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In Situ Preparation and Modification of Supported Lipid Layers by Lipid Transfer from Vesicles Studied by QCM-D and TOF-SIMS

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The study of lipid transfer between lipid membranes is of high interest for the fundamental understanding of this complex and important process[1] and, furthermore, for providing a new avenue for the *in situ* modification of supported lipid bilayers (SLBs).[2−4] SLBs of some (but not all) compositions are conveniently formed by vesicle spreading onto a solid support. However, this method is limited to conditions (i.e., combination of vesicle lipid composition, surface chemical properties, and buffer)[5] such that the vesicles break spontaneously upon adsorption to the surface and also fuse to a coherent bilayer. Many SLB compositions are therefore not accessible by this approach. In the present study, we demonstrate how this limitation can be circumvented by using lipid transfer between a preformed SLB and vesicles added in bulk to form lipid layers with new compositions and striking new features, notably with respect to stability. After lipid transfer between negatively charged vesicles and a positively charged SLB on TiO_2, an SLB is obtained, which, upon exposure to an ionic detergent, leaves behind a lipid monolayer. It is shown how this monolayer can be used for creating new SLBs. The multiple steps in this preparation are monitored in real time by the quartz crystal microbalance with dissipation (QCM-D) technique, and the lipid composition is analyzed for each step in postpreparation spectroscopic analyses using time-of-flight secondary ion mass spectrometry (TOF-SIMS). The QCM-D technique has been shown to be a unique tool to characterize SLB formation and the quality of bilayers,[5,6] and also lipid exchange between two contacting bilayers.[4] The possibility to use SIMS for chemical characterization of SLBs has recently been demonstrated.[7,8] The QCM-D + TOF-SIMS combination has not been used before.

Observation of lipid transfer between charged SLBs on SiO_2 and oppositely charged vesicles by QCM-D was recently reported by our group.[4] In the present work, similar experiments were carried out on TiO_2-coated crystals using positively charged POEPC vesicles, which form bilayers on TiO_2, and negatively charged D31-POPS vesicles (see Figure 1).

Figure 2 shows the frequency and dissipation shifts (Δf and ΔD) versus time curves together with schematic illustrations of the different lipid layers formed during the experiment. In the first step (i), a positively charged SLB (100% POEPC, A) is formed on a TiO_2 surface by adsorption and spontaneous rupture/fusion of vesicles via a critical surface coverage of vesicles ($\Delta f = -26$ Hz and $\Delta D < 0.5 \times 10^{-6}$).[6] Next, the SLB is (ii) exposed to negatively charged vesicles (100% D31-POPS). A decrease of Δf and an increase of ΔD are observed, indicating that vesicles attach to the SLB. The attached vesicles start to exchange lipids with the SLB until this exchange eventually counterbalances the disequilibrium between oppositely charged lipids, and the vesicles detach from

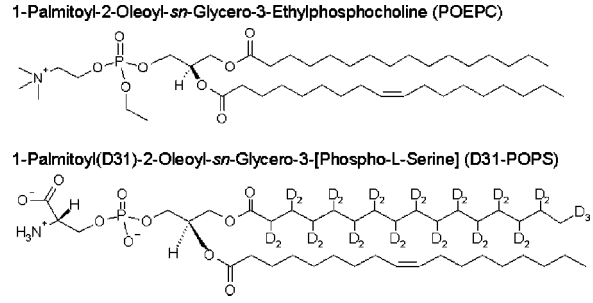

Figure 1. Chemical structure of phospholipids used in this study.

