P-26

Preparation of cubosomes and elucidation of their stability, phase transitions and interaction with giant unilamellar vesicles

［１］Organization
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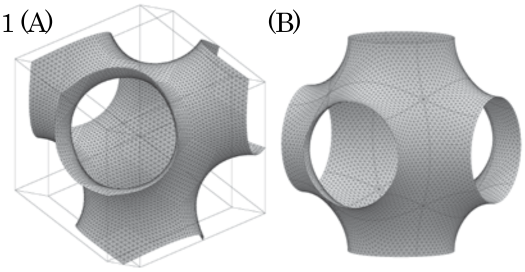
［２］Purpose of research

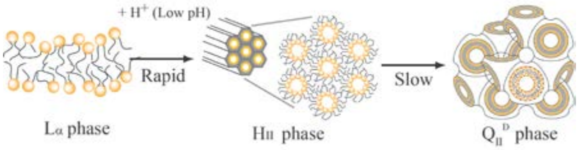
Biomembranes/lipid membranes are usually in the lamellar liquid-crystalline (L_α) phase, but under certain conditions they form inverse bicontinuous cubic (Q_{II}) phases, where the membranes are connected in 3-dimensional space with a cubic symmetry. The Q_{II} phases such as the double-diamond Q_{II}^D (or Q^{224}) (Fig. 1A) and the primitive Q_{II}^P (or Q^{229}) (Fig. 1B) have an infinite periodic minimal surface consisting of bicontinuous regions of water and hydrocarbon.

Fig.1 (A) (B)



Yamazaki and colleague found that the modulations of electrostatic interaction (EI) due to surface charges were able to induce the phase transitions between the L_α and the Q_{II} phases and intercubic phase transitions for the first time. Among these EI-induced phase transitions, there is the low pH-induced L_α to Q_{II}^D phase transition in dioleoylphosphatidylserine (DOPS) /monoolein (MO) membranes (Fig. 2). To reveal the mechanism behind the L_α/Q_{II} phase transitions, the kinetic pathway of the low pH-induced L_α to Q_{II}^D phase transition in DOPS/MO was investigated using time-resolved small-angle X-ray scattering (TR-SAXS). At the initial step, the L_α phase was directly transformed into the hexagonal II (H_{II}) phase and subsequently the H_{II} phase slowly converted into the Q_{II}^D phase.

Fig. 2



In this project, to elucidate the mechanism of low pH-induced L_α to Q_{II}^D phase transition in DOPS/MO membranes and to develop its application, we tried to construct cubosomes with a diameter of 50–200 nm containing cubic phase membrane, which can be stably suspended in aqueous solution, and also investigated the temperature dependence of the kinetic pathway of the low pH-induced L_α to Q_{II}^D phase transition.

［３］ Outcome

（３－１）Results of the research

(1) Construction of cubosomes

We tried to prepare cubosomes of monoolein (MO) in HEPES buffer (pH 7.0) using two methods. In the first method, a dry thin film of MO was prepared after drying MO chloroform solution, then a solution of an amphipathic polymer, Pluronic F127, in HEPES buffer was added onto the dry film, and a resulting suspension was ultrasonicated several times. The measurement using dynamic light scattering (DLS) indicates that the average particle size of the suspension ranged from 68 to 71 nm, depending on F127 concentration. This result indicates that a large MO membrane in the Q_{II}^D phase decreased to smaller particles. However, SAXS measurement of this suspension indicates that well-defined SAXS peaks corresponding to the Q_{II}^D phase were not detected. This may be due to a damage of membrane by sonication. In the second method, first a dry thin film of MO and F127 mixture was prepared after drying MO and F127 mixture in chloroform solution. Then HEPES buffer was added onto the dry film, and a resulting suspension was ultrasonicated several times. The measurement using DLS indicates that the average particle size of the suspension ranged from 78 to 90 nm, depending on the ratio of F127 to MO. However, SAXS measurement of this suspension indicates that well-defined SAXS peaks corresponding to the Q_{II}^{D} phase were not detected.

We also tried to prepare hexasomes of dioleoylphosphatidylethanolamine (DOPE) in HEPES buffer. Using both the methods, we succeeded in preparing the hexasomes of DOPE with an average diameter of ~130 nm which shows strong SAXS peaks corresponding to the H_{II} phase.

In near future we have to improve the method to prepare cubosomes of DOPS/MO based on the above results.

(2) Temperature effect on the low pH-induced L_{α} to Q_{II}^{D} phase transition in DOPS/MO

We investigated the effect of temperature on the low pH-induced L_{α} to Q_{II}^{D} phase transition in DOPS/MO using TR-SAXS with a stopped flow apparatus. First we investigated the temperature effect of the phase transition at final pH 2.6. At 20 °C, a SAXS peak corresponding to the H_{II} phase started to appear at 10 s. The intensities of the peaks of the H_{II} phase increased with time up to 50 s and then decreased, whereas those of the L_{α} phase became low at 50 s. At 100 s, two weak peaks corresponding to the Q_{II}^{D} phase appeared, then their intensities increased with time. In contrast, at 35 °C, a SAXS peak corresponding to the H_{II} phase started to appear at 3 s, but the SAXS peaks of the Q_{II}^{D} phase started to appear at ~1000 s. Even at 1 h after the mixing, only small peaks of the Q_{II}^{D} phase were observed, indicating that the rate of the transition from the H_{II} to the Q_{II}^{D} phase was very slow, i.e., the membranes almost trapped in the intermediate H_{II} phase.

To obtain the rate constants of the initial step (i.e., the L_{α} to the intermediate H_{II} phase transition) and of the second step (i.e., the H_{II} to Q_{II}^{D} phase transition) quantitatively, we analyzed the time course of the intensity of the (10) peak of the H_{II} phase. The rate constant of the initial step increased with temperature. By analysing this result, we obtained the values of its activation energy, which did not change with temperature but increased a little with a decrease in pH. In contrast, the rate constant of the second step decreased or kept constant with temperature, indicating that the activation energy of the second step increased with temperature. Based on these data, we discuss the mechanism of this phase transition.

（３－２）Impacts and Perspective

The experimental results on the construction of the cubosomes and the hexasomes are valuable for future improvement of the method of their preparation.

By analyzing the results of the temperature effect on the low pH-induced L_{α} to Q_{II}^{D} phase transition in DOPS/MO, information on activation energy of the elementary processes of this phase transition was obtained. To our knowledge, the values of the activation energies of any kinds of phase transitions of lipid membranes have never been published, thereby this result provides the first values of the activation energy of phase transitions of lipid membranes. The information on activation energy of the elementary processes is indispensable data to elucidate the mechanism of this EI-induced phase transition.

［４］Publication lists
(the list of papers which contains the above results)

（１）T. Oka et al., The Effect of Temperature on the Low pH-Induced Lamellar to Bicontinuous Cubic Phase Transition in Dioleoylphosphatidyl-serine/ Monoolein, Submitted for publication.