# Dynamical study of initial stage of lysozyme protein crystallization by liquid-cell transmission electron microscopy

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Crystallization from solution has a key to understand geochemical processes of the earth and to synthesize functional materials. The primary stage of crystallization, called nucleation, has an important role to generate a new phase in an ambient phase. The following process is called crystal growth, which a formed crystalline nuclei grow continuously. To understand these processes in molecular scale, one of the powerful methods is direct observation of their dynamics in real-time by various microscopies. Crystal growth is well examined by in-situ observation using various advanced optical and scanning microscopies. On the other hand, nucleation process have been studied by indirect methods such as dynamic light scattering and spectroscopic methods, because nucleation process occurs at nano-scale and finishes within a few seconds and, therefore, nucleation is difficult to visualize by microscopy.. These indirect measurements suggest that nucleation is more complex process, for example, prenucleation clusters and dense liquids are formed before crystals appear. These primary particles may have critical role for nucleation of crystals, however, direct evidence is still poor because of difficulties to observe it. Recent advancement of nano-fabricate technique provides us the cells to observe liquids by transmission electron microscopy (TEM). This technique give us a chance to unveil the complex process of nucleation of minerals in nano-scale spatial resolution and real time. Here we adopt this technique to observe the nucleation process of protein lysozyme crystals. The dynamics of protein crystallization is relatively slower than that of inorganics due to their large molecular weight.
Optimizing the crystallization condition of lysozyme for TEM observation, we succeeded in observing three phases of lysozyme, amorphous and two crystalline phases. In addition, we caught the moments of nucleation of orthorhombic crystal which is the most stable phase in our crystallization condition. We measured the size evolution of nucleated particles with time, and found that the growth rate of the particle immediately after nucleation is surprisingly rapid, several hundred nm $s^{-1}$, which is two-three orders of magnitude faster than that of bulk lysozyme crystals. After this, growth rate is suddenly decreased, and facet faces appeared. Within several seconds from the particle appeared, growth rate became several nm $s^{-1}$ which is the same as that of the bulk crystal at same supersaturation. Direct analysis of the nucleated particle suggests that the initial state of the nucleated particle is non-crystalline phase, and it transforms into the crystalline phase.

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