Anomalous X-ray Reflectivity of Monolayers at the Air/Water Interface

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Introduction

The distribution of charged ions at cell membrane surfaces influences the function and conformation of nearby molecules, and plays a role in inter- and intracellular transport and recognition processes.[1,2] Moreover, charge patterns at membrane interfaces may control directed growth of crystals in biomineralization.[3,4] As model systems for studying charge distribution and growth processes at the organic/inorganic interface, Langmuir monolayers (LM) have been widely employed in recent years.[3-6] For example, the charge distribution that eventually directs the morphology of product crystals, can be controlled by the choice of functional headgroups, their density and the subphase pH, ionic strength, and temperature. In biomimetic models, molecular surface layers can be formed from protein-lipid mixtures or other molecules of biological or physiological relevance. Such a "bottom up" approach for biomineralization has already shed light on some of the mechanisms involved.[3-6] X-ray reflectivity (XR), and diffraction at grazing angles of incidence (GIXD) are well suited for structure determination of interfaces on the molecular length scale.[7] GIXD is a superior characterization technique when it comes to the in-plane arrangement of molecules; however, it requires that the layer be crystalline, which is only rarely the case in biological systems. There is no evidence so far that the head groups of lipids are ordered, and therefore, it is more likely that the first layer of adsorbed ions also lack long-range order.

Several x-ray fluorescence methods probe adsorbed ions at solid and liquid interfaces. In the near-total-external fluorescence (NTEF) technique, introduced by Bloch et al.,[8] the Kα fluorescence from an illuminated sample is monitored as a function of the incident angle with respect to the surface. Although powerful, this technique is insensitive to the actual location of the ions with respect to the surface. The x-ray standing wave (XSW) technique[9] is similar to NTEF but may also probe the location of an ionic layer with respect to the substrate. It requires, however, that the ionic layer be exterior to the substrate surface.[9] Neither technique provides a global structural picture of an absorbed layer with respect to other constituents of the film.

Here we employ anomalous scattering by collecting x-ray reflectivities at different energies, both at and away from the absorption edge of the probed ions. In this technique, the effective electron density (ED) profile of the surface film at each energy is determined. From their differences, the contribution of the adsorbed ions can be extracted. The advantage of the present technique is that it determines specifically the location of the ionic layer irrespective of its position with respect to the surface. Experimentally, this approach is similar to the method developed by Sanyal et al.[10] for model-independent reconstruction of ED profiles of thin films on Ge substrate.

Methods and Materials

DMPA (1,2-dimystroyl-*sn*-glycero-3-phosphate, Avanti Polar Lipids, Fig. 1) monolayers were spread from chloroform on aqueous $BaCl_2$ (0.1 and 10 mM, Sigma, purity: 99+%) solutions with ultrapure water (NANOpure, Barnstead, Dubuque, IA) in an encapsulated Teflon Langmuir trough kept under He atmosphere at T = 18°C. X-ray reflectivity experiments were performed on a new liquid surface diffractometer at beamline 6-ID of the APS at Argonne National Laboratory. A downstream Si double-crystal monochromator selects the x-ray beam at the desired energy. The highly monochromatic beam is deflected onto the liquid surface to the desired angle of incidence with Ge(111).[11] For each angle of incidence, the θ_M and $2\theta_M$ angles of the Ge(111) crystal and diffractometer are adjusted.[12]

1,2-Dimyristoyl-*sn*-Glycero-3-Phospahte (DMPA)

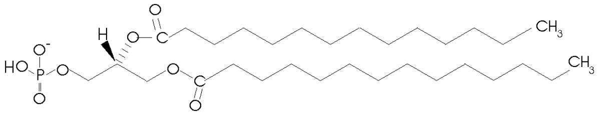

FIG. 1. Chemical structure of DMPA, used in this study.

At x-ray energies significantly higher than the absorption edges of any constituents in the sample, the electrons are practically free. In this case, the reflectivity is determined by the refractive index, $n(z) = 1- r_0\rho(z)\lambda^2/2\pi$, where $\rho(z)$ is the ED across the interface and r_0 is the classical electron radius. One then may use the standard matrix method[13] or a recursion method[14] to calculate the reflectivities numerically. In the vicinity of absorption edges the refractive index is a complex number

$$\delta(z)+i\beta(z)=\frac{r_0}{2\pi}\left(\frac{hc}{E}\right)^2\sum_j N_j(z)Z_j\left(f_j^{'}(E)+if_j^{''}(E)\right)$$

where E is the x-ray energy and Nj the number density of ions of type j with Zj electrons. To calculate the reflectivity near an absorption edge, we introduce the *effective* number of "free" electrons $Z_{j,eff}(E) \equiv Z_j f_j'(E)$ (see Fig. 2) and apply the standard techniques for calculating reflectivity in the high-energy limit, i.e. free electrons.[13,14]

