Elucidating the early stages of protein crystallization by TEM

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To obtain a clearer picture of the formation process of the early stages of crystallization, we believe that understanding the difference between bulk material and nanometer sized particles is crucial. Physical properties that appear exclusively in nanoparticles induce significant phenomena that depend on the particle size, called size effects. For instance, surface free energy of nanoparticles is different from that of bulk [1, 2] and even in the melting point decreases to be 1100 K for a particle with 5 nm in diameter [3] and to be ~700 K for a particle with 2 nm in diameter [4] from 1337 K in case of gold.

The ideal to understand crystallization would be atomic-scale in-situ direct observation of the clustering process of growth units. Then, how such size effects of nanoparticles affect to the process should be observable. Unforutunately, however, the processes are in too small and too rapid to investigate by traditional techniques. To overcome the difficulties, we started and optimized real time in-situ observation of crystallization processes of a protein, lysozyme, from a solution by transmission electron microscopes (TEM).

Lysozyme is a protein most extensively studied as a model protein molecule and has a relatively larger growth unit compared with inorganic crystals. Size of the unit cell and number of molecules/atoms in it are 2.4×10^5 $Å^3$ (79.1 × 79.1 × 37.9 Å for tetragonal) and 8 for lysozyme, and 68 $Å^3$ (d = 4.08 Å) and 4 for gold, respectively. Gold nanoparticles with 2 or 5 nm in diameter consist of ~250 or ~3900 atoms. In case of lysozyme, 250 or 3900 molecules are able to make a crystal with ~24 nm or ~60 nm in diameters, which are obserbable size scale using TEM. Then, more than 10% of molecules/atoms located particle surface, i.e., the particle has large surface to volume ratio, which induces characteristcs of tiny particles. Since the size of critical nuclei can be several nanometers to tens of nanometers, there is a chance to observe lysozyme nuclei and the processes including significant phenomena caused by its characteristic physical properties. Here, we show the resent results of the crystallization of lysozyme based on the concept.

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