Biological Science

15A/2008G652

Cyclodextrin-induced modulation of the phase behaviour of the dipalmitoylphosphatidylglycerol/cholesterol mixed liposome

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Introduction

Cholesterol (chol) is one of the key modifiers of the structure and function of biomembranes; It affects not only the membrane 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 addition of chol restricts the mobility of lipid hydrocarbon chains and consequently gives rise to the raft-like rigid domain called the liquid ordered (L_o) phase [1].

Previously, we examined the influence of the cyclodextrin (cyd)-induced extraction of chol on the lipid packing structure in phosphatidylcholine (PC)/chol mixed multi-lamellar liposomes: Our wide angle X-ray diffraction experiments showed that the addition of cyd brings forth only small effects on the structure of the PC/chol mixed liposome. Speculatively speaking, sufficient amounts of cyd may not be supplied in the inner layers of the PC/chol multi-lamellar liposome because the interbilayer space, in which cyd molecules are localized, is limited (~7 nm). In this study, we employed anionic phospholipid dipalmitoylphosphatidyl-glycerol (DPPG) to supply sufficient cyd into interbilayer spaces; the anionic lipid liposome has relatively large interbilayer spaces due to electrostatic repulsion.

Experimental

DPPG/20 mol% chol multi-lamellar liposomes were prepared by a conventional method. Briefly, appropriate amounts of DPPG and chol dissolved in organic solvent were dried under a flow of nitrogen and then under reduced pressure overnight. The resulting lipid film was dispersed into a 50 mM cyd solution and kept at 60ºC for ~1 hour. The final DPPG concentration was 20 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. Small angle X-ray diffraction (SAXD) measurements were performed at BL-15A in the Photon Factory. The wavelength of the X-ray beam λ was 0.15 nm and the camera length was 160 cm. We used a position sensitive proportional counter (PSPC) with 512 channels as a detector.

Results and Discussion

Fig. 1a shows a DSC heating thermogram and corresponding temperature-resolved SAXD patterns of DPPG/chol mixed liposomes. Below the main transition temperature (T_m=41.0ºC), there appeared peaks corresponding to the lamellar periodicity of 15.4 nm. As expected from electrostatic interbilayer interaction, anionic PG liposomes had much larger lamellar periodicity than PC ones. When the sample was heated, the lamellar periodicity decreased at the main transition temperature (12.5 nm above T_m). Fig 1b shows a DSC thermogram and temperature-resolved SAXD patterns of DPPG/chol liposomes in the presence of 50 mM cyd. The DSC measurement showed that there appeared a new transition immediately below T_m (arrow in Fig. 1b). The lamellar periodicity once decreased to 11.4 nm at the new transition, suggesting formation of a new phase with a smaller interbilayer space. A preliminarily DSC measurement of the DPPG/cyd binary system showed no sign for the new transition (data not shown). Thus, it is likely that the formation of the new phase is provided by the interaction between cyd and chol embedded in DPPG/chol mixed bilayers.

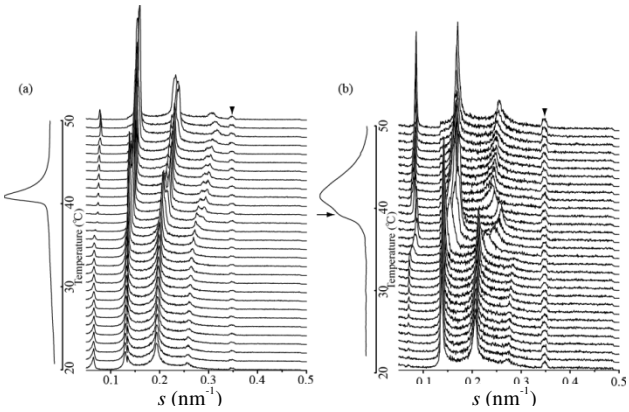

Figure 1. DSC heating thermograms and corresponding temperature-resolved SAXD patterns of the DPPG/chol liposome (a) in the absence and (b) in the presence of cyd. The arrow in (b) indicates the newly appeared transition (see Text). The scattering vector is defined as $s=2\sin\theta/\lambda$, where 2θ is the scattering angle. Arrowheads indicate the reflection from the kapton film.

References

[1] D. Meder and K. Simons, 2006. Lipid rafts, Caveolae and membrane traffic. In C. J. Fielding (Ed), Lipid rafts and caveolae. pp. 3-5, Wiley-VCH.

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