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Soft X-ray Absorption Spectroscopy of Phospholipid Films Supported on Au Substrates by Different Casting Techniques

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Lipids are one of the major constituents of living organisms. In particular, lipids self-assemble into oriented amphiphilic bilayers, which are the basic structure of the cell membranes. Lipid bilayers not only play an important protective role in maintaining cells, but also serve as sites for information transfer and protein reactions. Therefore, understanding the properties of lipid membranes is fundamental to studying biological mechanisms in detail, and to this end, attempts have been made to reproduce artificial cell membranes by supporting lipids on metal substrates. Lipid membranes supported on solid substrates (Figure 1), such as LB (Langmuir-Blodgett) and vesicle fusion methods, resemble natural cell membranes, and the information obtained are known to be biologically reliable. We have found that even a drop of an organic solution of phospholipid molecules onto a gold substrate can form a highly oriented multi lipid-bilayers. In this study, we prepared multi lipid-bilayer membranes by spin-casting method of lipid solutions, aiming to create more uniformly oriented bilayers and to evaluate the state of the bilayers.

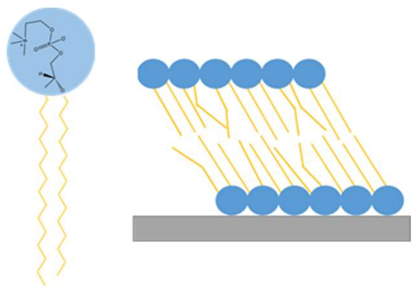

FIGURE 1. Molecular structure of DPPC phospholipids and schematic bilayer membrane.

DPPC shown in Figure 1 was used as the phospholipid and dissolved in chloroform to make a lipid solution. The solution was dropped onto Au substrates and dried under a nitrogen stream to create a multi lipid-bilayer membranes. The spin-casting method was also used to create a more uniform bilayer films on the substrates.

The prepared lipid films were characterized by NEXAFS (near edge X-ray absorption fine structure) measurements at HiSOR BL13 and AFM (atomic force microscopy) measurements at the laboratory. NEXAFS spectra were obtained by irradiating soft X-rays in the carbon K-edge region, and the emitted electrons were detected as drain current. The polarization angle dependence of the NEXAFS spectra was measured by changing the incidence angle of horizontally polarized X-rays on the sample surface, and the orientation angle of the organized lipid molecules was thereby determined to evaluate the differences in the multilayer films due to the different preparation methods.

In this study, soft X-ray absorption spectroscopy measurements were performed at different incident angles of soft X-rays to investigate the orientation angles of lipid films prepared by drop and spin-casting methods. Figure 2 shows NEXAFS spectra in the C K-edge region measured at different sample positions for lipid films prepared by drop and the spectrum for the lipid film prepared by spin-casting. The obtained NEXAFS spectra show characteristic resonant excitation peaks at specific energies and different spectral shapes with respect to the incident angle of soft X-ray. In other words, both lipid membranes are highly oriented, despite being multilayered.

Each spectrum can be fitted with a Gaussian function to extract each resonant excitation component. From the area intensities of fitted functions, the orientation angle of the transition dipole moment at each transition was determined. As a result, there is a clear difference between the orientation angles of the lipid films prepared by the drop and spin-casting methods. This is thought to be due to the difference in the method used to prepare the lipid films and the difference in the thickness of the films.

From the Rydberg and σ*(C-C) transitions, the orientation angle of the hydrocarbon chains, which determines the overall shape of the lipid molecules, can be derived specifically. In lipid films prepared by the spin-casting method, the orientation angle is closer to 55 degrees, which is called the "magic angle", than in films prepared by drop-casting. Based on previous studies [1], it is thought that the orientation ordering is reduced in lipid films obtained by spin-casting due to the random structure of some of the hydrocarbon chains. In addition, the NEXAFS spectra of the lipid films obtained by spin-casting are different from those obtained by the drop-casting method in their detailed shape, and are in good agreement with the spectral shape of the single bilayer films in the previous study [1]. These results suggest that the spin-casted lipid films are sufficiently thin to be considered single bilayer films, unlike the drop method, and that the degree of freedom of the molecules constituting the films is increased in this ultra-thin state.

REFERENCES

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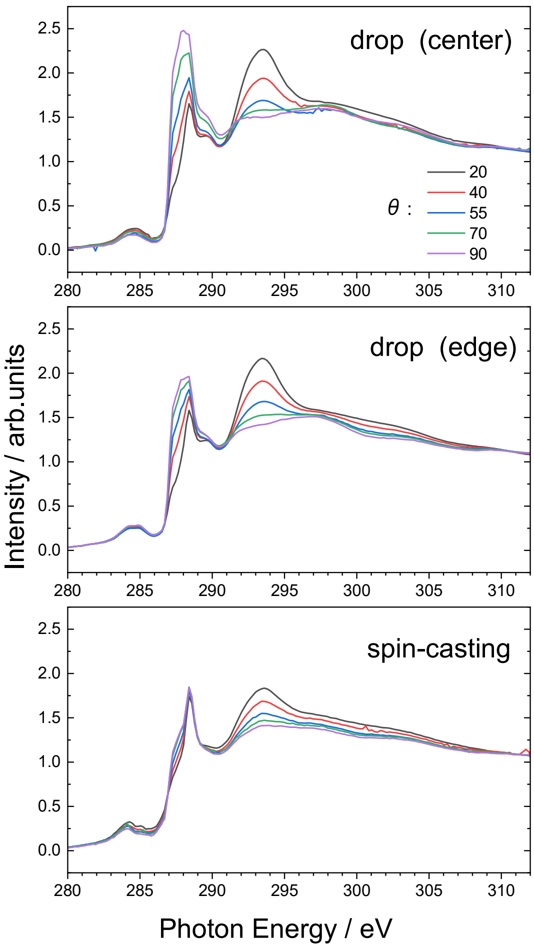

FIGURE 2. Polarization dependent NEXAFS spectra of (upper) center and (middle) edge positions of DPPC drop-casting films and (c) spin-casting film measured at C K-edge. θ: incident angle from the surface, and therefore the angle of electric vector from the surface normal.

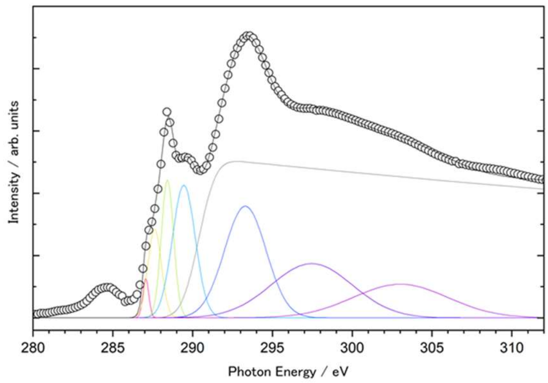

FIGURE 3. Typical result of Gaussian fitting obtained for drop-casting film at 55-degree incidence angle.