Structural phase transitions of lipid monoolein cubic phases induced by protein lysozyme molecules confined in nano-channels of the cubic phases.

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脂質の一種であるモノオレインは、過剰な水の存在下で $Pn3m$ の対称性をもつ立方相を形成する。この立方相はナノメートルサイズの「水路」を内部にもち、そこにタンパク質を取り込むことができる。我々は、このような微小空間に拘束されたタンパク質溶液における結晶化が、通常の溶液よりも促進されることを見いだしてきた [1]。本発表では、タンパク質の存在によって立方相が受ける構造変化を X 線小角散乱法によって調べた結果を報告する。

Lipidic cubic phases consist of nanoscopic water channels which are periodically interwoven. The size of the channels for the case of monoolein is about 2-10 nm in diameter. On the other hand, a protein lysozyme has the size about 3.5 nm in diameter. Therefore, when lysozyme molecules are confined in the channels of the cubic phase, there will be strong steric interaction between them (figure 1). We found that this interaction eventually drives lysozyme molecules to crystallize at lower concentrations than the normal crystallization conditions [1]. In other words, the crystallization is enhanced by the confinement. In this study, we focus on the structure of the cubic phase where lysozyme molecules are confined, using small angle X-ray scattering. Information on how lysozyme molecules are confined is necessary to elucidate the molecular mechanism of the enhanced crystallization, which may be useful for the crystallization of other proteins.

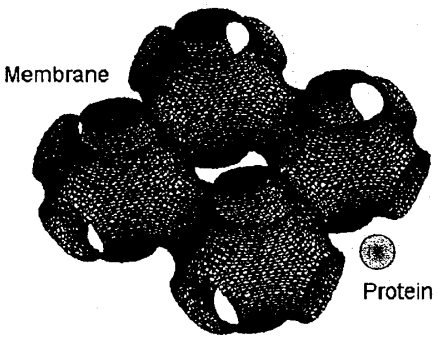

Figure 1: Schematic representation of the cubic phase and a lysozyme molecule.

Small angle X-ray scattering (SAXS) was conducted with a beamline BL40B2 at a synchrotron radiation facility SPring-8. The details of the experiments are given at the presentation. Briefly, an excess amount of lysozyme solutions containing NaCl is mixed with melted monoolein. A transparent cubic

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phase is formed almost instantly. It is known that adding NaCl to a lysozyme solution induces attraction between (otherwise repulsive) lysozyme molecules.

Figure 2 shows the change of SAXS profile when increasing lysozyme concentration without NaCl. The peak positions of the profile at 0% lysozyme suggests that the cubic phase has a $Pn3m$ symmetry as expected. As increasing lysozyme concentration, new peaks characteristic of an $Im3m$ symmetry appear and grow. For the case of monoolein cubic phase, $Im3m$ phase has a wider space in the water channels than that of $Pn3m$ phase [2]. Therefore, the result shown in figure 2 confirms that lysozyme molecules widen the nanoscopic space in the water channels.

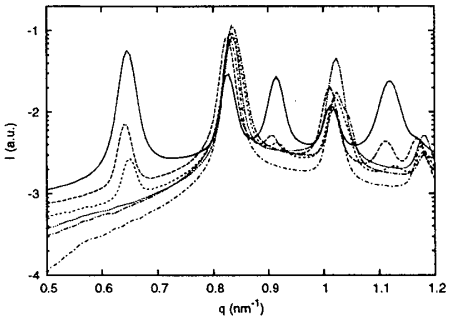

Figure 2: SAXS from the cubic phases containing a lysozyme solution without NaCl. Lysozyme concentration is 0%(w/v) to 5% from the bottom to the top at the left side of the graph.

Figure 3 demonstrates the effect of interaction between lysozyme molecules on the cubic phase structure. As increasing NaCl concentration, which is equivalent with increasing attraction between lysozyme molecules, a new peak at $q \simeq 0.6$ (nm^{-1}) appears and grows. The position of the peak is characteristic to $Im3m$ phase, and is the same as the peak appears in response to the increase of lysozyme concentration (figure 2). This result shows that intermolecular attraction also widens the water channels, and induces the structural phase transitions.

When lysozyme molecules are confined in the narrow water channels, lipid membranes are forced to bend to accommodate them. This effect will be strong when lysozyme concentration is high, and/or lysozyme molecules have a tendency to aggregate. Our results are consistent with this picture. Moreover, the results show that when frustrated by the presence of lysozyme, lipid membranes do not bend randomly (in other words, melt) but form another stable (crystalline) cubic structure. The free energy for the system must be needed to do a detailed analysis for this transition. The possible relation between the structural phase transition and the enhanced crystallization will be discussed in the presentation.

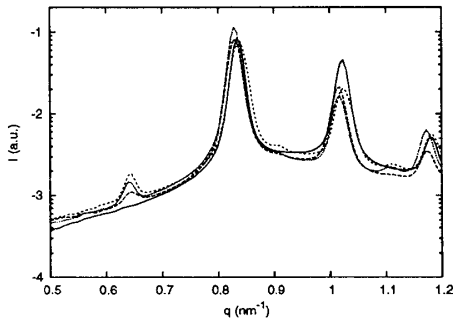

Figure 3: SAXS from the cubic phases at a fixed lysozyme concentration (2%). NaCl concentration is 0 M to 0.3 M from the bottom to the top at the left side of the graph.

References

[1] S. Tanaka, S. U. Egelhaaf, and W. C. K. Poon, Phys. Rev. Lett. **92** (2004), 128102.

[2] V. Razumas, Z. Talaikytė, J. Barauskas, K. Larsson, Y. Miezis, and T. Nylander, Chem. Phys. Lipids **84** (1996), 123.