Proc. Int. Symposium on Advances in Neutron Scattering Research, Tokai, 2000
J. Phys. Soc. Jpn. **70** (2001) Suppl. A pp. 341–343

# Neutron Diffraction on Polymorphic Phases of Phospholipids

Tomohiro ADACHI*, Michihiro FURUSAKA, Toshiya OTOMO and Ichiro HATTA[1]

*Institute of Material Structure Science, High Energy Accelerator Research Organization, Tsukuba, Ibaraki 305-0801, Japan*

[1]*Department of Applied physics, Graduate School of Engineering, Nagoya University, Nagoya 464-8603, Japan*

Small angle neutron diffraction experiments were performed in DPPC and DPPC/cholesterol systems. We investigated the DPPC-d62 bilayers without cholesterol and the DPPC-d75 bilayers with 5 and 15 mol% cholesterol. For DPPC-d62 systems, in the gel and fluid phase, the reflections up to third order from lamellar structure were observed. Scattering length density profiles of these systems were generated. They show that the packing density of hydrocarbon chain in gel phase is higher than in fluid phase. We show that the neutron diffraction experiment is effective on observing the packing and the scattering length density of the hydrocarbon chain. On the other hand, for DPPC-d75/cholesterol systems, only the reflection from the ripple structure was observed. It shows that cholesterol is periodically localized in accordance with ripple structure forming a periodic bandlike structure parallel to a ridge of the ripple structure.

KEYWORDS: DPPC, phospholipid, phosphatidylcholine, small angle neutron diffraction, ripple phase

## §1. Introduction

A ripple phase is well known in phospholipid systems, especially in dipalmitoylphosphatidylcholine (DPPC). The ripple structure exhibits a periodic undulation of a bilayer, but its detailed microscopic structure has not yet been determined. Incorporation of cholesterol into a phospholipid bilayer affects the nature of the bilayer. It has been reported that the periodic spacing of the ripple structure increases as cholesterol concentrations rise.[1)] Although several explanations of this mechanism have been proposed,[2–4)] it has still not elucidated. The most important point to understand the cholesterol effect on the ripple structure is to determine the distribution of cholesterol in the bilayers. Neutron diffraction is effective in an experiment focused on hydrocarbon chain because the scattering length density can be changed by isotope substitution, especially hydrogen and deuterium, keeping a molecular formula and a chemical nature. It means that a contrast can be given between cholesterol and DPPC. Thus, in order to make clear the cholesterol distribution in the ripple structure, small angle neutron diffraction experiments were performed in a DPPC/cholesterol system.

## §2. Materials and Methods

Synthetic, 1,2-dipalmitoyl-d62-*sn*-glycero-3-phosphocholine (DPPC-d62) and 1,2-dipalmitoyl-d62-*sn*-glycero-3-phosphocholine-d13 (DPPC-d75) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL, USA). Deuterium oxide (99.9 %) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA). Cholesterol was product of Nu-Chek-Prep, Inc. (Elysian, MN, USA). All these were used as delivered.

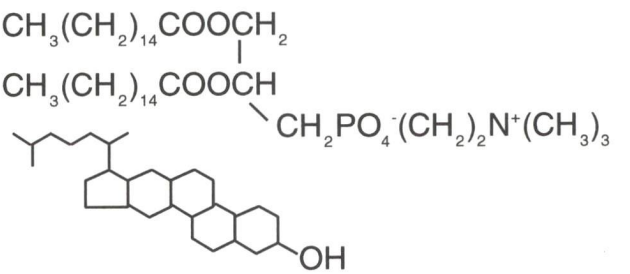


Fig. 1. Moleculer structure formulas of DPPC (above) and cholesterol (below).

For preparation of multilamellar liposomes, lipid powder was first dissolved in spectroscopic-grade chloroform. For a sample of lipid/cholesterol, a desired amount of chloroform solution of cholesterol was added to the lipid solution and sufficiently mixed. After chloroform was evaporated under a stream of nitrogen gas, the sample was dried under vacuum overnight to remove the trace of the solvent. They were hydrated with deuterium oxide and incubated in a water-bath at about 50 °C for 4 h with vortexing for intervals so as to make homogeneous dispersions. The lipid content was 18 wt%.

The pretransition and the main transition temperatures of DPPC decrease 6 °C by deuteration.

