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Time course of the ordered phase formation in lipid bilayers caused by sphingomyelin hydrolysis

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1 Introduction

Sphingomyelin (SM) is a representative sphingolipid, distributed widely in plasma membranes. SM is known to form an ordered domain together with cholesterol (chol) and the SM/chol-rich domain has been extensively studied as a model for the biological raft [1]. SM/ceramide (Cer) mixed bilayer also forms an ordered domain similar to that in the SM/chol mixed bilayer [2].

Considering that Cer is produced by hydrolysis of SM in biological systems, Cer generation may preferentially occur in the SM-rich raft domain and involved in the raft formation. Although there are some reports on the physicochemical properties of the Cer-rich domains, the process/time course of the ordered phase formation still remains unknown. In this work, we tried to feed Cer molecules into SM bilayers as in biological systems; Cer molecules were generated in situ by hydrolysis of membrane-constituting SM. Cer formation was monitored by ordering in chain packing.

2 Experiments

SM derived from chicken egg and SMase were purchased from Avanti Polar Lipid and Sigma Aldrich, respectively. Other chemicals were purchased from Wako pure chemicals. The powder SMase was dissolved in the 5 mM HEPES buffer (20 mM $MgCl_2$ and 20 mM $CaCl_2$). The powder SM was dispersed in the buffer and incubated at 60ºC for 30 min with intermittent voltexing. We prepared ULVs by extruding sample through the membrane filter (Φ200 nm) for 29 times. Then the sample dispersion was centrifuged for 3-4 hours at 20,000G to obtain ULVs concentrated enough for wide angel x-ray (WAXD) measurements (~10 wt%). The sample was enclosed in the sample cell immediately after the addition of the SMase solution and the sample cell was put into the temperature controller kept at 43ºC.

The WAXD measurements were carried out at BL-6A with the imaging plate detector. The wave length was 0.15 nm and the camera length was estimated to be 19 cm by using cholesterol.

3 Results and Discussion

Fig. 1a shows a broad peak centered at 2.31 nm^{-1}, which is a typical WAXD pattern of SM bilayers in the fluid phase. The SM-ULV/SMase mixture after incubation at 43ºC for 5 min shows two peaks centered at 2.37 nm^{-1} and 2.46 nm^{-1} (arrowhead and arrow in Fig. 1b) overlapped by the broad peak. Considering that the main transition temperatures of the Cer-rich ordered domains are higher than that of pure SM bilayers (DSC data not shown), these peaks suggest the formation of the Cer-rich domains in the gel phase. Increase in the incubation time enhances the peak areas, suggesting the growth of the Cer-rich domains (Fig. 1b-f). After 8 hours about 70% of all domains transformed into the Cer-rich domains, judging from the area of the broad peak. Thus, we succeeded in detecting the dynamical change in bilayer structures induced by in situ introduction of Cer molecules as degradation products of SM.

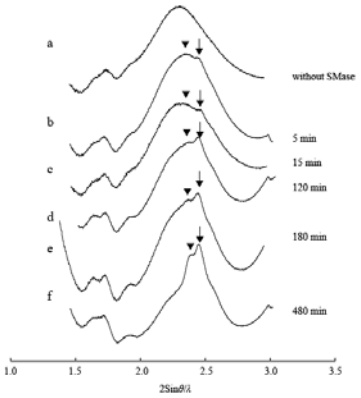

Fig. 1: WAXD patterns of SM-ULVs in the absence and presence of SMase (3 units) at 43ºC. The incubation time at 43ºC after the addition of SMase was directly indicated in the figure.

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References

[1] J. R. Silvius, Biochim. Biophys. Acta **1610** (2003) 174.
[2] J. Sot *et al.*, Biophys. J., **90** (2006) 903.
[3] B. Boulgaropoulos *et al.*, Biophys. J. **99** (2010) 499.

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