Results and Discussion

Figure 3A shows normalized reflectivities from a DMPA monolayer on 0.1mM $BaCl_2$ at x-ray energies E = 5247 eV (Ba L_{III} absorption edge) and off the edge at 5100 and 8000 eV. In qualitative terms, all three reflectivities exhibit sharp minima at Q_z ~ 0.21 Å^{-1}, and are characteristic of highly organized monolayers, as previously shown.[15] However, the reflectivity at the Ba edge has a sharper and deeper minimum that is slightly shifted to higher Q_z. Also, the overall reflectivity at the edge is small-

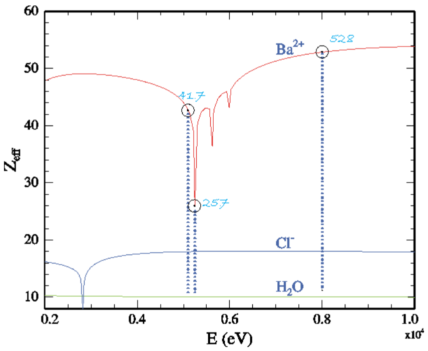

FIG. 2. Effective number free electrons (Z_{eff}, as discussed in the text) for the ions used in this study (Z_{eff} for the constituents of DMPA do not vary in the energy range shown.)

er than those off the edge. This is an indication for a lower effective ED compared to those away from the absorption edge. To fit the data, we applied a box model,[6] where the minimum number of slabs that gives the best fit to the data is used. In the present case, a two-slab model is used in which two layer thickness values, two electron densities and one conformal roughness (i.e., five free parameters) are refined. In the refinement we have constrained the slab close to the air compartment, associated with the alkyl tails, to be the same for the three reflectivities. Thus, the ED models for the three energies are different only with regard to the two parameters that characterize the headgroup slab. Figure 3b shows the electron densities extracted for the corresponding reflectivities in Fig. 3a. Similar results were obtained for DMPA monolayers on 0.1 mM and on 10 mM $BaCl_2$ at a variety of surface pressures. It is evident from the models (Fig. 3b) that the ED at the headgroup region is lowest for the reflectivity measured at the Ba edge (5247 eV), as expected.

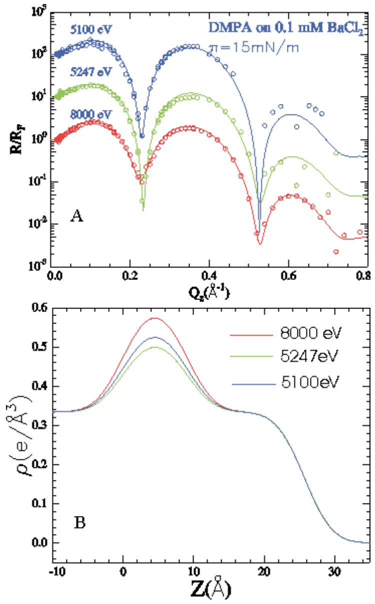

FIG. 3. Normalized reflectivities from DMPA on BaCl2 solution at three different x-ray energies (A). Corresponding electron densities that best fit the reflectivity data (B).

The number, N_{Ba}, of Ba ions per lipid molecule (occupying an area *A)* is determined by integrating over the difference between two ED profiles at two energies as follows:

$$A\int[\rho(z,E_1)-\rho(z,E_2)]dz=N_{Ba}[Z_{eff}(E_1)-Z_{eff}(E_2)]$$

where the difference in the effective number of electrons is taken from Fig. 2. Using this approach, we find that for DMPA spread on 10 mM BaCl2 solution there are on average 0.7 to 0.9 Ba^{2+} per lipid. The Ba^{2+} layer is localized within the head group slab with no indication of gradual charge decay across the interface. This confirms recent findings that Ba^{2+} binds in quantities to anionic monolayers that are larger than stoichiometric, which has been attributed to cobinding of Cl^- species.[15,16]

Conclusions

Using anomalous reflectivity, the location and density of Ba ions bound at an anionic surface monolayer were determined. For the pH at which our experiments were conducted (pure water), there is one negative charge per DMPA molecule, which could be compensated by 0.5 Ba^{2+}. However, the present results show that more than 0.5 Ba ions per DMPA are bound at the interface. Assuming, Ba^{2+} at the interface, this finding implies charge overcompensation with strong repulsion among positive Ba^{2+} cations which can in turn be compensated by Cl^- ions. Since lone Cl^- ions are initially repelled by the negative interface, we propose that the $BaCl^+$ complex binds to the negative headgroups. Such a model could shed light on one of the central problems in biomineralization.[6]

Acknowledgments

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