

of arthritis, cancer and asthma and there are correlations between expression levels and disease prognosis. However, the function of these secreted proteins is completely unknown. This project is aimed at uncovering the physiological roles of the chilectins, using a combination of structural biology and cell biological studies.

Keywords: chilectin, chitinase, arthritis

P04.01.06

Acta Cryst. (2008). A64, C232

A structural investigation in to the basis of Celiac disease

Sophie E Broughton¹, Hugh Reid¹, Kate N Henderson¹, Stuart Mannering², Zhenjun Chen³, Jason Tye-Din², Timothy Beissbarth², James McCluskey³, Robert Anderson², Jamie Rossjohn¹

¹Monash University, Biochemistry and Molecular Biology, Wellington Rd, Clayton, Victoria, 3800, Australia, ²The Walter and Eliza Hall Institute, 1G Royal Pde, Parkville, Victoria, 3050, Australia, ³Melbourne University, Grattan St, Parkville, Victoria, 3010, Australia, E-mail : sophie.broughton@med.monash.edu.au

Celiac disease (CD) is a common T cell mediated disease in which the body develops an inappropriate immune response to dietary gluten. Gluten ingestion by CD affected individuals' result in symptoms such as bloating, diarrhoea, and malabsorption. The only available treatment against these symptoms is strict adherence to a lifelong gluten free diet. Major Histocompatibility Complex (MHC) class II proteins HLA-DQ2 and/or HLA-DQ8, are critical for the development of a CD4+ T cell response towards gluten. The structures of both HLA-DQ2 and HLA-DQ8 were previously solved in complex with an antigenic gluten peptide. HLA-DQ2 was solved in complex with an α -gliadin-I gluten peptide, one of two strongly antigenic registers that overlap within an optimal peptide. The 2nd register, α -gliadin II, has been shown by functional studies to be a stronger antigen. Crystallization studies are underway to assess the functional and structural properties of this immunodominant epitope. The structure of HLA-DQ2/ α -gliadin II gluten peptide complex will aid in our understanding of CD4+ T cell recognition of gluten epitopes in CD. No structures of a TcR specific for any HLA-DQ2/DQ8-gliadin peptide have been reported. Crystals have been optimised for solution of an apo gluten specific TcR, and I am attempting to crystallize a HLA-DQ2/DQ8-peptide-TcR complex. This project aims to structurally characterise the interactions between HLA-DQ8-gluten peptide complex, and an HLA-DQ8/peptide specific TCR. The ternary complex will identify important contact residues in the peptide that allow TcR recognition and hence may aid in the development of new treatments (e.g. peptide vaccine), and provide significant insight into the structural mechanism that results in the activation of CD4+ T cells in CD.

Keywords: MHC proteins, T cell receptor, X-ray crystallography of proteins

P04.01.07

Acta Cryst. (2008). A64, C232

Purification, crystallization and preliminary X-ray analysis of photosystem II dimer from a red alga

Hideyuki Adachi¹, Yasufumi Umena², Isao Enami³, Nobuo Kamiya², Jian-Ren Shen¹

¹Okayama University, Division of Biosciences, Graduate School of

Natural Science and Technology, Tsushima-Naka, 3-1-1, Okayama, Okayama, 700-8530, Japan, ²Graduate School of Science, Osaka City University, 3-3-138, Sugimoto, Sumiyoshi, Osaka 558-8585, Japan, ³Department of Biology, Tokyo University of Science, Kagurazaka 1-3, Shinjuku-ku, Tokyo, 162-8601 Japan, E-mail: pwyfw306@yahoo.co.jp

Photosystem II (PSII) is a membrane protein complex performing light-induced electron transfer and water-splitting reactions, leading to the evolution of molecular oxygen which is required for all oxygenic life on the earth. The central part of photosystem II is highly conserved from prokaryotic cyanobacteria to eukaryotes; however, there are some apparent differences in the extrinsic proteins involved in oxygen evolution among different organisms. So far, the crystal structure of PSII from cyanobacteria has been reported, whereas no reports have been published on the structure of any eukaryotic PSII. Red alga is one of the eukaryotic algae, and its PSII differs from that of cyanobacteria in that the former contains a 20 kDa protein, a unique, fourth extrinsic protein. In order to elucidate the structure of red algal PSII and its differences with cyanobacterial PSII, we purified and crystallized PSII from an acidophilic, thermophilic red alga *Cyanidium caldarium*. In order to obtain pure PSII dimer suitable for crystallization from the red alga, we improved the purification procedure published previously, which yielded a highly purified PSII dimer preparation with high oxygen-evolving activities comparable with that of thermophilic cyanobacterial PSII. We succeeded in the crystallization of red algal PSII dimer and obtained two types of crystals with different space group crystals. One type of the crystal had a space group of $P222_1$ with unit cell dimensions of $a = 146.8\text{\AA}$, $b = 176.9\text{\AA}$, $c = 353.7\text{\AA}$. Another type of the crystal had a space group $P2_12_12_1$ with unit cell dimensions of $a = 209.2\text{\AA}$, $b = 237.5\text{\AA}$, $c = 299.8\text{\AA}$. Multiple data sets of native crystals have been collected and processed to 3.7\AA , which may enable us to resolve the structure of red algal PSII.

Keywords: photosynthesis, membrane proteins, crystallization

P04.01.08

Acta Cryst. (2008). A64, C232-233

Structural analysis of ATP:Cob(I)alamin adenosyltransferase

Ae-kyung Park, Jin-Ho Moon, Eun-Hyuk Jang, Young-Min Chi
Korea University, College of life sciences & biotechnology, Korea University, Seoul, Korea, 136-713, Seoul, 136-713, Korea (S), E-mail: wc_2002@korea.ac.kr

B12 is an important nutrient for maintaining life in all animals, lower eukaryotes, and prokaryotes, but is only synthesized by a prokaryotes. In mammalian, B12 should be converted to two biologically active cofactors, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), and these cofactors are used by enzymes, the functions of which are needed for the Acetyl-CoA synthesis, methyl transfer, ribonucleotide reduction, fermentation process, and methionine synthesis. Because B12 synthesis is limited within the micro-organisms, the adenosyltransferases that are able to transfer the 5'-deoxyadenosyl moiety from ATP to the cobalt atom of cob(I)alamin is needed to construct the complete biosynthetic pathway of AdoCbl in higher order organisms. Until now, two crystal structures of PduO enzyme with Mg-ATP are available in the RCSB protein data bank (2nt8, 2idx), and these structures indicates that the enzyme is a trimer and each subunit consists of five helix-bundle. Further, co-crystal structure with Mg-ATP reveals that unseen ATP binding motif at the N-terminal of the protein in native crystal structure is visible in substrate-protein complex crystal

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). A64, C233

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

P04.01.10

Acta Cryst. (2008). A64, C233

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

P04.01.11

Acta Cryst. (2008). A64, 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 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

P04.01.12

Acta Cryst. (2008). A64, 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