Monolayer properties of a novel polymerizable phosphatidylcholine, 1,2-di-(9*Z*,11*E*-octadecadienoyl)-*sn*-glycero-3-phosphocholine

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Monolayers formed from the title phospholipid have been deposited on graphite substrates, in monomeric form or after UV-induced polymerization, and studied by atomic force microscopy to reveal the uniform morphology and domain organization of the monomeric and polymeric films.

Polymerizable phospholipids are currently the subject of extensive studies. Owing to their tendency to form supramolecular assemblies, combined with their capacity for cross linking, they provide a challenging opportunity for creating fairly stable monolayer and bilayer structures.[1] Many synthetic polymerizable lipids have been studied in aqueous dispersions as bilayer membranes. However, much less is known about their behaviour in monolayers at the air/water interface and especially about the deposition and properties of the polymeric monomolecular lipid films on solid substrates.[2]

In this paper we introduce a new polymerizable phospholipid, 1,2-di-(9*Z*,11*E*-octadecadienoyl)-*sn*-glycero-3-phosphocholine (9,11-DODPC), describe its properties in monolayers on the water surface before and after polymerization, and report on the atomic force microscopy (AFM) characterization of the films deposited on graphite substrates.

9,11-DODPC has conjugated dienic groups, the polymerizable fragments, in the middle of both hydrocarbon chains. In this regard, among the polymerizable phospholipids reported so far, 9,11-DODPC most closely resembles natural unsaturated phosphatidylcholines. Therefore, the molecular organization of 9,11-DODPC monomers in the layered structures should not differ significantly from the organization of native phospholipids.

9,11-DODPC was synthesized *via* acylation of *sn*-glycero-3-phosphocholine with the anhydride of 9*Z*,11*E*-octadecadienoic acid.[3] The latter was derived from ricinoleic acid by the bromination-dehydrobromination sequence.[4†]

When spread on the water surface, 9,11-DODPC gives the liquid-expanded monolayers typical of unsaturated phosphatidylcholines. The isotherms of monomeric films exhibit collapse at a surface pressure of *ca.* 50 mN m^{-1} and a molecular area extrapolated to zero surface pressure of *ca.* 70 Å^2.

UV-irradiation of 9,11-DODPC monolayers at a constant surface pressure leads to a decrease in the monolayer area (Figure 1). This, apparently, is not due to a photo-oxidative degradation since monolayers of a non-polymerizable unsaturated phosphatidylcholine, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, retain their area unaltered under the same irradiation conditions (data not shown). Taken together with the fact that no monolayer contraction is observed without UV-treatment, even at the highest surface pressure applied [40 mN m^{-1}, Figure 1(*a*)], the above suggests that UV-irradiation does induce polymerization of 9,11-DODPC monolayers. This conclusion is supported by the fact that multilayer 9,11-DODPC films deposited on quartz slides show a steady decrease in the absorption of conjugated diene groups at 232 nm during UV-irradiation. Although the extent of monolayer contraction depends noticeably on the surface pressure value (Figure 1), in all experiments the polymerization process is completed in less than 10 min. This agrees well with the data reported for polymerization of 2,4-DODPC monolayers.[5] The isotherms of polymerized and monomeric 9,11-DODPC monolayers are similar, except for the slightly steeper slope of the former that implies a less compressible film.

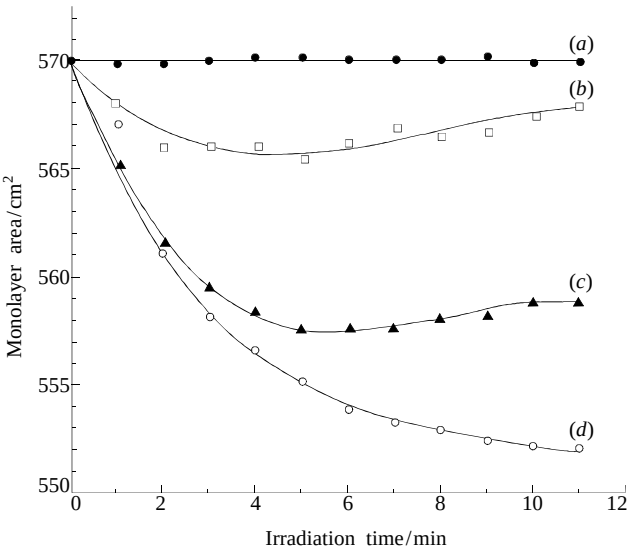
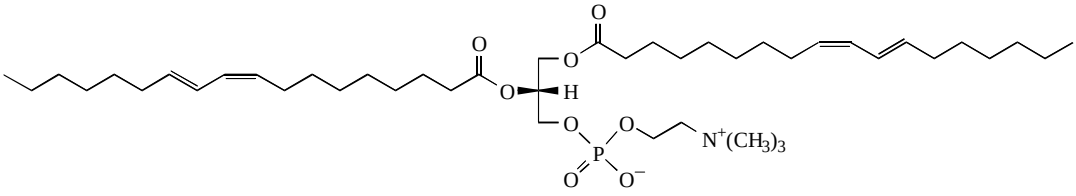


