透過型電子顕微鏡を用いたタンパク質の結晶化における準安定相の直接観察

Direct observation of metastable phase in protein crystallization using transmission electron microscopy

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A thermodynamically metastable phase, such as amorphous and dense liquid, has an important role in a crystallization process. In a nucleation process, amorphous particles appear before nucleation of a crystalline phase, and those serve as nucleation sites for more energetically favorable crystalline phases [1]. In crystal growth processes, a dense and liquid-like cluster most likely assists formation of a macro-step on a crystal surface [2]. To demonstrate these crystallization processes, *in situ* observation using a microscope is one of the powerful methods because it can directly visualize these processes in real time. However, it is difficult to visualize behavior of such metastable particles because those sizes are normally submicron, sometimes in nanoscale. Recently developed liquid cells adapting to high-vacuum environments of transmission electron microscopy (TEM) provide nanoscale views of nanoparticles and crystallization processes in aqueous solutions [3]. We developed the fluid-reaction transmission electron microscopy (FR-TEM) system for *in situ* observation of crystallization process in aqueous solutions. Using this system, we performed *in situ* observation of a protein crystallization, for investigating its nucleation and crystal growth processes.

Hen-egg white lysozyme was used as a protein sample without further purification and was crystallized using NaCl as a precipitant in a sodium acetate buffer solution at pH = 4.5. For observation of crystals in a solution under TEM, we used a “Poseidon” TEM holder (Protochip, Inc.) combined with a liquid cell. The liquid cell consists of a pair of semiconductor-based plates with an amorphous silicon nitride window and 150 or 500-nm-thick spacer to form a flow path of a crystallization solution.

We succeeded in observing two crystalline phases of orthorhombic and tetragonal in addition to an amorphous phase of the lysozyme [4]. Orthorhombic is the most stable of phases in our experimental solution. In this presentation, we present recent results of *in situ* TEM observation of its crystallization process including behaviors of metastable phases.

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