

structure. Determining the N-terminal ATP binding motif at the native crystal structure provides detailed understanding of the molecular mechanism employed by cob(I)alamin adenosyltransferase enzymes.

Keywords: adenosyltransferase, MgATP complex, *Bacillus cereus*

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Expression, purification and crystallization of phosphoketolase from *Lactococcus lactis*

Georgiana Petrareanu, Mihaela Camelia Balasu, Rodica Aura Badea, Stefan Szedlaczek

Institute of Biochemistry, Enzymology, Spl. Independentei, 296, Bucharest, Romania, 060031, Romania, E-mail : georgianapetrareanu@yahoo.com

Xylulose 5-phosphate (X5P) phosphoketolase (PK) is a central enzyme of the pentose phosphate pathway and in the same time, the largest representative of the thiamin diphosphate-dependent enzymes. In the presence of inorganic phosphate this enzyme converts X5P into glyceraldehyde 3-phosphate and acetyl phosphate. The *ptk* gene of PK from *Lactococcus lactis* was amplified by PCR and introduced into a prokaryotic expression vector. The enzyme was expressed as a fusion protein and purified by affinity and gel filtration chromatography. The purified protein thus obtained was electrophoretically homogeneous. The crystallization trials were performed using both hanging drop and sitting drop vapor diffusion techniques. Hanging drop procedure proved to be more efficient than sitting drop alternative procedure. Initially, crystal clusters were obtained; further optimization of protein purification as well as of crystallization conditions led to single crystals. X-ray diffractive tests on the crystals thus obtained evidenced that the crystals diffracted to approximately 3 Å. However the quality of the diffractive data still needs further improvement.

Keywords: phosphoketolase, thiamin diphosphate-dependent enzyme, protein crystallization

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Try to solve abscisic acid (ABA) receptor's structure and learn how ABA signal is transduced

Yuanyuan Li, Sheng Fu

Institute of Biophysics, Chinese Academy of Sciences, E-mail : liyy@xtal.tsinghua.edu.cn

Abscisic acid (ABA) is a vital phytohormone that regulates mainly stomatal aperture and seed development, but ABA receptors involved in these processes have yet to be determined. Our collaborator identified from broad bean an ABA-binding protein (ABAR) potentially involved in stomatal signalling, the gene for which encodes the H subunit of Mg-chelatase (CHLH), which is a key component in both chlorophyll biosynthesis and plastid-to-nucleus signalling. Here we try to solve the abscisic acid receptor's structure and try to understand the transport of ABA signal. Now we have cloned the ABAR gene to PET48b plasmid, purified ABAR and crystallized this protein.

Keywords: ABAR, seed development, CHLH

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Polymer and co-polymer surface modifying effects in the protein crystallization

Jindrich Hasek, Jan Dohnalek, Jarmila Duskova, Tereza Skalova, Petr Kolenko, Tomas Koval, Andrea Stepankova

Inst. of Macromolecular Chemistry, X-ray diffraction, UMCH AV CR, Heyrovskeho nam.2, PRAHA 6, Czech Republic, CZ-16206, Czech Republic, E-mail : hasek@imc.cas.cz

Non-covalent interactions of polymers with protein surface are observed by standard tools of protein crystallography. Basing on several hundreds of protein-polymer complexes from PDB, we classified the typical interactions, according to the dominating residue involved in the complex formation [1], the polymer fold and the position of interaction on the polymer chain. The knowledge is useful for growing crystals of better quality and for a design of the space group. It is well known that macromolecular precipitants (i.e. water soluble polymers) show much better performance than their low molecular equivalents. Also, different polymers and/or co-polymers behave differently in the process of crystallization. Our study includes polymers and co-polymers of the type A-Poly1-B-Poly2-C. Polymer conformations dissolved in crystal cavities, those deposited on the protein surface [1] and those in crystalline state (PSD [2]) differ greatly. Changing the preferences of various competitive metastable protein complexes formed temporarily on the surface of the growing crystal, one can protect deposition of incompatible protein complexes into the crystal lattice influencing thus the diffraction quality of crystals. Interaction energy profiles calculated for some model cases give a glimpse of the relative stabilities of different complexes formed on the protein surface during crystallogenesis. Review of special features of polymer adhesion on protein surface give a tool for growing crystals of better diffraction quality and for preparation of crystals in different space groups. The project is supported by GA CR IAA500500701 and GA AV 305/07/1073.

[1] Hasek J. *Zeitschrift fur Kristallogr.* (2006) 23, 613-619

[2] Hasek J. et al, *Database of polymer structures-PolyBase* (1995) CSCA Praha

Keywords: protein crystallography, polymers, precipitants

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Structural basis for CD44 recognition by ERM proteins

Tomoyuki Mori, Ken Kitano, Shin-ichi Terawaki, Ryoko Maesaki, Yayoi Fukami, Toshio Hakoshima

Nara Institute of Science and Technology (NAIST), structural biology laboratory, 8916-5 Takayama, Ikoma City, Nara, 630-0192, Japan, E-mail : t-mori@bs.naist.jp

Hyaluronan receptor CD44 is an important adhesion molecule, which regulates cell-cell and cell-matrix interactions. Increasing evidence has indicated that CD44 is assembled in a regulated manner into protein complexes at the membrane-cytoskeletal junction mediated by scaffolding proteins such as ERM (Ezrin/Radixin/Moesin) proteins, which link membrane proteins to the actin cytoskeleton. Formation of this protein complex serves to focus downstream signal transduction events that regulate cell growth and development and to play structural and regulatory roles at the polarized cell cortex. ERM proteins consist of three functional domains; an N-terminal FERM (Four point one, Ezrin, Radixin, Moesin) domain, an extended coiled-coil region, and a short C-terminal domain that binds F-actin. The