Evaluation of hydration repulsive force of acylamino-phospholipid from X-ray diffraction measurement

Hiroshi TAKAHASHI*, Satoru ABE ¹Gunma Univ., Aramaki 4-2, Maebashi, Gunma, 371-8510, Japan

Introduction

Hydration repulsive forces play an important role in various phenomena: stability of lyotropic liquid crystals, fusion of lipid vesicles and biological membranes, etc. Recently, for lipid multilamellar vesicles systems, it has been reported that the hydration repulsive forces can be evaluated by combining the inter-lamellar distance estimated from X-ray diffraction with the chemical potential of ice formation [1-3].

Using this method, we have measured the hydration repulsive force of a synthetic acylamino-phospholipid (1,2-dimyristoylamido-1,2-deoxyphosphatidylcholine

 $(D_{14}DPC)$) at subzero temperatures and have compared it with that of dipalmitoylphosphatidylcholne (DPPC)).

The reason why we studied acylamino-phospholipids is as follows. Recently, so-called raft, microdomain in biomembrane, has greatly attracted attention from the viewpoint of cell signaling. The raft domain contains a large amount of sphingolipids having an amide group. Thus, in order to clarify the effect of the amide group on the physical properties of membranes containing sphingolipids, we studied the hydration force of $D_{14}DPC$ having two amide groups.

Materials and Methods

DPPC was obtained from Avanti Polar Lipids, Inc (Alabaster, AL, USA) and D14DPC was kindly given to us by Prof. J. Sunamoto. Those lipids were used without further purification. X-ray diffraction measurements were performed at the beamlines 9C and 15A of the Photon Factory. The temperature of the samples was controlled using a modified calorimeter (FP84, Mettler).

Results and Discussion

Figure 1 shows a plot of observed lamellar repeat spacing of multi-lamellar $D_{14}DPC$ vesicles against temperature. At subzero temperature region, the values of the lamellar spacing are gradually increased with increasing temperature. This behavior has been commonly observed for neutral lipid multilamellar vesicle systems [2,3].

Based on the observed X-ray diffraction data, we calculated the strength of repulsive interaction of $D_{14}DPC$ as the repulsive pressure between adjoining bilayers. The result is shown as a function of interbilayer spacing in Fig.2, with that of DPPC. It can be seen from the figure that there is significantly difference of hydration repulsive force between D14DPC and DPPC multilamellar vesicles. However, the physical origin of the difference has yet to be appreciated.



Fig.1 Temperature dependence of lamellar spacing of D_{14} DPC.



Fig.2 Plot of hydration repulsive pressures of $D_{14}DPC$ and DPPC as a function of interbilayer spacing.

References

[1] Z. Yan et al., J.Chem. Soc. Frarady Trans. **89**, 2583 (1993).

[2] J.T. Gleesen et al., Biophys.J., 67, 706 (1994).

[3] H.Takahashi and P.J.Quinn, *Mol.Cryst. Liq.Cryst.* **367**, 427 (2001).

* htakahas@fs.aramaki.gunma-u.ac.jp