Neutron diffraction measurements were made at the small and wide angle diffractometer (SWAN) at the High Energy Accelerator Research Organization.[5)] The samples were filled in flat square-shaped quartz cells which have window thickness of 1 mm and path length of 2 mm. This was installed in an aluminum holder which temperature was controlled by the temperature-controlled water supplied from a temperature constant bath. The temperature of the sample was monitored with a chromel-alumel thermocouple situated on the surface of quartz cell. Each measurement was of duration varying in the 6-8 h.

* Present address: Division of Image Information, Advanced Computing Center, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan

## §3. Results

### *3.1 DPPC*

Figure 2a shows a pattern of small angle neutron diffraction at 15 °C in gel phase of DPPC-d62. The intensity was normalized by the vanadium incoherent scattering intensity. Three reflections up to the third order were observed. The lamellar repeat period is 6.2 nm. One dimensional scattering length density distribution profile could be generated from the integrated intensities of three reflections by a Fourier method. The scattering length density distribution profile of DPPC-d62 in gel phase at 15 °C is shown in Fig. 2b. Two deep valleys represent the hydrated headgroups which is shown by filled circles in a superimposed schematic view. A shallow valley at the center represents the methyl trough which is a gap between the methyl ends of the deuterated carbon chains. Two hills on both sides of the shallow valley at the center represent the deuterated carbon chains which are shown by lines in a superimposed schematic view. Figure 3a shows a pattern of small angle neutron diffraction at 39 °C in fluid phase of DPPC-d62. Three reflections up to the third order were also observed. The lamellar repeat period is 6.4 nm. The scattering length density distribution profile of DPPC-d62 in fluid phase at 39 °C is also generated and shown in Fig. 3b. The main difference of the scattering length density distribution between gel and fluid phase is behavior around the hill representing the deuterated carbon chains.

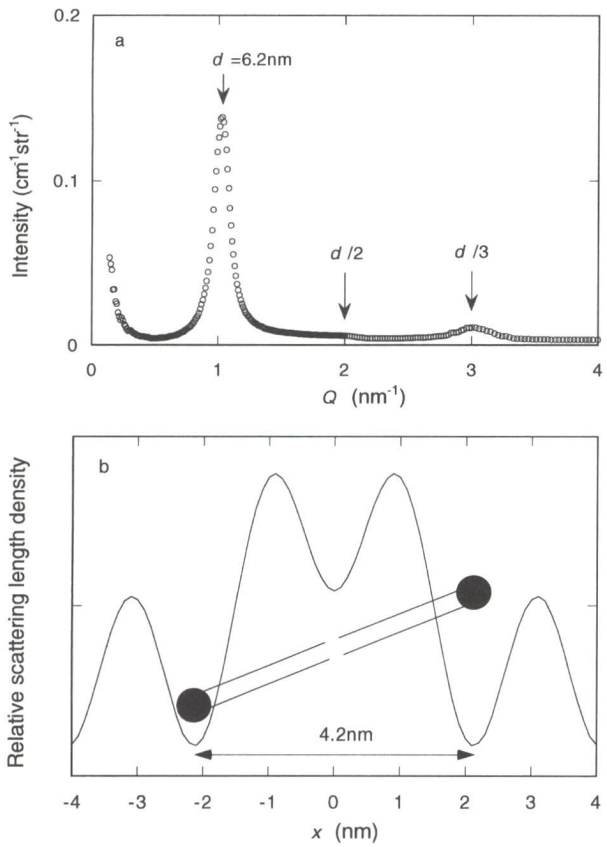


Fig. 2. The observed pattern of small angle neutron diffraction (a) and the generated scattering length density distribution profile (b) of DPPC-d62 in the gel phase at 15 °C. Filled circles with two lines in (b) show a schematic view of DPPC-d62 molecule.

