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*

P04.01.09

Acta Cryst. (2008). A**64**, C233

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

Georgiana Petrareanu, Mihaela Camelia Balasu, Rodica Aura Badea, Stefan Szedlacsek
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 diffractional tests on the crystals thus obtained evidenced that the crystals diffracted to approximately 3 Å. However the quality of the diffractional data still needs further improvement.

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

P04.01.10

Acta Cryst. (2008). A**64**, C233

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

Yuanyuan Li, Sheng Fu
Insitute 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

P04.01.11

Acta Cryst. (2008). A**64**, C233

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 crystalogenesis. 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

P04.01.12

Acta Cryst. (2008). A**64**, C233-234

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

FERM domain associates with the plasmamembrane and directly binds the juxtamembrane region of the cytoplasmic tail of CD44, whereas precise details concerning with the FERM-CD44 interaction remain unclear. Recently, we have successfully crystallized the radixin FERM domain bound to the CD44 cytoplasmic tail peptide (Mori *et al*.,2007). The structure revealed the CD44 binding to the groove between helix α1C and strand β5C of FERM subdomain C. The CD44 peptide forms a short β strand that associates with strand β5C by anti-parallel β-β interactions. In addition, the C-terminal tail of the peptide binds to the hydrophobic groove between helix α1C and strand β5C. The binding mode found in the present structure is compared with those of the previously determined structures of the FERM domain bound to ICAM-2 (Hamada *et al*.,2003) and NEP (Terawaki *et al*.,2007).

Hamada *et al*., ***EMBO J.*** **22**, 502-514 (2003).
Terawaki *et al*., ***JBC*** **282**, 19854-19862 (2007).
Mori *et al*., ***Acta Crystallogr. F*** **63**, 844-847 (2007).

Keywords: CD44, ERM proteins, FERM domain

P04.01.13

Acta Cryst. (2008). A**64**, C234

Expression, purification and crystallization of Aurora kinase C

Masami Isogai[1], Takayoshi Kinoshita[1], Tetsuko Nakaniwa[1], Asako Yamaguchi[1], Masaki Gouda[2], Koichi Yokota[2], Hiroshi Ishiguro[2], Toshiji Tada[1]
[1]Osaka Prefecture University, Graduate School of Science, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]Carna Biosciences, Inc., KIBC 511, 5-5-2 Minatojima-Minamimachi, Chuo-ku, Kobe, 650-0047, Japan, E-mail：isogai06@b.s.osakafu-u.ac.jp

The Aurora family of serine/threonine kinases plays key roles in cell division. Three Aurora kinases (Aurora A, Aurora B and Aurora C) are expressed in mammals and have highly conserved catalytic domains. Despite these similarities, they differ in function, subcellular localization, and timing of activity during cell division. Aurora A and Aurora B are present in mitosis. In contrast, the expression of Aurora C is restricted to meiosis. Although Aurora C is indicated to work as a key regulator in meiosis, its function is poorly understood. We attempted to determine the structure of Aurora C in order to obtain the insight into its function. Active-Aurora C as a C-terminal His6-tagged fusion protein was overexpressed in *E. coli* and purified by Ni-NTA column chromatography. The purity was confirmed by SDS-PAGE. Initial crystallization trials using 1-2 mg/ml of purified protein gave small crystals. Optimization of crystallization conditions for X-ray crystallography is currently in progress.

Keywords: aurora, kinase, crystallization

P04.01.14

Acta Cryst. (2008). A**64**, C234

Structural insights into the SM protein-syntaxin interactions

Shu-Hong Hu[1], Michelle Christie[1], Catherine F. Latham[1], David E. James[2], Jennifer L. Martin[1]
[1]Institute for Molecular Biosciences, QBP, Level 2, IMB, University of Queensland, Brisbane, QLD, 4072, Australia, [2]Garvan Institute of Medical Research, 384 Victoria Road, Darlinghurst, Sydney, New South Wales 2010, Australia, E-mail：s.hu@imb.uq.edu.au

The Sec1/Munc18 (SM) proteins and the soluble N-ethymaleimide-sensitive factor attachment protein receptors (SNAREs) play an essential role in vesicle docking and fusion. The SNARE complex is composed of the target membrane SNAREs (t-SNAREs Sx and SNAP) and the vesicle membrane SNARE (v-SNARE VAMP). The formation of the SNARE complex leads to membrane fusion. Three mammalian SM proteins (Munc18a, Munc18b and Munc18c) are suggested to regulate vesicle transport to the plasma membrane. Munc18a is a predominantly neuronal protein, which binds to Sx1-3. Munc18b interacts with the same Sxs as Munc18a but is expressed mainly in epithelial cells. Munc18c binds to Sx2 and Sx4, involved in insulin-regulated trafficking of glucose transporter (GLUT4). Previous structural studies showed that neuronal Munc18a binds to cognate Sx1a in a closed conformation that is incompatible with SNARE complex formation (1). We recently showed that Munc18c binds to SNARE complex as well as Sx4 alone (2). Furthermore, we found that Munc18c required the N-terminal 29 residues (N-peptide) of Sx4 for its interaction and determined the crystal structure of Munc18c bound to Sx4 N-peptide (3). Taken together, our findings suggest that the N-terminal binding mode is a conserved molecular mechanism that mediates membrane fusion.

References
1. Misura K, Scheller R, Weis W. (2000) Nature 404, 355-62.
2. Latham, C.F., Lopez, J.A., Hu., S.-H., Gee, C.L., Westbury, E., Duncan Blair, D., Armishaw, C., Alewood, P.F., Bryant, N.J., James, D.E. and Martin, J.L. (2006) Traffic 7, 1408-1419.
3. Hu, S.-H., Latham, C.F., Gee, C.L., James, D.E., and Martin, J.L. (2007) PNAS 104, 8773-8778.

Keywords: protein crystallography, vesicle trafficking, protein-protein interactions

P04.01.15

Acta Cryst. (2008). A**64**, C234

Nucleant-mediated protein crystallization with microporous zeolite showing heteroepitaxial growth

Michihiro Sugahara, Yukuhiko Asada, Yuko Morikawa, Yuichi Kageyama, Naoki Kunishima
RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：msuga@spring8.or.jp

Protein crystallization is a major bottleneck in X-ray crystallography to determine the three-dimensional structure which is indispensable for understanding the chemical mechanism of protein function. Because the current methodology of protein crystallization is a kind of screening, medically or biologically important targets such as human membrane proteins are usually difficult to be crystallized due to their poor crystallizability. The hetero-epitaxial growth from the surface of mineral crystal as a nucleant had been thought to be effective to enhance the chance of protein crystallization. However, generally applicable hetero-epitaxial nucleants for protein crystallization have never been found. Here we introduce the first candidate of universal hetero-epitaxial nucleant, microporous zeolite, which is a synthetic aluminosilicate crystalline polymer with regular micropores and generally promotes a form-selective crystal nucleation of proteins as a crystallization catalyst. The most successful zeolite nucleant was Molecular Sieves 5A with pore size of 5 Å and with bound calcium ion, suggesting that the versatility of the microporous zeolite is likely to be derived from a general regularity in proteins such as secondary structures, or the high frequency of calcium ion recognition on the protein surface.

Keywords: X-ray crystallography, protein crystallization, epitaxial growth