[1] H. Adachi et al., Jpn. J. Appl. Phys. 42 (2003) L798.
[2] H. Adachi et al., Jpn. J. Appl. Phys. 43 (2004) L1376.

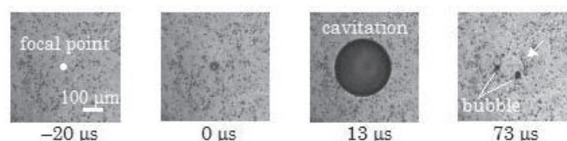

Fig.1 The movement of microbeads by femtosecond laser irradiation

Keywords: protein crystallization, nucleation, laser technology

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Single-molecule visualization on a protein crystal surface

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During elementary growth processes of crystals, such as surface diffusion, adsorption and desorption of molecules at a solution-crystal interface, the behavior of individual molecules that constitute a crystal plays a key role. A single-molecule visualization (SMV) technique allows us to track dynamic behavior of individual molecules. Since SMV requires a fluorescent label attached to a target molecule for visualization, target molecules have to be large enough so that a fluorescent label does not affect their dynamic behavior. Hence, we adopted fluorescent-labeled protein and protein crystals as a model system. We have used hen egg-white lysozyme (HEWL) crystals and fluorescent-labeled HEWL (F-HEWL) [1], and reported the intrinsic picture of diffusion at a solution-crystal interface [2]. In this study, we demonstrate intrinsic pictures of adsorption. First we observed F-HEWL molecules adsorbed on a crystal surface by SMV, and also observed elementary steps in the same field of view by laser confocal microscopy. We found that F-HEWL adsorbed preferentially on steps, showing that F-HEWL molecules behave like solute HEWL molecules, because of very small size of the fluorescent label compared to that of HEWL. Next we tracked the adsorption kinetics, and found that the amount of adsorbed F-HEWL increased after a certain “induction period”. This phenomenon clearly indicates that the adsorption proceeds through successive multiple elementary processes. In addition, we also found that F-HEWL molecules that stayed on a crystal surface for longer period adsorbed faster. This result supports the successive adsorption that proceeds gradually on a crystal surface.

[1] T. Matsui, et al., J. Crystal Growth, 293, 415-422 (2006).
[2] G. Sazaki, et al., Cryst. Growth Des., in press.

Keywords: single-molecule visualization, adsorption kinetics, protein

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Wavelength dependence of the crystallization by the laser irradiation

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Introduction : Light-induced crystallization have attracted attention as an application of temporal and spatial control of crystallization. We have proposed a new nucleation technique using a femtosecond laser at a wavelength of 780 nm and succeeded in producing high-quality protein crystals [1]. In order to optimize a laser condition, we investigated wavelength dependence of the crystallization by the laser irradiation about nucleation probability. Experiment : We compared the probability of nucleation, when focused femtosecond laser beams were irradiated in protein solutions, such as Lysozyme and Glucose Isomerase, with various laser conditions (wavelength:260 nm, 390 nm, 780 nm, energy: 13.5-94μJ/pulse). Trials were carried out using a batch method at 23 ℃ . At the same time we measured the strength of the impulse wave with shock wave sensor, and estimated deformations of solution. Result: In each wavelength, nucleation was promoted by femtosecond laser irradiation with certain energy level. However, nucleation probabilities were almost same in all wavelength of laser irradiated. These energy levels were comparable with threshold values of deformation of solution. Accordingly, nucleation was not dependent on wavelength of laser, but deformations of solution by the laser irradiation. From these results, we conclude that 780 nm laser is suitable for nucleation, because there is little denaturation of the protein by the laser irradiation at a fundamental wavelength of commercial femtosecond laser and there is no absorption to a plastic crystallization plate and a tape for sealing.

[1] H. Adachi, et al., Jpn. J. Appl. Phys. 42 (2003) L798.

