Keywords: CBS domains, cystathionine-beta-synthase, surface entropy reduction

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Crystal structure of DNMT3A ADD domain

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DNA methylation is a major epigenetic mark associated with a condensed state of chromatin and transcriptional repression. DNMT3A, one of de novo DNA methyltransferases, conducts methylation onto initially unmethylated DNA in genome, and plays an important role in developmental processes. The accessory protein, DNMT3L is required for DNMT3A to exert its DNA methyltransferase activity. These proteins share two structural motifs, the C-terminal methyltransferase fold and the ADD domain in the N-terminal regulatory region. Recently, the ADD domain of DNMT3L has been shown to recognize unmethylated Lys4 of histone H3 (H3K4) through its PHD finger motif, implying an important role of the ADD domain in determination of the DNA methylation site. To address the question how specific patterns of DNA methylation are established, we examined structural and functional properties of the ADD domain of DNMT3A. We have solved the crystal structure of the ADD domain of human DNMT3A at 2.3 Å resolution using a multi-wavelength anomalous dispersion method with intrinsic zinc atoms. The overall structure of the ADD domain is folded into a single globular domain similar to those of DNMT3L and ATRX, which is composed of GATA-1 like and PHD type zinc fingers. The ADD domain of DNMT3A has an acidic pocket in the PHD motif, the structural feature of which is similar to the H3K4 recognition pockets of other PHD finger proteins. In the crystal structure, the acidic pocket is occupied with the Arg residue side chain from the symmetry-related molecule, mimicking the H3K4 recognition. Combined with the biochemical data, our structural data suggest that the ADD domain of DNMT3A is involved in determination of the DNA methylation sites in genome by reading out the methylation state of H3K4.

Keywords: DNA methylation, PHD domain, histone

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The observation of individual protein molecules on a protein crystal under forced solution-flow

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A high-quality crystal of protein is indispensable for determining molecular structure of protein. We have proposed a crystallization method by which protein crystals are grown under forced solution-flow condition, and succeeded in improving crystal quality of several proteins[1]. However, to apply this method generally to many proteins, it is essential to clarify how forced solution flow benefits the quality of protein crystals. In this study, to reveal correlation of solution-flow with behavior of solute molecules on a crystal surface, we tried to observe individual protein molecules on a protein crystal surface under forced flow condition. We used single-molecule visualization of a thin-solution-layer type [2], monoclinic crystals of hen egg-white lysozyme (HEWL) and fluorescent-labeled HEWL (F-HEWL) [3], which can be regarded as solute HEWL. The utilization of the monoclinic HEWL crystals of several 10 μm thickness enabled us to visualize individual F-HEWL molecules on a crystal surface under forced flow conditions. We compared two single-molecule images taken with a time interval of 1 s, and determined the number density of F-HEWL molecules adsorbed on a crystal surface for 1 s. We traced the temporal change of number density of adsorbed F-HEWL molecules. The experiments carried out with and without forced solution flow clearly demonstrated that the net adsorption rate of F-HEWL under forced flow was significantly faster than that without forced flow. From this result, we conclude that forced flow increases the incorporation rate of solute molecules into a crystal, i.e. the crystal growth rate.

[1] H. Adachi, et al., Jpn. J. Appl. Phys. 41 (2002) L1025. [2] G. Sazaki, et al., Cryst. Growth Des., in press. [3] T. Matsui, et al., J. Cryst. Growth, 293 (2006) 415.

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Study on femtosecond laser-induced nucleation dynamics of proteins

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We have previously developed a new technique for control of nucleation by femtosecond laser irradiation. On the basis of this technique, we have succeeded in obtaining high quality crystals such as water-soluble protein lysozyme [1], membrane protein AcrB [2]. In this work, we tried to clarify the nucleation mechanism with femtosecond laser irradiation in order to exploit more effective laser irradiation condition. We observed the laser focal point with high speed CCD camera, and found that laser irradiation induced cavitation, shockwave and bubbles on a time scale of microseconds to seconds. These phenomena moved the microbeads (4um) distributed in the solution(Fig.1). Additionally, we conducted direct observation of molecular movement with fluorescence labeled lysozyme. We found strong fluorescence signal around cavitation. From these results, we conclude that cavitation move molecules and produce high concentration area which promote nucleation.

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

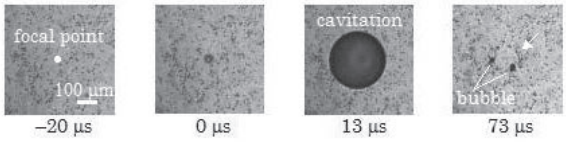

Fig.1 The movement of microbeads by femtosecond laser irradiation

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

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

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