Order analysis of supported lipid bilayers on gold substrates by soft X-ray absorption spectroscopy

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Keywords: Phospholipids, XAS (X-ray absorption spectroscopy), NEXAFS(near edge X-ray absorption fine structure), Linear polarization

In this study, supported phospholipid bilayers formed on gold substrates were used as model biological membranes, and the molecular orientation and ordering of lipid molecules within the membranes were evaluated at the molecular level using near-edge X-ray absorption fine structure (NEXAFS) spectroscopy and X-ray photoelectron spectroscopy (XPS).

Lipid bilayers are the fundamental structural units of biological membranes and consist of lipid molecules arranged in opposing layers. Their molecular orientation and membrane order are closely related to physical properties such as membrane stability and barrier performance. In highly ordered membranes, hydrocarbon chains are densely packed, resulting in high barrier properties, whereas in less ordered membranes, packing is loose and the membranes exhibit high fluidity. In living systems, membranes with different properties are formed even though they share the same bilayer structure—for example, flexible cell membranes and barrier-function intercellular lipids in the stratum corneum. Accordingly, the dominant lipid species and the number of stacked bilayers vary depending on the biological environment.

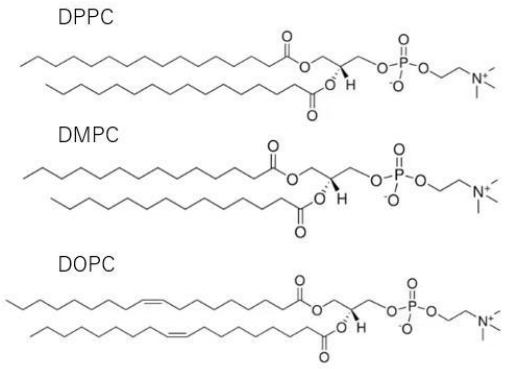

FIGURE 1. Schematic illustration of lipid molecules, DPPC (16:0), DMPC (14:0), and DOPC (18:1). The values in parentheses indicate hydrocarbon chain length and degree of unsaturation.

In this study, a model system of substrate-supported lipid membranes was employed to quantitatively clarify how lipid molecular species and membrane layer number affect membrane structure and order.

DPPC, DMPC, and DOPC phospholipids (Fig. 1), which differ in molecular length and degree of unsaturation, were used as samples, and multilamellar lipid bilayers were prepared on gold substrates by spin coating. Single lipid bilayers were also prepared by hydration treatment of the multilamellar. Film thickness was evaluated by XPS to confirm formation of single bilayers.

NEXAFS measurements were mainly conducted at the carbon K-edge. As shown in Fig. 2, differences in stacking and membrane constituent molecules resulted in different changes in spectral intensity at each X-ray incidence angle. By analyzing this angle dependence and using Rydberg and σ*(C–C) transitions, the tilt angle of the membranes was evaluated. Furthermore, focusing on the difference in orientation between these two transitions, this difference was interpreted as reflecting random components within the hydrocarbon chains, and thus the ordering of hydrocarbon chains in the lipid membranes was assessed.

As a result, in multilayers, DPPC and DMPC containing saturated hydrocarbon chains exhibited high ordering of approximately 80%, whereas DOPC containing unsaturated hydrocarbon chains showed a lower

ordering of about 70%. This is attributed to reduced packing in DOPC membranes due to double bonds. In addition, comparison between single bilayers and multilayers revealed that ordering of hydrocarbon chains decreased by about 10% in single bilayers. The higher ordering observed in multilayers is considered to result from suppression of vertical membrane fluctuations due to stacking. These findings reflect the enhancement of membrane order caused by multilayer formation in biological environments.

This study demonstrates that the effects of lipid molecular unsaturation and membrane layer number (single vs multilayer) on hydrocarbon chain ordering in lipid bilayers can be evaluated using NEXAFS with a consistent metric. The results clarify how factors such as molecular composition and stacking influence membrane order and contribute to understanding biological membrane.

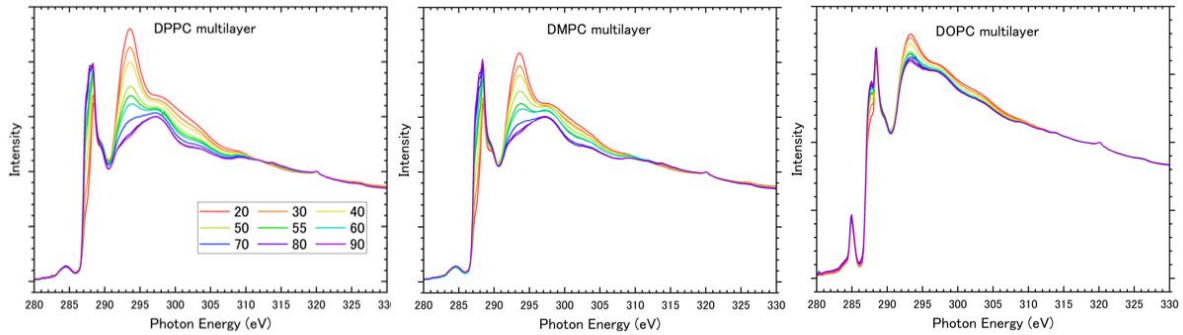

FIGURE 2. NEXAFS spectra measured for DPPC, DMPC, and DOPC multilayers with varying incidence angles from 20° to 90°. Differences in angular dependence are observed.