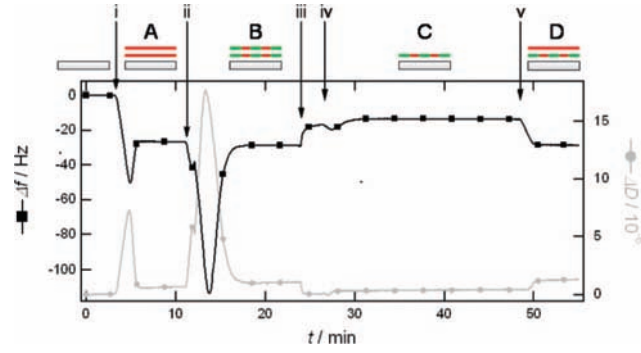

Figure 2. QCM-D data and schematic illustration of the lipid transfer experiment where (i) a POEPC SLB (A, red) is formed on TiO_2 and (ii) exposed to D31-POPS vesicles. The resulting, modified SLB (B, red/green) is (iii) rinsed with SDS and (iv) buffer, resulting in a lipid monolayer, and (v) exposed to POEPC vesicles, resulting in a new SLB.

the SLB.[4] After detachment is completed (Δf and ΔD have then regained the same values as those for the initial bilayer), the new SLB (B) is exposed to 10 mM sodium dodecyl sulfate (SDS, anionic detergent) followed by continuous rinsing with buffer (marked by iii and iv, respectively). SDS is routinely used to remove all lipid molecules from the surface of the quartz crystal indicated by the final QCM-D shifts; $\Delta f = 0$ Hz and $\Delta D = 0$. Interestingly, however, we observe in this particular case a frequency shift, $\Delta f = -13$ Hz, corresponding to a monolayer of phospholipids.[6] In other words, these data indicate removal of only one lipid leaflet, leaving, after (iv), a lipid monolayer on the surface. In the final step (v), POEPC vesicles are injected and exposed to the (assumed) monolayer (C). This leads to a drop of Δf and a slight increase of ΔD until both values stabilize at values typical for a bilayer. The reassembly of the bilayer (D) at (v) shows a kinetic behavior that is similar to that of lipid monolayers forming on hydrophobic surfaces[6] but distinctly different from bilayer formation on the bare TiO_2 surface, supporting the interpretation that a monolayer of phospholipids was left on the TiO_2 after rinsing with SDS. Additional support is provided by high resolution TOF-SIMS images, which show a

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homogeneous lipid layer in C (see Supporting Information). Since such a highly stable layer could not be observed performing a similar experiment on SiO_2 or for bilayers which had not been subjected to the lipid transfer step, we conclude that the existence of this monolayer is specifically related to an interaction between the mixture of the two lipids and TiO_2 (a likely contributing mechanism is specific interactions between the carboxylic acid groups on the PS headgroup with the TiO_2 substrate as observed for long chain carboxylic acids[9]). The preparation of this layer was only possible using the lipid transfer protocol, where the composition of the POEPC SLB is changed upon lipid transfer between the SLB and added POPS vesicles. Note that all lipid material on the surface can be removed by Triton X-100 (nonionic detergent), even after lipid transfer, indicating that the remaining lipid layer on the surface after SDS treatment was stabilized not only by the specific interaction with TiO_2 but also to a high degree by hydrophobic (nonionic) interactions.

Although these and earlier presented QCM-D data provide indirect evidence for lipid exchange between the vesicles and the SLB,[4] direct measurement of the lipid composition in the SLBs would add important information. For this reason, TOF-SIMS was employed. The lipid layers for spectrometric analysis in vacuum were prepared on the TiO_2-coated QCM-D sensors, followed by plunge-freezing and freeze-drying.[8] To obtain a sensitive and unambiguous measure of the amount of POPS in the lipid layers, the vesicles used for lipid exchange were prepared from D31-POPS with fully deuterated palmitate fatty acid groups (Figure 1). The presence of D31-POPS in the lipid layers could then be monitored by measuring the characteristic signal from deuterated palmitate (d-C16:0) ions in the TOF-SIMS spectra. Similarly, the TOF-SIMS signal from undeuterated palmitate (C16:0) could be used to monitor POEPC, while the oleate (C18:1) signal includes contributions from both D31-POPS and POEPC. Reference spectra of bilayers with known D31-POPS/POEPC contents (25% and 50% of D31-POPS), prepared directly on SiO_2, were recorded to allow for determination of the D31-POPS/POEPC composition in the lipid layers.

