Poster Sessions

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Novel approaches in protein crystallization
Lanfen Li, Wei Mi, Xiao-dong Su
Peking University, College of Life Sciences, College of Life Sciences, Peking University, Beijing, 100871, China, Beijing, Beijing, 100871, China, E-mail: lif@pku.edu.cn

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 diffraction 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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The crystal structure of lipase a from Candida Antarctica
Anna-Maria Brandt, Yvonne Nympalm-Rejstrom, Tomi Airenne, Tiina Salminen
Abo Akademi University, Department of Biochemistry and Pharmacy, Tykistokatu 6 A, Turku, -, FI20750, Finland, E-mail: anna.brandt@abo.fi

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-acetylation 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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A preliminary crystallographic study of CDCP2 from Arabidopsis thaliana
Byung-Cheon Jeong, Kyoung Shin Yoo, Jeong Sheep Shin, Hyun Kyu Song
Korea University, School of Life Sciences and Biotechnology, #408, School of Life Sciences and Biotechnology, Korea University, Anam-Dong, Seongbuk-Gu, Seoul, none, 136-701, Korea (S), E-mail: lovejbc@korea.ac.kr

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, α=β=90° and γ=120°. 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.