Molecular Mechanisms of Phosphatidylcholine Monolayer Solidification due to Hydroxyl Radicals

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The reaction of L-\(\alpha\)-1,2-dipalmitoylphosphatidylcholine (DPPC) with hydroxyl (HO\(^-\)) radicals (Fenton solutions) was investigated using monolayer techniques: isotherms, infrared absorption reflection spectroscopy (IRRAS), grasing incidence X-ray diffraction, X-ray reflection and fluorescence microscopy.\textsuperscript{[1]} The DPPC monolayer is attacked with different \(\cdot\text{HO}^-\) concentrations produced by the Fenton reaction (\(2\cdot\text{HO}^- + \text{Fe}^{3+} \rightarrow \text{OH}^- + \text{OH}^+ + \text{Fe}^{2+}\)).\textsuperscript{[2]} The decrease of the lateral pressure was used as a measure of the efficiency of the HO\(^-\) attack. With increasing HO\(^-\) concentration, the plateau region in the isotherm was shifted to a lower surface pressure; eventually it disappeared. Fluorescence microscopy during the HO\(^-\) attack showed that new domains in the condensed phase nucleate immediately. With isotherms and X-ray diffraction we found that the monolayer can be compressed to smaller molecular areas (cf. Fig. 1).

Figure 1: Grazing incidence X-ray diffraction measurements of a DPPC monolayer on a 2 mM Fenton solution, before and after the HO\(^-\) attack (details see text). Left: The fresh DPPC monolayer was characterized by two diffraction peaks (top to bottom) the peak positions shifted to larger \(Q_{xy}\)-values indicating smaller lattice spacing. Simultaneously, the peak at large \(Q_z\)-positions moved to smaller values, a sign of a decreased tilt angle. Furthermore, the peaks distorted and showed a banana-like shape which is attributed to a distribution of the tilt azimuth \textsuperscript{[3]}. Right: Monolayer compression after the HO\(^-\) attack: Already at almost zero lateral pressure (top), the out-of-plane peak occurred at larger \(Q_{xy}\) and lower \(Q_z\)-positions. The lines are fits, the intensity is colour coded (note the almost logarithmic colour coding). For each measurement, the lateral pressure and the molecular area as deduced from the isotherm are indicated.
The reduced tilt angle and increased translational order (cf. Fig. 2) showed decreased inter- and intramolecular repulsion. IRRAS experiments indicated a decomposition of the head group. We conclude that a reduced head group size improves the ordering of the alkyl tails.

Figure 2: Parameters deduced from the analysis of the X-ray diffraction peaks as function of the molecular area A from the isotherm. Before the HO⁻ attack, the freshly prepared monolayer was characterized (red, peaks shown in Fig. 3, left). Two series of experiments were performed, one DPPC monolayer was subject to a weak HO⁻ attack (open symbols), the other to a strong attack (full symbols). In the first case, the lattice parameters were changed only little (green), in the second case more (blue, peaks shown in Fig. 3, right) Top: tilt angle \( t \) with respect to the surface normal. After the strong HO⁻ attack, smaller molecular areas are obtained in the isotherm are accompanied by smaller tilt angles. Dashed line: tilt angle calculated with \( \cos t = 40.4 \ \text{Å}^2 / A \). Centre: Tilt azimuth distribution. On monolayer compression, the distribution increased. After the HO⁻ attack, the azimuth angle distribution was small and stayed so. Bottom: Correlation length determined from the peak at low \( Q_{xy} \). For the freshly prepared layer, the correlation length was constant (ca. 110 Å), after the HO⁻ attack it increased to 150 Å. Dotted lines are guide to the eye.

We attribute the solidification we observe to a preferential radical attack in the hydrophilic region of the monolayer. Summarizing, the subtle interplay of inter- and intramolecular interactions lead to the following effects: (i) immediate formation of liquid-condensed phase on HO⁻ attack, (ii) improved crystalline order in the liquid condensed phase, (iii) lower molecular areas feasible, and (iv) a decreased intermolecular repulsion which reduces the phase transition pressure from the fluid to the liquid condensed phase. The implications of the observed solidification of the monolayer for biological systems and membranes will addressed in future collaborative research with scientists from biology and medicine.

References