Title	Lamellar-lamellar phase separation in phospholipid/salt/water system(Poster session 1, New Frontiers in Colloidal Physics : A Bridge between Micro- and Macroscopic Concepts in Soft Matter)
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Citation	物性研究 (2007), 89(1): 97-98
Issue Date	2007-10-20
URL	http://hdl.handle.net/2433/110926
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Lamellar-lamellar phase separation in phospholipid/salt/water system

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リン脂質と一価塩を一定の割合で混合して形成したマルチラメラベシクルにおいて観測されるラメラーラメラ相分離(別の面間隔を持つ2種類のラメラ構造が共存する)の要因を明らかにするため、DPPC と NaCl の混合系でのそれぞれのラメラ相の面間隔の温度変化に対する応答を、X 線小角散乱をもちいて観測した。その結果、この相分離は一価塩濃度の濃いラメラ相と薄いラメラ相に相分離することにより引き起こされることが示唆された。

Introduction

Phospholipid molecules are the main constituent of biomembranes. These molecules spontaneously assemble into bilayers in water to form vesicles. The equilibrium structure of this assembly is a multi-lamellar vesicle with a characteristic repeat distance on the order of nm. The equilibrium repeat distance between bilayers is uniquely determined by the balance between attractive and repulsive interaction such as van der Waals interaction, hydration repulsion, electric interaction and steric repulsion (Helfrich repulsion). It has been known that the addition of a salt with divalent cation affects the electrostatic interaction between bilayers. On the other hand, monovalent salts have been assumed to have no direct interaction with neutral phospholipid bilayer.

Rappolt et al. observed that two or three multi-lamellar structures with different repeat distances coexist when certain concentration of monovalent salt is added in the suspension of neutral phospholipid multi-lamellar vesicle [1][2]. These results could be the evidences of the lamellar-lamellar phase separation induced by the monovalent salts. However, the origin of such the structure has not been clarified yet.

In order to investigate the effect of monovalent salt and the origin of the lamellar-lamellar phase separation, we performed small-angle X-ray scattering (SAXS) on the mixture of DPPC and NaCl.

Results and Discussion

Figure 1 shows a typical SAXS profile at [DPPC]/[NaCl] = 1/10 [mol/mol] and at 50℃. The profile can be interpreted by two Bragg peaks with different lamellar

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repeat distances. This is the evidence of the lamellar-lamellar phase separation (the coexistence phase) in the liquid crystalline phase of lipid bilayers. Fig.2 shows temperature dependences of the repeat distances of the coexistence phase at [DPPC]/[NaCl] = 1/10 [mol/mol] just above the main transition temperature where the region of the anomalous swelling behavior compared with that of pure DPPC aqueous solution. The temperature dependence of the larger repeat distance in the coexistence phase follows the similar temperature dependence of the pure DPPC aqueous solution in the region of the anomalous swelling. On the other hand, the repeat distance with smaller repeat distance changes drastically by changing temperature. The same tendencies are observed in the other concentration of NaCl against DPPC. These behaviors could be interpreted that the concentrations of the salt are different in the phase-separated domains with different repeat distances of lamellar considering the results by Korreman et al. [3] These results suggest that the phase separation should be induced by the heterogeneous distribution of salt molecules in lipid bilayers.

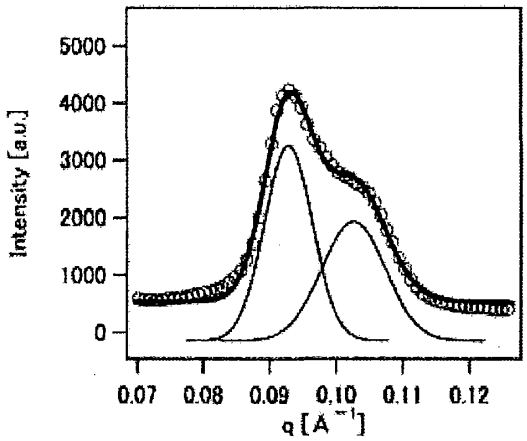

Fig.1 Typical SAXS result of DPPC multi-lamellar vesicle with NaCl (○). The result is well fitted by superposition of two Gaussian functions.

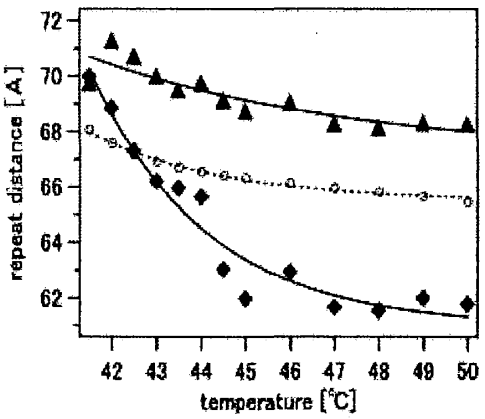

Fig.2 The repeat distances of separated two lamellar phases of DPPC with NaCl (▲ and ◆), and DPPC-only multi-layer (○). The lines are obtained by fitting with exponential.

Acknowledgement

This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for JSPS Fellows, 19-2774, 2007.

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