Direct TEM observation of nucleation processes in a solution

Yuki Kimura
Institute of Low Temperature Science, Hokkaido University
My recent works

Dissolution process by TEM

Nucleation process by TEM

Microgravity

Protein

Dying star

Drug

Lunar base

Food

Star formation region

Bio-mineralization

Interferometry

Analysis of meteorite

In-situ IR spectroscopy

Magnetic remnant by electron holography TEM


K. Kimura et al., Chemistry of Materials, 28 (2016) 8732.

In the **classical view**, nucleation is a **simple** process; single growth unit attaches to an $n$-mer to be an $(n+1)$-mer, which process progresses sequentially from a formation of dimer.

Nevertheless, we cannot explain a real process and predict results of crystallization.

Motivation

Nucleation theories give us nucleation rates, \( J \), but having large difference with that by experiments or MD simulations.

\[
\frac{J_{\text{theory}}}{J_{\text{(experiment or MD)}}} = \text{Several orders (H}_2\text{O, Methanol)} \text{ to 20 orders (Ar)}
\]

Why?

- Limitation of the theories
- Heterogeneous nucleation
- Multistep nucleation

We don’t know why nucleation rates are so different.
Steps in early stages of crystallization by dissolution & precipitation or solid-solid phase transition

Direct Nucleation

Nucleation processes always pass through the size of meso-scale. I believe this makes one of difficulties to understand nucleation.

Methods

- Poseidon holder (Protochips Inc.)

- **EDS** Compatible Tip

- Liquid Flow

- Electrodes

- Flow in (PEEK tube, φ100 µm)

- Flow out (PEEK tube, φ150 µm)

- Liquid Heating

- Poseidon holder (Protochips Inc.)
Methods

- Preparation sequences of a liquid cell

Drop a solution (~0.5 – 1 μL)

Covered by a large E-chip

Enlarged, tilted
Final amount of a solution in a liquid cell with 500 nm spacer is ~2 nL.
Methods

- Preparation sequences of a liquid cell
  - Check a leakage
  - Introduced into a TEM
    - In case of flow, PEEK tube is connected with a syringe pump
Lysozyme

- Molar weight: 14300
- Size: $45 \times 30 \times 30 \, \text{Å}$

Methods

Protein sample: Hen egg white lysozyme
Crystallized using NaCl as a precipitant in sodium acetate buffer solution (pH = 4.5).

**Lysozyme 15 [mg ml⁻¹]**
**NaCl 50 [mg ml⁻¹]**

Solution was continuously flowed in the cell.

Thickness of the liquid layer is 150-500 nm.
TEM images of protein particles

**a:** An elongated orthorhombic lysozyme crystal. The crystal zone axis is $[103\ 14]$.

**b:** A tetragonal lysozyme crystal viewed from its (110) face. The crystal zone axis is $[110]$. 

**c:** Cluster of spherical amorphous particles.

Thickness of E-chips spacer: 500 nm

Amorphous and tetragonal particles are incorporated into most stable orthorhombic crystals via dissolution rather than attachment or fusion.
In-situ observation of growing crystal

Very small growth rates (~1-2 nm/s) of the growing crystal can be determined in relatively shorter time (<10 s). In-situ TEM observation of an orthorhombic lysozyme crystal. Play speed of the video is 5 times faster.

Growth rates of lysozyme crystals as a function of supersaturation $\sigma = \ln(C/C_e)$ ($C$: lysozyme concentration, $C_e$: solubility at certain temperature) measured under optical microscopy. There is the threshold of electron dose not to affect the crystallization.

Dissolution of a lysozyme crystal

- Increasing flux of electron

There is a threshold of electron flux, which can be neglected to discuss crystallization of lysozyme protein

At the moments of crystallization, orthorhombic lysozyme crystal nucleated separately from the amorphous particles. The amorphous particles did not transfer into a crystal.

At the moments of crystallization 2 × faster

Sample : Lysozyme crystal
E-chips : Flow
Spacer : 500 nm
Flow rate: 2 μL/min
TEM : H-8100
Acc. V : 200 kV
E. Gun : LaB₆

Scale bar: 500 nm

Orthorhombic lysozyme crystal nucleated on the amorphous particle. The amorphous particles did not incorporated into the crystal.

**Crystallization of protein (Pattern A)**

Still micrographs of the nucleation processes as observed by TEM. There are two amorphous particles at 0 s. The yellow triangles indicate a pre-existing amorphous particle. The red triangle shows a spherical particle that nucleated at 0.17 s near the amorphous particle indicated by the yellow triangle. This nucleated particle grew and transformed into an orthorhombic lysozyme crystal.

The role of amorphous phases in the nucleation of orthorhombic lysozyme crystals. (Left) Time resolved in situ TEM images. A spherical cluster, indicated with a yellow arrowhead, forms at 0.17 s near an amorphous solid particle (ASP) and transforms into an orthorhombic crystal. The scale bar is 200 nm. (Right) The size evolutions of particles reveal decreasing, on the average, growth rates. (Inset) Zoom-in of times 0 – 10 s. Dotted lines are logarithmic fits to each data set.

Frozen particle with a crystal in its center observed by cryo-TEM.

(A) The particle has a different contrast than the faceted faces, indicating a crystalline structure. (Scale bar: 50 nm)

(B) The corresponding diffraction pattern of the particle shows a Laue spot (indicated by a triangle). (Scale bar: 2 nm$^{-1}$)

Schematic of the nucleation pathway of lysozyme crystals. Crystals are not directly nucleated by assembly of molecules from the bulk solution (A) or transformation of the ASP (B). Instead, orthorhombic crystal nucleate within disordered protein-rich clusters (C), which heterogeneously nucleate on the surface of the ASPs or the container walls.

Summary

- Competition is an actual event before nucleation.
- Amorphous protein particles with 150-200 nm in size is not dense liquid, but solid, and not precursor of lysozyme crystal.
- Lysozyme crystals seem to be nucleated via dense liquid phase.
- Even in protein, physical properties of nanoparticles may play important roles in nucleation processes.

Acknowledgements

Grants-in-Aid from KAKENHI, for Scientific Research (S) (Y. Kimura; 15H05731)

Contact information

ykimura@lowtem.hokudai.ac.jp