Figure 1 Decrease in the area of 9,11-DODPC monolayers during UV-irradiation at different surface pressure values: (*a*) 40 mN m^{-1} (a control without UV-irradiation), (*b*) 15 mN m^{-1}, (*c*) 30 mN m^{-1}, (*d*) 40 mN m^{-1}. The source of UV-irradiation, a 15 W high-pressure mercury lamp, was placed at a distance of 7.5 cm from the water surface.

The morphology of 9,11-DODPC monolayers before and after UV-irradiation was examined by AFM.[‡] Typical AFM images of monomeric and polymeric films are shown in Figures 2 and

† After purification by column chromatography, 9,11-DODPC showed a single spot in TLC analysis (R_f 0.3, silica, $CHCl_3$:CH_3OH:H_2O = 65:25:4) and a single peak in the ^{31}P NMR spectrum (δ –0.86 ppm, relative to 85% H_3PO_4), indicating the high purity of the material. ^1H NMR (300 MHz, CD_3OD) δ: 0.86 (m, 6H, $2CH_3$), 1.29 (m, 32H, $16CH_2$), 1.58 (m, 4H, $2OCOCH_2CH_2$), 2.06 (dt, 4H, J_d 7.1 Hz, J_t 6.5 Hz, 2*trans*-C=CH–CH_2), 2.12 (dt, 4H, J 6.6 Hz, 2*cis*-C=CH–CH_2), 2.28 (t, 2H, J 7.5 Hz, CH_2COO), 2.30 (t, 2H, J 7.5 Hz, CH_2COO), 3.10 [s, 9H, $N(CH_3)_3$], 3.60 (m, 2H, CH_2N), 3.96 (t, 2H, J 5.7 Hz, CH_2OP), 4.13 (dd, 1H, J 6.9 and 12.0 Hz, CH_AH_BOCO), 4.24 (m, 2H, $POCH_2CH_2$), 4.39 (dd, 1H, J 3.0 and 12.0 Hz, CH_AH_BOCO), 5.21 (m, 3H, CHOCO and 2*cis*-C=CH–CH_2), 5.59 (dt, 2H, J_d 15.0 Hz, J_t 7.1 Hz, 2*trans*-C=CH–CH_2), 5.89 (dd, 2H, J 10.5 and 11.1 Hz, 2*cis*-C=CH–CH=), 6.22 (dd, 2H, J 10.5 and 15.0 Hz, *trans*-C=CH–CH=).

‡ All samples were prepared by Langmuir–Shaeffer deposition at a surface pressure of 25 mN m^{-1}. Highly oriented pyrolytic graphite (HOPG) substrates with an atomically flat surface were used, therefore the hydrocarbon chains of the transferred monolayers were directed to the hydrophobic surface of HOPG. Measurements were performed with a commercially available Scanning Probe Microscope (NanoScope II, Digital Instruments, Santa Barbara, CA) at room temperature in ambient conditions. The force of the cantilever against the surface was adjusted to a minimum (< 10 mN) to prevent the monolayer scratching.



