**IN-SITU OBSERVATION OF PROTEIN CRYSTAL GROWTH UNDER MAGNETIC QUASI-MICROGRAVITY CONDITIONS ON EARTH**

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Protein crystallization in space is assumed to proceed in a purely diffusional manner without gravity-induced convection [1]. As a result, protein crystals are expected to be formed free of defects and/or impurities. Proteins as well as protein solutions are usually diamagnetic, so that a similar effect on convection should be possible by magnetic force. Magnetic force \( F_m = \frac{1}{\mu_0} m \chi_m B \frac{dB}{dz} \) is proportional to the product of magnetic field \( B \) and magnetic field gradient \( \frac{dB}{dz} \), as given in the following equation (1):

\[
F_m = \frac{1}{\mu_0} m \chi_m B \frac{dB}{dz}
\]

where \( \mu_0 \) is the vacuum permeability, \( m \) is the mass, \( \chi_m \) is the mass susceptibility, \( B \) and \( \frac{dB}{dz} \) is the magnetic flux density and gradient of magnetic field, respectively, in vertical direction. A diamagnetic substance is levitated or its motion is impeded when \( F_m \) is equal or close to gravity. Therefore, a magnetic field and a magnetic field gradient have been utilized to obtain high quality protein crystals, in which magnetic orientation of crystals and reduction in natural convection contribute mainly to the crystal quality improvement [2]. Using this technology we have developed a high-throughput and high-quality protein crystal growth system with strong magnetic force that enables to cancel out the gravity of a water droplet. This system consists of a superconducting magnet, protein crystallization plates, a temperature controller, and equipment for in-situ observation (Fig. 1) [3].

The system developed contains two groups of superconducting coils; the top coil of Nb₃Sn generates magnetic fields downward, while the bottom coils of NbTi and Nb₃Sn generate magnetic fields upward; these coils are operated in persistent mode. The maximum magnetic field generated reaches 16 T. \( F_m \) and \( \frac{dB}{dz} \) become largest between the two groups of coils, creating a reduced-gravity zone where the crystallization plates are placed (Fig. 1).

Since the crystallization plates are placed in the magnet bore, we cannot know if crystallization has occurred until we examine the cell outside of the bore. Then, we not only interrupt the crystallization process, but also change the whole gravity conditions. In terms of efficiency, it is highly desirable not to interrupt the crystallization process by taking out the crystallization plates from the bore. It is direct observation that eliminates these problems.

We have made up an optical observation probe which can look into the crystallization plates in the magnet bore, enabling
in-situ observation of the crystallization process. The optical probe has a 3-D adjustable focusing mechanism with enough high resolution of a few microns (Fig. 1).

Protein crystallization has been carried out through the sitting drop vapour diffusion method. In the system, we can stack 10 crystallization plates, each having 12 solution reservoirs and 24 crystallization drop wells, enabling us to examine 240 conditions in one experiment. The temperature around the crystallization plates is controlled by the dry air between 4 and 20 °C within ± 0.1°C.

In this study, we have conducted crystallization experiments using two protein samples, a GFP (green fluorescent protein)-like protein and a zinc protease. In the case of the GFP-like protein, triangular pyramid-shaped crystals appeared within 12–24 hours after the initiation of vapour diffusion, exhibiting magnetic orientation. X-ray diffraction experiments indicated that the crystallographic c-axis was parallel with the direction of the magnetic field. Needle-shaped thin crystals of the zinc protease also showed magnetic orientation (Fig. 2). The long side of crystals was aligned along the c-axis which is parallel with the direction of the magnetic field. The crystals were grown 1–2 days. X-ray diffraction measurements showed that these protein crystals obtained in the magnetic quasi-microgravity had better crystallographic quality and less variation in quality than crystals grown without the magnetic field.


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BIOPHYSICAL AND STRUCTURAL CHARACTERIZATION OF DIPEPTIDYL PEPTIDASE III FROM PORPHYROMonas GINGIVALIS

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Porphyromonas gingivalis is a gram-negative human pathogenic bacterium. It is found in the oral cavity and is able to break through human gingival fibroblasts causing difficult and painful diseases like gingivitis and periodontitis [1]. It also contains a group of enzymes which belong to the dipeptidyl peptidase III (DPP III) family. This group of enzymes, also known as enkephalinase B, is an enkephalin-degrading enzyme that cleaves dipeptides sequentially from the N-termini of its substrates. All DPPs III described thus far contain the unique zinc binding motif HEXXGH characteristic for the metallopeptidase family M49. DPP III play important role in the mammalian pain modulatory system. This is supported by the finding that low levels of DPP III activity were detected in the cerebrospinal fluid of individuals suffering from acute pain [2].