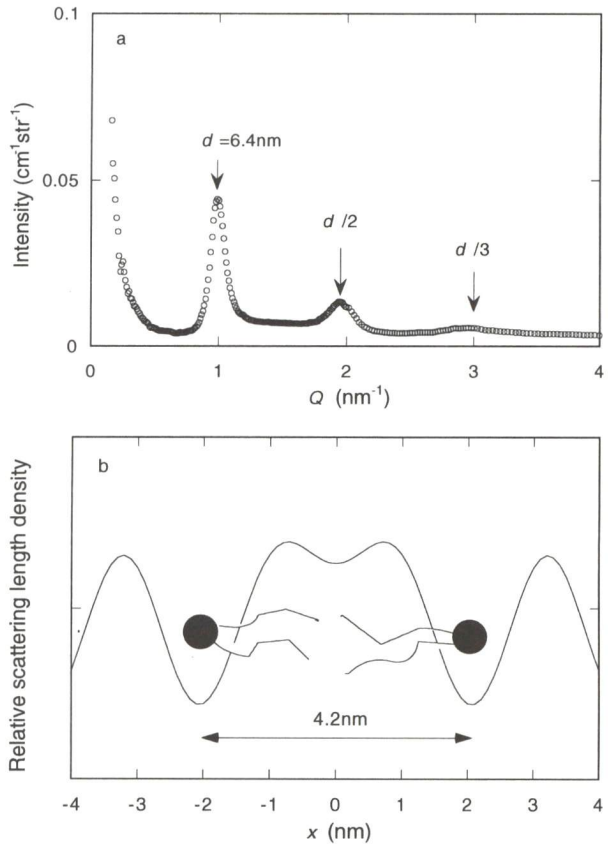


Fig. 3. The observed pattern of small angle neutron diffraction (a) and the generated scattering length density distribution profile (b) of DPPC-d62 in the gel phase at 39 °C. Filled circles with two curves in (b) show a schematic view of DPPC-d62 molecule. Scale of y-axis of the profile (b) is same as Fig. 2b.

### *3.2 DPPC/cholesterol*

Small angle neutron diffraction patterns of DPPC-d75 with 5 mol% and 15 mol% cholesterol at 33 °C in the ripple phase are shown in Fig. 4. Reflection peak due to the ripple structure was observed at $Q$=0.241 nm$^{-1}$ ($d$=26.1 nm) and $Q$=0.229 nm$^{-1}$ ($d$=27.4 nm) in the system with 5 mol% and 15 mol% cholesterol, respectively. These values of $Q$ were determined to fit by a Lorentz function with a power function as back ground, where $Q$ is a momentum transfer and $d=2\pi/Q$. Reflection peaks from lamella structure as seen in the gel or the fluid phase of DPPC-d62 were not observed clearly because of low contrast of the scattering length density.

## §4. Discussion

Information on a phase angle of each reflection is necessary in a structure analysis of a diffraction experimental result. There are some methods to determine the phase angle. The swelling method[6] is well-known and reasonable but it needs a series of swelling experiments. In a centro-symmetrical system, the phase angle is limited to either 0 or $\pi$. Therefore, there are eight possible combinations of the phase angle when the number of reflections is three. It is easy to test all eight cases for the scattering length density profiles against a predictable molecular model. We adopted this method, and the best combination of the phase angle was (0, 0, $\pi$) to fit the scattering length density of the molecular model, when

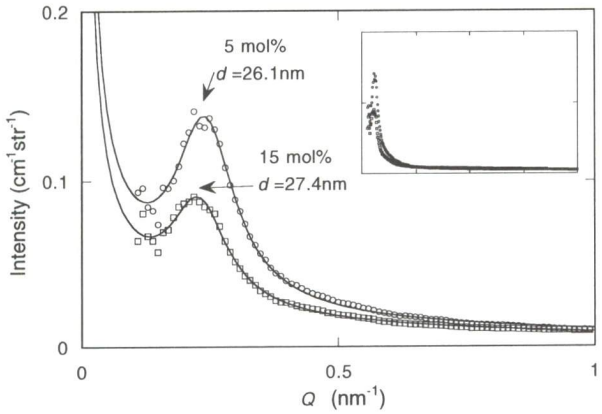


Fig. 4. Small angle neutron diffraction patterns of DPPC-d75 with 5 mol% (○) and 15 mol% (□) cholesterol in the ripple phase at 33 °C. Solid lines: fitted curve by a Lorentz function with a power function as back ground. Axes in the inset is same as figure except for full scale of $Q$-axis, which is from 0 to 4 nm$^{-1}$. The first and the second order reflection peaks from ripple structure were observed, but the intensity of the second order reflection was a little. Reflection peaks from lamella structure were hardly observed because of low contrast.

the methyl trough was fixed as a symmetrical center.