Keywords: wavelength, laser radiation, nucleation

P04.01.56

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Growth of large protein crystals for neutron crystallography by hanging a seed crystal

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P4

The biological function of proteins is dominated by hydrogen atoms. Neutron protein crystallography enables us to determine positions of hydrogen atoms, which allows rational drug design and the understanding of enzymatic processes. Although recent advances in proteins to crystallize, the bottleneck of neutron protein crystallography still remains the growth of large crystals (-1 mm³). To overcome the difficulties in obtaining a mm-sized crystal, we have developed a new method that we call the Pendant Technique. This method has improved the Floating And Stirring Technique (FAST) and seeding techniques. As shown in Fig.1, a seed crystal was hanged in a protein solution to prevent poly-crystallization by separating additional nucleated crystals. With a long-term growth and promotion of growth speed by solution stirring, we have successfully grown a 5.9×3.8×3.7 mm single hen egg-white lysozyme crystal. Using this method, we have also grown HIV protease up to 3.2×1.7×0.5 mm. Therefore, this technique gives us a suggestion for production of huge crystals which are enough for neutron protein crystallography.

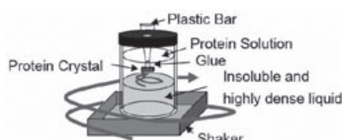

Fig.1. Schematic illustration of the method. Protein crystals can be grown hanging in solution.

Keywords: protein crystallization, crystallization methods, neutron crystallography

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Crystallization and biochemical analysis of eIFA protein

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eIFA(eIF4GI analogue) participates in nonsense mediated mRNA decay (NMD) which controls mRNA quality in eukaryote. Although down-regulating of eIFA is thought to abrogate NMD, its function in NMD is not clear yet. And eIFA is found to interact with CBP80, a member of heterodimeric nuclear cap-binding complex (CBC). CBC binds to 5' cap of mRNA and then involves in mRNA modification and elimination. eIFA protein fused with 6-His tag was expressed in E.coli and purified with affinity, ion exchange and gel filtration column chromatography. The crystals of eIFA were obtained at 290K by microbatch method. Biochemical assay on translation suggests that eIFA has a direct role in initiation of translation.

Keywords: eIFA, NMD, translation

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The scottish structural proteomics facility

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The Scottish Structural Proteomics Facility (SSPF) is a cooperative of researchers with interests in structural biology, enzyme chemistry, structure-based inhibitor discovery, virology, glycobiology and microbial pathogenesis established with funding from the Scottish Funding Council and BBSRC (UK). Our objectives are: 1. To establish a sustainable centre of excellence in Structural Proteomics. 2. To exploit post-genomic sciences and technologies to increase our understanding of pathogen physiology at the molecular level. 3. Establish a Computational Chemistry group and exploit opportunities for the rational design of novel antimicrobial agents. 4. Provide new drug leads to combat emerging multiply-resistant infectious agents. 5. Forge links with local, national and international biotechnology-pharmaceutical companies. The funding available to us is considerably less than provided to structural genomics centres in the USA, Japan or in Europe and we have adopted a focused approach to our targets closely tied to existing experience and research interests. To date we have determined numerous structures that inform about unusual aspects of enzyme chemistry, essential biosynthetic pathways in pathogens, pathogenicity, DNA repair, viral proteins and carbohydrate transport. We have established the technologies to drive computational chemistry approaches to drug discovery where a small subset of our targets is relevant. We have been testing high-throughput methods, novel expression protocols and systems. Our targets include proteins from viruses, archea, bacteria, protozoan parasites and higher eukaryotes; small cytosolic enzymes through to multi-subunit complexes and membrane bound proteins.

Keywords: structural proteomics, Scottish, drug targets

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A dynamic light scattering system combined with a conventional chromatography for sample preparation

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Recent progress in structure determination of proteins by x-ray crystallography allows us to understand the important and complicated mechanism of biological reaction in vivo. However, crystallization process of protein molecules is still the rate determining step caused by difficulty and uncertainty of the sample preparation. Measurement of dynamic light scattering (DLS) is well known as an effective method for evaluation of the sample quality, because DLS results show good correlation between dispersity of the protein solution and possibility of crystallization in many cases. However, it takes a couple of minutes for each samples to measure the polydispersity of molecules, and also, the measurements must be performed in batch mode. If a real-time DLS system is equipped to a conventional chromatography system as a detector for polydispersity of each peak, it is quite useful for evaluation of sample quality. It can be possible to recognize which fraction should be used for crystallization. Therefore, we developed a flow-type cell for a real-time DLS system which can be used as an on-line detector system for a conventional chromatography during the preparation and purification of protein samples. In this study, we report the results of the DLS measurement of alpha amylase from *Aspergillus oryzae* and other proteins by using FPLC system equipped with the real-time DLS system with the flow-type cell.

Keywords: dynamic light scattering, protein purification crystallization, protein crystallization development