The function of the DPP III homolog from Porphyromonas gingivalis is still unknown, but it could be involved in pathogenicity. We aim at determining potential substrates of this enzyme as well as its three-dimensional X-ray structures in order to obtain information about its potential function. Here, we represent and discuss ITC and SAXS data together with predicted structural model. We also present findings about potential inhibitor of the enzyme using conformational changes calculated from SAXS measurements. Additionally, we briefly investigate C-terminal end of the enzyme since it possesses structural simi-
THE EFFECT OF AUDIBLE SOUND ON PROTEIN CRYSTALLISATION

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The successful crystallisation of proteins is important because their molecular three dimensional structures can be obtained through X-ray diffraction when in their crystalline form [1]. Investigation of the crystallisation process is beneficial for this purpose. We have reported that protein crystallisation is sensitive to audible sound, which is commonly present but is often ignored. Here we investigate the effect of audible sound parameters, especially frequency, on a protein crystallisation. We show a significant facilitation of protein crystallisation using 5000 Hz audible sound (Figure 1), possible mechanism was also tried to be clarified [2]. Suitably controlled audible sound can be beneficial for promoting protein crystallisation. Therefore, audible sound can be used as a simple tool to promote protein crystallisation. In addition, the processing of other types of materials may also be affected by audible sound.


\textbf{Figure 1}. Crystallisation screening results with and without audible sounds. (a) Ratio of crystallisation screening hits with audible sound irradiation to that without sound irradiation. The initial concentrations for lysozyme and concanavalin A were both 10 mg/ml. (Error bar: standard deviation, n=4). (b) Crystallisation hits at 1000 Hz. (Error bar: standard deviation, n=4). (c) Crystallisation hits at 5000 Hz. (Error bar: standard deviation, n=4). (d) Number of crystallisation hits at 1000 Hz (red circles) and 5000 Hz (blue triangle) normalized to the number of hits without sound irradiation.
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CRYSTALLOGENESIS STUDIES OF BACTERIAL AMINOACYL-tRNA SYNTHETASES AS POTENTIAL TARGETS FOR DRUG DESIGN

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Aminoacyl-tRNA synthetases (aaRSs) are ubiquitous enzymes that catalyze the first step of protein biosynthesis or translation. In the presence of ATP, they activate the amino acids as adenylates and subsequently bind the amino acid moiety onto the 3’ CCA end of transfer RNAs [1]. The resulting aminoacyl-tRNAs are then carried by the elongation factors to the ribosome to be incorporated into nascent polypeptide chains. AaRSs are a target of choice for drug design because they are essential enzymes having a high specificity for their substrates.

Our study is focused on bacterial aspartyl-tRNA synthetases (AspRSs) that bind specifically L-aspartate. We have initiated the structural characterization of the binding mode of two families of inhibitors. One is a natural antibiotic produced by E. coli strains that targets the catalytic site of AspRSs (collaboration with Prof. S. Rebuffat, Museum National d’Histoire Naturelle, Paris) and the other a series of chemically synthesized peptides that were selected against an AspRS from the opportunistic human pathogen Pseudomonas aeruginosa (collaboration with Prof. Hiroaki Suga, University of Tokyo).

We apply various crystallogenesis approaches to prepare crystals that are suitable for the X-ray diffraction analysis. They involve the optimization of crystal production either by cocrystallization or by soaking of native crystals with ligands. In the case where the enzyme of one bacterial species does not yield exploit able crystals, the protein is either chemically methylated to change its surface properties and crystallizability, or a close structural homolog with a conserved active site is substituted to take advantage of genetic diversity. In the final step crystallization systematically takes place in an agarose gel with a low gelling temperature to improve crystal quality, stability during the soaking with inhibitors, and handling [2]. The rationale of our crystallogenesis strategy will be presented and illustrated with examples.