Phosphatidylcholine Monolayer after Attack of Hydroxyl Radicals: Conditions of Solidification

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Under aerobic conditions, biological stems are constantly exposed to reactive oxygen radicals (ROS). Furthermore, ROS play an important role in many pathological pathways, in cancer development, aging, etc. One of the hallmark features of cellular life is the presence of membranes that separate the interior of cells from the outside environment. We study phosphocholines because they constitute 10 – 20 % of eukaryotic membranes.

The reaction of L-α-1,2-dipalmitoylphosphatidylcholine (DPPC) with hydroxyl ($HO^{\bullet}$) radicals (Fenton solutions) is investigated using monolayer techniques: isotherms, infrared absorption reflection spectroscopy (IRRAS), gracing incidence X-ray diffraction, X-ray reflection (for the latter BW1 with the liquid surfaces set-up) and fluorescence microscopy [1]. The DPPC monolayer is attacked with different $HO^{\bullet}$ concentrations produced by the Fenton reaction [2]:

$$H_2O_2 + Fe^{2+} \longrightarrow OH^- + OH\bullet + Fe^{3+},$$
$$H_2O_2 + Fe^{3+} \longrightarrow Fe^{2+} + HO_2\bullet + H_2O$$
$$H_2O_2 + HO_2\bullet \longrightarrow O_2 + HO\bullet + H_2O$$

The subphase beneath the monolayer contains $(NH_4)_2Fe(SO_4)_2$ and EDTA (molar ratio 4:5). The concentration of these solutes is denominated as "Fenton concentration". To induce the radical attack, H_2O_2 is injected by a syringe. The molar ratio Fe^{2+} : H_2O_2 is 1:3.

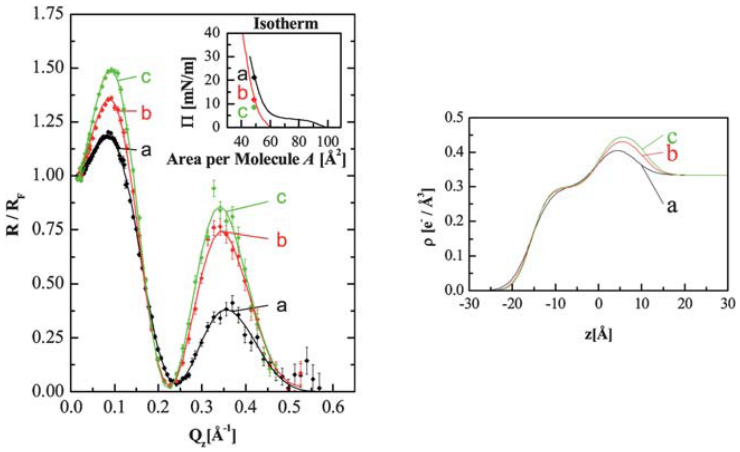

Figure 1: X-Ray reflectivity normalized to the Fresnel reflectivity (left) and corresponding electron density profiles (right) of a DPPC monolayer on a 2 mM Fenton solution, before (measurement a at 23 mN/m, black), during (b, red) and after (c at 8 mN/m, green) the $HO^{\bullet}$ attack. The isotherm is shown in the inset, with the surface pressure of each reflectivity measurement indicated. Also shown is a compression isotherm after completion of the Fenton attack (inset: red). Right: the electron density profiles indicate an unchanged hydrophobic region, while the head group increase in length and electron density. $z = 0$ is set at the interface between the hydrophobic and head group region.

During the Fenton attack a decrease of the lateral pressure is observed. It is used as a measure of the efficiency of the $HO^{\bullet}$ attack. With increasing Fenton concentration, the plateau region in the isotherm is shifted to a lower surface pressure; eventually it disappears ([1], see also Fig. 1). Fluorescence microscopy during the $HO^{\bullet}$ attack shows that new domains in the condensed phase nucleate immediately [1]. Furthermore, both isotherms and X-ray diffraction show that the monolayer can be compressed to smaller molecular areas (cf. Fig. 1, inset).The X-ray reflectivity curves in Fig. 1 show that at a constant molecular

area, the hydrophobic region of the monolayer remains unchanged, however the electron density and the thickness of the head group region increase. This can be explained by binding of Fe^{2+} ions to the head group. This finding is consistent with the reaction scheme depicted in Fig. 2.

