Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Developing novel methods for protein crystallization
（新規タンパク質結晶化法の開発）

The protein crystallography is a powerful technique in which scientists use the x-ray or neutron to determine the 3-D structure of the proteins. The structure of the proteins is essential for the understanding of the structure-function relationship of proteins, which plays an important role in biological and medical science, such as elucidating the mechanism of biological phenomena, drug design, and industrial enzyme engineering, etc. However, the preparation of protein crystals good enough for the diffraction experiment is still the main problem in the current, such as the rarity of nucleation, small crystal size or low crystal quality.

Proteins can be prompted to form crystals when protein solution becomes supersaturated. The process of crystallization can be divided into three steps: (1) the nucleation, (2) crystal growth, and (3) termination. In this study, we focus on developing novel methods to control the nucleation and growth of protein crystals, thus improving the success rate to obtain high quality crystals which are more appropriate for structure determination.

I. Enhancement of protein crystallization through lattice-ledge-directed heterogeneous nucleation

Nucleation is classified into homogeneous and heterogeneous cases. Homogeneous nucleation occurs without preferential nucleation sites. Many approaches have been developed to control homogeneous nucleation, such as the robotic systems and high-throughput methods, magnetic/electric field, microgravity, host-guest crystallography, and the modification of the target protein molecules. However, none of them have been proved as an efficient method in x-ray/neutron crystallography.

Unlike homogeneous nucleation, heterogeneous nucleation happened at the surface of nucleants. A variety of nucleants such as minerals, horse and human hair, thin films, charged surfaces, molecularly imprinted polymers, and mesoporous materials has been reported in preparing protein crystals. Although surface structural characteristics and chemical characteristics of the nucleant are thought to be important, the role of nucleant surfaces in heterogeneous nucleation, and phase transition on nucleant surfaces are still unclear. So far, the development of efficient nucleants is still underway.

Considering the heterogeneous nucleation should start by initial molecular packing on nucleant surfaces, we chose the lattice-surface of nucleants for initializing molecular packing, thus inducing the nucleation of protein crystals. Based on this idea, eight nucleants were selected and designed. Crystallization of two model proteins with nucleants was carried out. As the result, we found that crystalline networked-cages facilitate the nucleation of the protein crystal. We applied Co-cage-1 to the crystallization of 13 proteins. Except for two proteins, the crystallization and partly crystal structure determination of 11 proteins (11kDa~110kDa) confirmed the validity of Co-cage-1. To explore how crystals formed on the surface of Co-cage-1, in situ observations are performed using cryo-transmission electron microscopy (Cryo-TEM) and high-speed atomic force microscopy (HS-AFM). Based on the results, we proposed a lattice-ledge-directed heterogeneous nucleation mechanism. The ledge is the starting point for the formation of the protein aggregates. Accumulation of these aggregates leads to form the dense liquid clusters on the surface of nucleants. The lattice-surface initiates the protein molecules packing in a stable situation at the interface between the dense liquid clusters and nucleants. Then, the crystalline nucleus formed at the interface. The dense liquid is consumed by the growth of inner crystalline nucleus. The findings of this study open the door to rationally design the nucleants for crystallizing the proteins.

II. Growth of huge protein crystals for neutron protein crystallography

Neutron macromolecular crystallography (NMC) is a powerful method to obtain the accurate position of key hydrogen atoms in protein structures that are difficult to visualize by x-ray analysis alone. The most significant problem of NMC is the relatively low flux of available neutron beams, which requires large crystals (~ 1 mm^3) in order to obtain the measurable diffraction signal. However, growing large, high-quality protein crystals is very challenging.

Our target protein is glutamine amidotransferase CAB (GatCAB), in which the ammonia is proposed to transfer through the inner channel. To visualize the ammonia in the channel, neutron diffraction studies are indispensable. However, GatCAB is a challenge sample for growing large crystals because of its needle-like shape and slow growth rate. Various methods are failed to grow large GatCAB crystals, such as macro-seeding, double seeding, agarose gel, and surface mutation.

Inspired by the previous study that the crystal growth rate can be significantly slowed down or accelerated by rearranging structured water around both the surfaces of the protein molecules and the crystal, we adopted the temperature-responsive copolymer (TRCP) to adjust the crystal growth. As expected, GatCAB crystals were grown up to an approximate volume of 2.8 × 0.8 × 0.8 mm (1.8 mm^3) with TRCP within one month. Based on this result, we proposed that TRCP destruct the water shell on the surface of protein molecules, thus accelerating the crystal growth. To confirm this theory, we calculated the entropy of released water molecules during protein crystallization based on the experimental data of the temperature dependence of the solubility of GatCAB crystals. The results suggested additional water molecules should be released from proteins in the presence of TRCP.