Biological Science

15A/2008G625

Cyclodextrin-induced rearrangement of chain packing in dipalmitoylphosphatidylcholine/cholesterol mixed bilayers

Masanao Kinoshita*, Satoru Kato
Department of Physics, School of Science & Technology, Kwansei-Gakuin University, Gkuen2-1, Sanda, Hyogo, 669-1337, JAPAN

Introduction

The presence of cholesterol (chol) in biomembranes gives crucial influences on the biological functions; chol affects not only the protein activity but also the structure of the lipid bilayer. The addition of chol into the lipid bilayer brings froth extension of hydrocarbon chains of lipids, resulting in the increase in the bilayer thickness. In addition, it is known that the effects of chol on the lateral chain packing are different between in the disordered fluid phase and the ordered gel phase. When the rigid cholesterol molecules are added to the fluid phase membrane, they restrict the fluidity of lipid hydrophobic chains and promote the formation of the liquid ordered (L_O) phase, which has been extensively studied as a model for the raft membrane [1]. On the other hand, when chol molecules are added to the gel phase membrane, they work as steric destabilizers, disturbing the tight chain packing in the membrane [2].

In order to examine the influences of chol on the membrane structures, cyclodextrin (cyd) has been extensively used in the monolayer system. The cyd molecule can extract chol from lipid/chol mixed membranes and change the structural organization of lipids in the membrane (e.g. modification of the liquid ordered phase). On the other hand, in the bilayer system much less information is available on the influence of cyd-mediated chol-extraction. In this study, we examined the cyd-induced change in lipid lateral packing in the dipalmitoylphosphatidylcholine (DPPC)/chol mixed single bilayer vesicle by wide-angle X-ray diffraction (WAXD).

Experimental

DPPC/20 mol% chol vesicles were prepared by a conventional method. The multi-lamellar vesicles obtained were sonicated by a tip-type sonicator for 1 min, resulting in small unilamellar vesicles. An aqueous solution of cyd (6 wt%) was added to the sample immediately before the WAXD measurements. The final DPPC concentration was 15 wt%. The lipid dispersion was placed between kapton films kept parallel with a washer as a spacer and mounted on a DSC apparatus for an optical microscope, which was used as a temperature controller. The WAXD measurements were performed at BL-15A in Photon Factory. The wavelength of the X-ray beam λ was 0.15 nm and the camera length was 14.6 cm. The imaging plate was used as a detector with the exposure time of 2 min.

Results and Discussion

Fig. 1a shows a WAXD pattern of DPPC/chol mixed vesicles in the gel phase at 30°C. There appeared two peaks centred at 2.0 nm^{-1} (0.50 nm) and 2.3 nm^{-1} (0.43 nm), indicating coexistence of two domains. Considering that the DPPC bilayers in the gel phase give a sharp peak at 2.3 nm^{-1} with a shoulder in the wide angle side, the peak at 2.3 nm^{-1} in Fig. 1a may be attributed to the DPPC-rich domain in the gel phase. The other peak at 2.0 nm^{-1} is likely to be attributed to the DPPC/chol mixture domain in the more fluid L_O phase. Fig. 1b shows a WAXD pattern of DPPC/chol mixed vesicles incubated for 10 min at 30°C after the addition of cyd. The integrated intensity of the peak at 2.0 nm^{-1} seemed to decrease by about 20% during the incubation. These results suggest that the cyd-induced reduction of L_O phase via extraction of chol from the mixed bilayers. In addition, preliminary experiments of prolonged incubation showed that very slow structural change seemed to follow the initial rearrangement of chain packing. Thus, the cyd-mediated removal of chol from mixed bilayers may not be a single process.

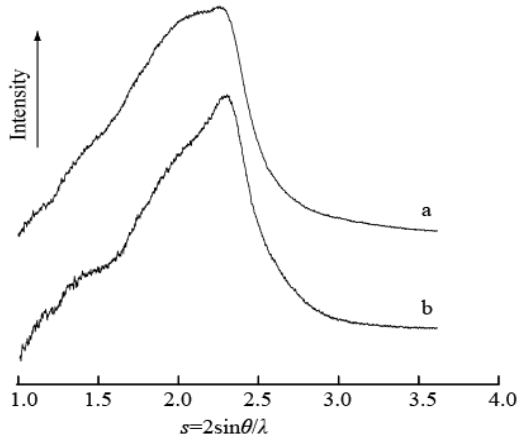

Figure 1. WAXD patterns of DPPC/cholesterol vesicles in the absence (a) and in the presence (b) of cyd at 30°C (see Experimental for detail). θ is the scattering angle and s is the modulus of the scattering vector.

References

[1] C. J. Fielding (Ed), 2006. Lipid rafts and caveolae. pp. 3-5.
[2] P. Yeagle (Ed), 1999. The structure of biological membrane. pp. 88-92.
* aul81636@kwansei.ac.jp