Basically, a positively charged ammonium group is replaced by a neutral, hydrophilic OH-group. Thus, the originally zwitterionic DPPC molecule becomes negative, and can bind Fe^{2+} ions electrostatically. This reaction scheme is based on the assumption, that the hydroxyl radicals do not only attack the lipid molecules but also the EDTA. Consequently, after the radical attack some Fe^{2+} ions can move freely in the aqueous solution and eventually bind to the monolayer. To verify this assumption, the EDTA concentration is increased by a factor two, to ensure that all Fe^{2+} remain bound to EDTA. As expected, the monolayer and the X-ray reflectivity curves are unchanged after a radical attack. Nevertheless, the radical attack reduces the size of the head group, and thus allows a reduction of the tilt angle as evidenced by the diffraction measurements (cf. Fig. 2, right). Note that due to the large volume of their head group the ordered phase of the phosphocholines is characterized by a pronounced tilt angle [3].

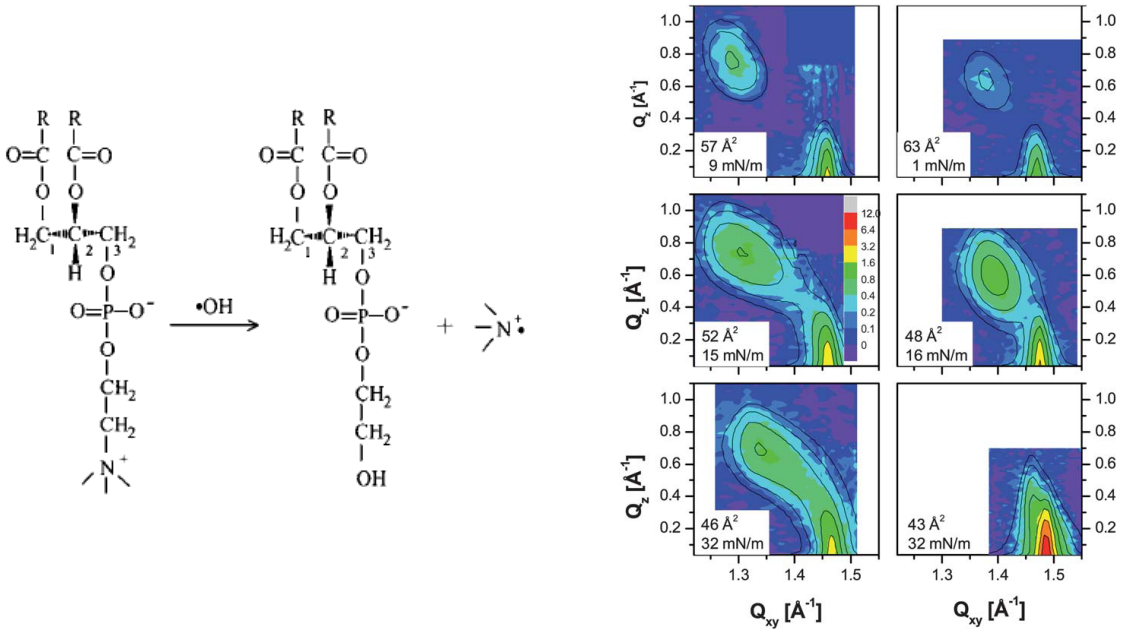

Figure 2, left: Suggested reaction scheme of a hydroxyl-radical with a DPPC molecule within a lipid monolayer. Right: Grazing incidence X-ray diffraction measurements of a DPPC monolayer on a 2 mM Fenton solution, before and after the $HO^{\bullet}$ attack (increased Fe^{2+} to H_2O_2 ratio: 1:5). The measurements are taken along the isotherm (top to bottom, lateral pressure and molecular area are indicated [3]). Left column: the fresh DPPC monolayer is characterized by two diffraction peaks, on compression the peak positions shift to larger Q_{xy}-values indicating smaller lattice spacing. Simultaneously, the peak at large Q_z-positions moves to smaller values, a sign of a decreased tilt angle. Right column: monolayer compression after the $HO^{\bullet}$ attack: already at almost zero lateral pressure (top), the out-of-plane peak occurs at larger Q_{xy}- and lower Q_z-positions. The lines are fits, the intensity is colour coded (note the almost logarithmic scale).

Therefore, we attribute the solidification of the monolayer as shown by the isotherms to a preferential radical attack in the hydrophilic region of the monolayer, and subsequent Fe^{2+} binding. To verify the solidification. Indeed, a decreased tilt angle is found.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

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