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# The bilayer morphology and the subgel phase formation of anionic phospholipid, dimyristoylphosphatidylglycerol

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## **Introduction**

Phosphatidylglycerol (PG) is one of major anionic phospholipid components for bacteria cell membranes. The interaction between antimicrobial peptides and PG bilayer membranes has been extensively studied to clarify the action mechanism of the peptides. In such studies, dimyristoyl-PG (DMPG) has been mainly used. However, the details of morphology and phase behavior of DMPG bilayers under low ionic conditions are not well understood. It has been pointed out that phase behavior of DMPG bilayers depends strongly on ionic strength of the buffer solution [1].

By using freeze-etch electron microscopic technique, we revealed that DMPG bilayer assemblies form flat bilayer sheet structures in the presence of low concentration of NaCl (~2mM) below the chain melting transition temperature, differing from usual vesicular structures (Fig.1) [2]. In addition, we also found that under low NaCl concentration conditions, a subgel phase (crystalline-like ordered phase) forms rapidly (~10min) when the DMPG samples are incubated at -5°C [2].

Here, we investigated the structure of the rapidly forming subgel phase of DMPG bilayer.

### Materials and methods

DMPG (1,2-Dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)]) was purchased from Avanti Polar Lipids and used without further purification. The lipid was dispersed into a buffer containing 10mM HEPES (pH 7.4) and an appropriate concentration of NaCl.

X-ray diffraction (XRD) measurements were carried out at the beamline 15A of PF, using both long and short camera length settings.

#### **Results and discussion**

Small angle X-ray scattering data indicated that the incubation at -5 °C hardly affects the thickness of the DMPG bilayers under low NaCl concentration conditions. On the other hand, the hydrocarbon chain packing structure of the DMPG bilayer was changed after incubation at -5 °C. Figure 1 shows the XRD patterns of the subgel phase of DMPG bilayer and the subgel phase of dipalmitoyl-phoshatidylcholine (DPPC) bilayers. PC is a major neutral lipid of animal cell plasma membranes. The data might indicate the formation of a new type of the subgel phase. The further detailed studies are required.



**Fig. 1** Schematic representation of the morphology of DMPG bilayers.



**Fig. 2** Wide-angle XRD profiles of the subgel phase of DPPC bilayers (upper) and the subgel phase of DMPG bilayers incubated at  $-5^{\circ}$ C for 10 min in the presence of 2 mM NaCl (lower).

## **References**

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