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In higher plants, algae, and cyanobacteria, phytobilins are utilized for photosensing and/or light harvesting. Phytobilins are synthesized by ferredoxin-dependent bilin reductases (FDBRs) from biliverdin IXα (BV) derived from heme by hemeoxygenase. Phycocyanobilin:ferredoxin oxidoreductase (PcyA), one of FDBRs, sequentially reduces the vinyl group of D-ring and A-ring of BV to produce 18^1,18^2-dihydrobiliverdin (18EtBV) and phycocyanobilin. We reported the crystal structures of PcyA from *Synechocystis* sp. PCC 6803 and its complex with BV at 2.5 Å and 1.51 Å resolutions, respectively. These structures revealed that Glu76, His88 and Asp105 are located near the U-shaped BV [1] and that upon BV binding induced-fit conformational changes occur in such a way that the substrate entrance is narrowed [2]. Focusing on the structural changes in PcyA during the sequential reduction of BV, we prepared the crystals of PcyA in complex with 18EtBV and BV13, an analog of 18EtBV. These pigments were chemically synthesized. The crystal structures of PcyA-18EtBV and PcyA-BV13 were determined at 1.48 Å and 1.04 Å resolutions, respectively, revealing PcyA conformation after the reduction of D-ring vinyl group in BV. The side chain of Glu76 rotates away from D-ring to form hydrogen-bonds with both Asn62 and Tyr238. On the basis of these structures, we discuss the sequential reduction mechanism of PcyA.

[1] Y. Hagiwara *et al*. PNAS, 103, 27-32 (2006)
[2] Y. Hagiwara *et al*. FEBS Lett. 580, 3823-3828 (2006)

Keywords: photosynthesis-related proteins, redox enzymes, reactive intermediates

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The crystal structure of lipase a from *Candida Antarctica*

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Lipases are widely used as catalysts in industrial applications due to their thermostability and enantioselectivity. *Candida antarctica* lipase A (CAL-A) is highly thermostable in organic solvents and has therefore become a frequently used catalyst in chemical and pharmaceutical industry. CAL-A shows some unusual properties, which makes it a highly attractive enzyme. CAL-A is the only known lipase to have Sn2-preference towards triglycerides. It is able to hydrolyze sterically hindered alcohols, both secondary and tertiary alcohols. In addition, it shows a high chemoselectivity for the N-acylation of beta-amino esters, which makes CAL-A an important catalyst in the production of enantiopure amino acids. We have determined the crystal structure of CAL-A at 2.1 Å resolution. CAL-A exhibits a typical alpha/beta hydrolase fold, consisting of a central beta-sheet and surrounding alpha-helices. The active site pocket is formed like a deep L-shaped tunnel covered by a lid that regulates the interfacial activation. Residues Ser184, Asp334 and His366 form the catalytic triad at the bottom of the pocket.

Keywords: lipases, enzyme structure, crystallography

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Novel approaches in protein crystallization

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Structure genomics (SG) studies usually include target selection, gene cloning, protein expression, purification, crystallization and structure determination. Among them, protein production and crystallization are the rate-limiting steps. We have developed novel methods in facilitating protein crystallization on our SG platform at Peking Univ. One example is that during the human chloride intracellular channel protein 2 (CLIC2) study, we have found that 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB) modification of the surface cysteines significantly improved the crystal quality and crystals diffracted to 2 Å were obtained. We have further explored the application of DTNB modification for other protein crystallization trials. We have also made statistical analyses of crystallization condition parameters which impact protein crystallization processes from hundreds of different proteins grown in our lab for the SG projects. During the efforts, we optimize and further develop strategies of protein crystallization and are trying to change it from arts to science.

Keywords: DTNB modification, protein crystallization strategy, protein crystallization method

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A preliminary crystallographic study of CDCP2 from *Arabidopsis thaliana*

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CBS (cystathionine-beta-synthase) domain is a small intracellular module, mostly found in two or four copies within a protein, which has been identified in many divergent proteins in all kingdoms of life. Tandem pairs of CBS domains can act as binding domains for adenosine derivatives and may regulate the activity of attached enzymes or other domains. In some cases, CBS domains may act as sensors of cellular energy status by being activated by AMP and inhibited by ATP. Many proteins with CBS domain are easily detected in plant genome. However, their exact physiological functions need to be resolved. One of these, CDCP2 (CBS Domain Containing Protein 2) from *Arabidopsis thaliana* has been cloned and analyzed. It encodes 246 amino acid residues which contain two tandem CBS domains. CDCP2 protein was overexpressed heterologously in E. coli and purified it as homogeneity. As an initial step toward three-dimensional structure determination, crystals of recombinant CDCP2 protein have been obtained using hanging drop vapor diffusion methods. The crystals diffract to 2.4 Å resolution using Synchrotron sources and belong to trigonal space group, $P3(1)21$ or $P3(2)21$ with unit cell parameters of $a=b=56.12$ Å, $c=82.44$ Å, $\alpha=\beta=90^\circ$ and $\gamma=120^\circ$. To obtain more high-quality crystals of CDCP2, high-entropy side chains were removed from the surface of the protein. The new crystal form has been obtained using entropy-reduced CDCP2 protein and subsequent experiments for solving phase problems are underway.

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.

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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 (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.