Difference between two scattering length density profiles, Figs. 2b and 3b, is mainly part of deuterated carbon chain. Occupied area per lipid is 0.473 nm$^2$ in the gel phase[7] or 0.62 nm$^2$ in the fluid phase[8] as shown in Table I. Packing density of deuterated carbon chain is proportional to inverse of the occupied area. The packing density is relatively high in gel phase and low in fluid phase. Therefore, the decrease of the packing density causes the decrease of the scattering length density. The scattering length density of a methylene group is $7.9 \times 10^{-17}$ cm/nm$^3$ in the gel phase and $6.1 \times 10^{-17}$ cm/nm$^3$ in the fluid phase. Postulating that these are the value of the summit corresponding to the hill of the deuterated carbon chain in Figs. 2b and 3b, the scattering length density of the summit corresponding to the deuterium oxide becomes $5.8 \times 10^{-17}$ cm/nm$^3$. The scattering length density of the bottom corresponding to the head group of DPPC becomes $3.4 \times 10^{-17}$ cm/nm$^3$ in a similar way. The scattering length density of the head group is higher than the value calculated from molecular formula, $1.2 \times 10^{-17}$ cm/nm$^3$. It would suggest that the head group is hydrated with about 50 vol% deuterium oxide. A difference of not only packing density but also scattering length density is observable by neutron diffraction study. It is a strong forte compared to the X-ray diffraction study observing electron density because scattering length density can be changed by isotope substitution, especially hydrogen and deuterium, keeping a molecular formula and a chemical nature. Electron density can not be changed keeping them. It was shown that neutron diffraction was effective in an experiment focused on hydrocarbon chain.

In Fig. 4, the intensity of diffraction peak caused by the ripple structure decreased as cholesterol concentration increased from 5 mol% to 15 mol%, due to the fact that the contrast decreases as a domain including cholesterol spread in the membrane. The experimental result that the periodic spacing of the ripple structure increased as cholesterol concentration rose consists with Copeland and McConnel.[1] If the ripple is symmetric like a sine curve, a periodic spacing of the ripple becomes a half in sight of the scattering length density. In these experiments, no peaks for a half periodic spacing were observed. The ripple would not be symmetric, in other words, the membrane would be heterogeneous in the ripple phase. Therefore, we concluded that a bandlike structure parallel to a ridge would be formed in the membrane. Cholesterol would prefer one of some domains to keep forming a bandlike structure. If the membrane becomes homogeneous, the ripple structure would fade away and no diffraction peak from the ripple structure would appears. Very weak diffraction peaks from ripple structure after the second order imply that the scattering length density profile could be roughly drawn by only a base sine wave of Fourier series. The ripple phase appears from lower temperature with cholesterol existence less than 20 mol%.[9,10] Cholesterol would promote microscopic phase separation in gel phase. Consequently, cholesterol would induce the ripple structure from lower temperature.

Table I. Values of structural quantities for DPPC.

| Phase | $D$ (nm) | $D_{HH}$ (nm) | $A_c$ (nm$^2$) | $\theta$ (°) | $A$ (nm$^2$) |
|---|---|---|---|---|---|
| Gel | 6.2 | 4.2 | 0.202[a] | 31.6[a] | 0.473[a] |
| Fluid | 6.4 | 4.2 | 0.31[b] | - | 0.62[b] |

[a]Reference 7 (25 °C*). [b]Reference 8 (50 °C*).
*For DPPC-d62, the transition temperature is about 6 °C lower than DPPC.

## Acknowledgements

This work was supported in part by Grant-in-Aid for Encouragement of Young Scientists from Ministry of Education, Science, Sports and Culture, Japan, and by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

1) B. R. Copeland and H. M. McConnel: Biochem. Biophys. Res. Commun. **599** (1980) 95.
2) S. Imaizumi and I. Hatta: J. Phys. Soc. Jpn. **53** (1984) 4476.
3) W. Knoll, G. Schmidt, K. Ibel and E. Sackmann: Biochemistry **24** (1985) 5240.
4) V. I. Gordeliy, V. G. Ivkov, Y. M. Ostanevich and L. S. Yaguzhinskij: Biochim. Biophys. Acta. **1061** (1991) 39.
5) T. Otomo, M. Furusaka, S. Sato, S. Ito, T. Adachi, S. Shimizu and M. Takeda: J. Phys. Chem. Solids **60** (1999) 1579.
6) J. Torbet and M. H. F. Wilkins: J. Theor. Biol. **62** (1976) 447.
7) W.-J. Sun, S. Tristram-Nagle, R. M. Suter and J. F. Nagle: Biophys. J. **71** (1996) 885.
8) J. F. Nagle: Biophys. J. **64** (1993) 1476.
9) R. D. Koynova, A. I. Boyanov and B. G. Tenchov: FEBS Lett. **187** (1985) 65.
10) A. I. Boyanov, R. D. Koynova and B. G. Tenchov: Chem. Phys. Lipids. **39** (1986) 155.