure 1: Contour plots of the corrected X-ray intensities as a function of the in-plane component Q_{xy} and the out-of-plane component Q_z of the scattering vector of DPPG monolayer at 25 mN/m on various subphases (from left to right): pH 2 adjusted with hydrochloric acid, pH 2 adjusted with hydrochloric acid and 0.1 M CsCl, phosphate buffer with pH 7, 0.1 M CsCl, EDTA, and phosphate buffer with pH 7, 0.1 M LiCl, EDTA

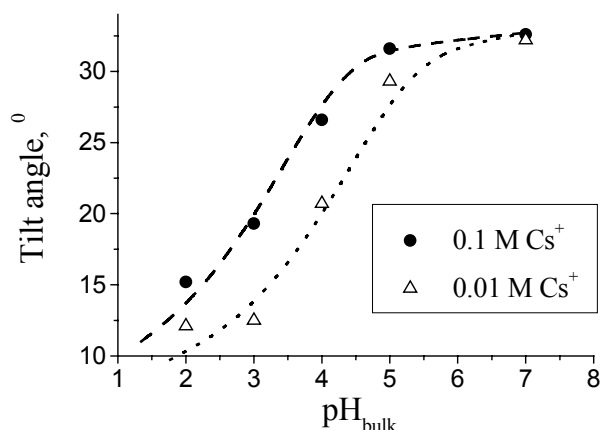


Figure 2: Tilt angle of DPPG chains versus subphase pH at 25 mN/m. The aqueous subphase contains CsCl in different concentrations (indicated)

Figure 2 shows the dependence of the tilt angle on pH at a surface pressure of 25 mN/m in the presence CsCl. The largest tilt angle of approximately 32° is observed at pH 7 and the smallest at pH 2 (12°). At the same pH, the tilt angle increases with increasing CsCl concentration. At pH 7, the observed tilt angle is independent of

the salt concentration. This indicates that DPPG is fully ionized at pH 7.

XR measurements were performed with DPPG monolayers on subphases containing Cs⁺ at different pH values. The reflectivity curves were fitted with a box model by varying roughness, thickness and electron density. The amount of excess electrons in the head group region was calculated by integrating the electron density profile curves in the headgroup region and multiplying with the area per molecule obtained from GIXD. The excess of electrons in the head group at different pH values (55 at pH 2 without Cs⁺ and 91 at pH 7 with Cs⁺) should be proportional to the ionization degree of DPPG molecules. Assuming an ionization degree α of 1 at pH 7, the obtained titration curve is shown in figure 3. The XR data reveal that the DPPG monolayer is partly charged even at pH 2 in the presence of 0.1 M CsCl in good agreement with the GIXD data. The best fit to the experimental data using the Gouy-Chapman theory was achieved for $pK_a = 1$. The obtained intrinsic pK_a is close to the previously estimated value ($pK_a = 1.2$ [1]).

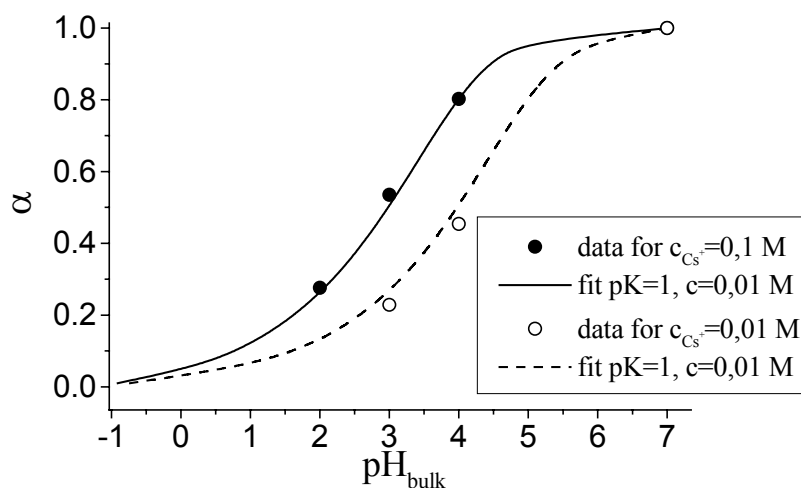


Figure 3: Ionization degree α versus bulk pH on aqueous subphases containing different CsCl concentrations. The data are fitted using the Gouy-Chapman theory with $pK_a = 1$

The higher ionization degree of DPPG increases the repulsion between neighboring molecules, which leads to the formation

of a more expanded layer. The possible penetration of counterions into the headgroup region might explain the small differences in the DPPG monolayer structures on LiCl and CsCl indicating a small ion size effect.

XR measurements in combination with IRRAS allow to obtain direct information about the ionization degree of a DPPG monolayer.

References

- [1] Sacre, M.M. and J.F. Tocanne, Chem. Phys. Lipids 18, 334 (1977)