9,11-DODPC

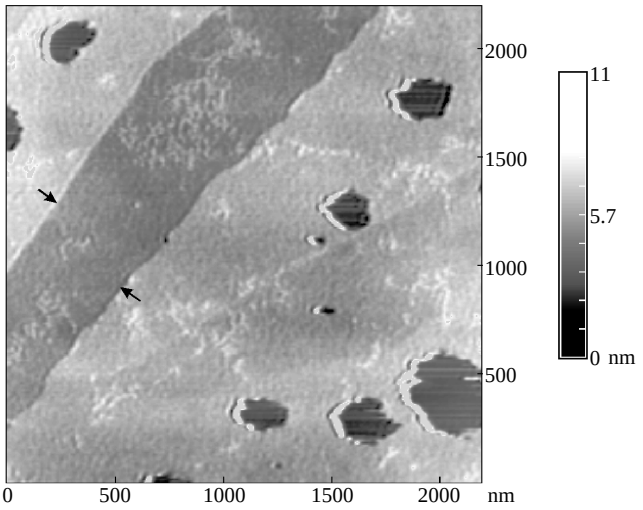


Figure 2 AFM image of monomeric 9,11-DODPC monolayer on HOPG, top view. The gray scale encodes the height (for nm scale see bar on the right side). Defects in the film appear dark while higher regions are light. Stretched step-like terraces observed on the AFM images are due to the HOPG surface of flat regions with discrete heights. Two lines indicated by arrows correspond to the edges of step-like terraces of HOPG. Fine web structure is clearly seen on all monolayer surfaces.

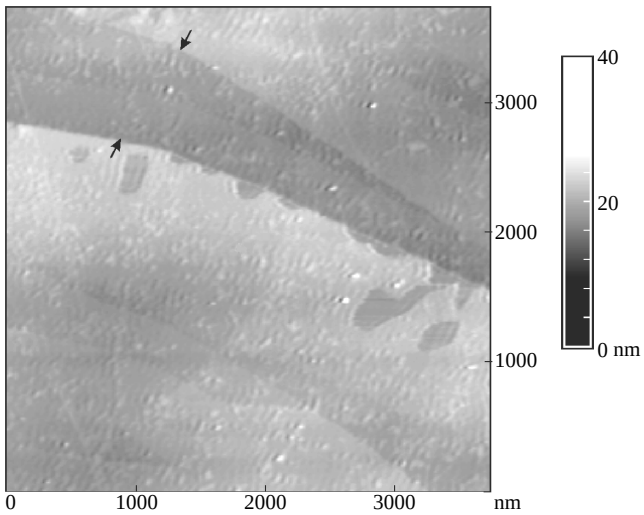


Figure 3 AFM image of polymeric 9,11-DODPC monolayer on HOPG (all details as in Figure 2).

3, respectively. Defects of different shape, basically rounded, are seen in both cases. For polymeric films some almost perfect areas are also seen, practically free of defects (see Figure 3).

The thickness of the films was measured from the cross-section profiles across the defects observed and the surface height histograms. For all samples tested, monomeric and polymeric, a fairly broad thickness distribution 2.8–4.0 nm was observed with two well defined heights ranges, 2.8–3.2 and 3.5–4.0 nm (mean values 3.0 and 3.8 nm). In both height ranges, the deposited films were clearly single monolayers. Since the height measurements might be influenced by the friction forces between the probing tip and the sample surface,[6] the heights were also measured with different scanning directions. Considering that the same ranges of height values were regained, possible instrumental effects can be neglected.

Well-defined web-like structures are also observed in some samples (Figures 2 and 3). Cross-sections of the film surface through the web-like domains reveal that they are *ca.* 1 nm higher than the nearby film areas. The mean roughness of the monolayer in these domains is *ca.* 0.5 nm, while this parameter does not exceed 0.2 nm for bare HOPG and smooth monolayer surface.

The discrepancy in monolayer thickness amounting to 0.8–1 nm does not result from polymerization since it is observable in both polymerized and monomeric films. Taking into account the dimensions of the 9,11-DODPC molecule, this diversity can be explained by various spatial orientations of the polar head groups relative to the monolayer surface. In fact, the height range of 2.8–3.2 nm is consistent with the length of the molecule with the head group lying flat in the plane of the monolayer.[7] Due to the transition of the phosphocholine group to a vertical orientation with the P–N axis normal to the monolayer plane, the total length of the 9,11-DODPC molecule increases up to 3.8 nm, which is well within the second range (3.5–4.0 nm) of the measured heights. The fairly broad distribution of thickness within both intervals may reflect some conformational dynamics of the polar head groups and probably fatty acyl chains around certain equilibrium states.

Clearly this explanation is a tentative one and needs further experimental verification. If it is true this implies that phospholipid monolayer films deposited on solid substrates may be organized in distinct domains, within which the polar head groups can have a fairly uniform conformation and orientation, different from those in the nearby monolayer areas.

In conclusion, the present study shows that monolayers of 9,11-DODPC at the air/water interface change their properties under UV-irradiation due to polymerization of the lipid. Transferred onto the hydrophobic surface of HOPG, the monomeric and polymeric films reveal similarities in their morphology and domain organisation. The discrepancy in monolayer thickness within different domains probably results from conformational changes of the lipid polar head groups.

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