Figure 3 (left panel) shows negative ion TOF-SIMS spectra from some of the investigated surfaces, in the mass range including the oleate (C18:1) peak at *m*/*z* 281.24 and peaks from deuterated palmitate (d-C16:0) ions at *m*/*z* 286.41 (completely deuterized), 285.41, 284.40, and 283.40 (containing 1, 2, and 3 ^{1}H atoms, respectively). The undeuterated palmitate (C16:0) ion originating from POEPC was observed at *m*/*z* 255.22 (not shown). The right panel shows the signal intensity ratios between deuterated palmitate (D31-POPS) and oleate (POEPC + D31-POPS) and between deuterated (D31-POPS) and undeuterated palmitate (POEPC), for the different lipid layers. Comparison of the measured signal ratios with those of the reference samples containing known fractions of D31-POPS directly shows that the relative concentration of D31-POPS is ~50% in the SLB after D31-POPS exchange (B), significantly higher in the monolayer prepared *in situ* by SDS rinse (C), and 20−25% after reassembly of the SLB using POEPC vesicles (D). The results thus provide unambiguous evidence for extensive lipid transfer between the initial POEPC SLB and D31-POPS vesicles in solution. The relative D31-POPS concentrations in the different lipid layers were calculated based on the assumption that each lipid contributes to the corresponding signal intensities at a magnitude that is proportional to the surface concentration of the lipid.

Although this is a simplified model, e.g. not taking into account possible matrix effects and bilayer asymmetries (different compositions in upper and lower leaflets), the results shown in Table 1, provide semiquantitatively accurate estimates of the D31-POPS fractions in the surface-supported lipid layers. Note that only very low traces of SDS were detected in the lipid monolayer after SDS rinse ($<10^{-3}$ of the signal intensity from an SDS reference sample).

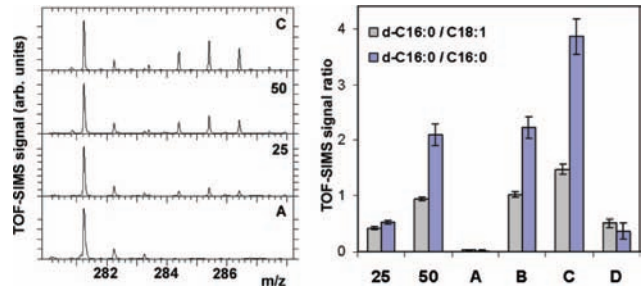

Figure 3. TOF-SIMS results from differently prepared lipid layers. "25" and "50" correspond to D31-POPS/POEPC SLBs prepared on SiO_2 from vesicles with 25% and 50% D31-POPS, respectively. "A" to "D" corresponds to the different stages described above and in Figure 2 (A: initial POEPC SLB, B: SLB after D31-POPS exchange, C: monolayer after SDS rinse, D: reassembled SLB after exposure to POEPC vesicles). Left panel: negative ion spectra containing peaks from the C18:1 fatty acid, originating from both D31-POPS and POEPC, and the deuterated C16:0 fatty acid, originating only from D31-POPS. Right panel: signal intensity ratios between deuterated C16:0 (D31-POPS) and C18:1 (D31-POPS + POEPC) and between deuterated C16:0 (D31-POPS) and C16:0 (POEPC). Averages and standard deviations based on 4−5 measurements.

Table 1. D31-POPS Fractions in the *in Situ* Prepared Lipid Layers

	D31-POPS fraction based on d-C16:0/C18:1 signal ratio	D31-POPS fraction based on d-C16:0/C16:0 signal ratio
A	0.016 ± 0.0025	0.013 ± 0.0021
B	0.53 ± 0.022	0.55 ± 0.022
C	0.71 ± 0.032	0.68 ± 0.018
D	0.29 ± 0.040	0.17 ± 0.056

In conclusion, QCM-D and TOF-SIMS were used to follow lipid transfer between charged SLBs and oppositely charged vesicles during the *in situ* preparation of surface-supported lipid layers that could not be formed by vesicle spreading. This approach was used to form a highly stabilized (SDS-resistant) lipid monolayer on TiO_2 and then used for the reassembly of an SLB. We propose this as a promising method for *in situ* preparation of asymmetric SLBs.

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Supporting Information Available: Experimental and calculation details. This material is available free of charge via the Internet at http://pubs.acs.org.

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