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], monolayer 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 monolayer 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.

Keywords: protein crystals, molecular imaging, fluorescent

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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 (4μm) 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.

Keywords: protein crystals, molecular imaging, fluorescent