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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.