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Application of the transmission electron microscopy to the study of the crystallization process in a solution

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One of the powerful methods to demonstrate the crystal growth and dissolution processes is in situ observation using a microscope, because it can directly visualize these processes in real time. The crystal growth and dissolution processes in a solution have been studied mainly using various optical microscopies as a noninvasive technique. Some high resolution optical microscopies is able to visualize the molecular-level structure on a crystal surface [1-3]. However, the lateral resolution of optical microscopy is sub-micron because it is limited by the wavelength of a light source [1]. To resolve the crystallization processes more clearly, the transmission electron microscopy (TEM), which has the nano-order resolution, is a strong method for in situ observation. Recently, in situ observations of the crystallization processes in solutions under TEM have been energetically performed using the liquid cells or an ionic liquid to adapt to the high-vacuum environment [4-6]. These observations allow us to see the behavior of nano-particles and crystallization process of inorganic materials in a solution. Here, using the liquid cell, we have performed in situ observation of the crystallization of a lysozyme protein, which is used to study the crystallization process in a solution as a model material, by the TEM for understanding the processes of crystallization.

We used two TEMs with LaB6 filament at an acceleration voltage of 200 kV (Hitachi H-8100) and with field-emission gun at an acceleration voltage of 300 kV (Hitachi HF-3300). For the observation of crystals in a solution, 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 the flow path of the crystallization solution. The lysozyme was crystallized using NaCl as a precipitant in a sodium acetate buffer solution at pH = 4.5.

As a result, we succeeded in observing the crystals and amorphous particles of lysozyme protein using the TEM and the liquid cell holder. In this presentation, we report the recent results of in situ observation of its crystallization process including the growth and dissolution.

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