surface identified by NMR was flat and included a strip of three solvent-exposed Trp residues flanked by acidic residues. The overall structure of the AD2 was a TIM-barrel fold, which is a common fold in family 18 chitinases. The active site of the AD2 was in the groove-like cleft and was open to the solvent due to the lack of an additional small domain, which is observed in other family 18 chitinases.

Keywords: catalysts, enzyme structure, domains

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Successful expression of archaeal STT3/AglB membrane protein in *E.coli* cells

Mayumi Igura, Nobuo Maita, Jun Kamishikiryou, James Nyirenda, Katsumi Maenaka, Daisuke Kohda
Medical Institute of Bioregulation, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka city, Fukuoka, 812-8582, Japan, E-mail : nmigura@bioreg.kyushu-u.ac.jp

Oligosaccharyltransferase (OST) is an enzyme that catalyzes the transfer of the oligosaccharide from a lipid donor to the side chain of an Asn residue within a consensus sequence of Asn-X-Thr/Ser, where X can be any amino acid residue except for Pro. Asn-glycosylation is widespread not only in eukaryotes but also in archaea and some eubacteria. OST catalyzes the co-translational transfer of an oligosaccharide to Asn residues in nascent polypeptide chains. The OST enzyme is a membrane-associated multisubunit protein complex in eukaryotes. STT3 is the most conserved subunit in the OST complex in the three domains of life. The STT3 is called AglB for archaea and PglB for bacteria. All STT3/AglB/PglB proteins are characterized by 11-13 transmembrane helices in the N-terminal half of the amino acid sequence and a globular domain in the C-terminal half on the luminal/out side of the membranes. We reported that the OST of *Pyrococcus furiosus*, a thermophilic archaeon, is composed of the STT3 protein alone, and catalyzes the transfer of a heptasaccharide onto peptides in an Asn-X-Thr/Ser-motif-dependent manner[1]. We determined the crystal structure of the C-terminal soluble domain of *P. furiosus* STT3 (PfSTT3) [1,2]. Here, we expressed the full-length PfSTT3 in *E. coli*, and found that the membrane fraction of *E. coli* cells had the OST activity. We then optimized *E. coli* strains and culture conditions to minimize the degradation of the full-length PfSTT3, and succeeded to obtain homogenous PfSTT3 after several purification steps, including heat treatment and His-tag affinity chromatography. The PfSTT3 is estimated to be monomeric from the elution volume of gel filtration in the presence of various detergents.

1) EMBO J., **27**, 234-43 (2008)
2) Acta Cryst., **F63**, 798-801(2007)

Keywords: membrane proteins, purification, quaternary association of proteins

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X-ray structure of HIV-1 protease-product peptides complex: Insights into the reaction mechanism

Vishal Prashar[1], Smita Mahale[2], Jean-Luc Ferrer[3], Madhusoodan Hosur[1]
[1]Bhabha Atomic Research Centre, Solid State Physics Division, V.N.Purav Marg, Trombay, Mumbai, Maharashtra, 400085, India, [2]National Institute for Research in Reproductive Health, Parel, Mumbai, India, [3]Institut de Biologie Structurale (IBS), CEA/CNRS/UJF, Grenoble Cedex 1, France, E-mail : vishalp@barc.gov.in

Inhibitors based on the correct mechanism are likely to provide an alternate set of drugs against AIDS. To investigate the mechanism of HIV-1 protease, we have undertaken crystal structure analyses of HIV-1 protease complexed with substrate oligo-peptides, corresponding in sequence to natural cleavage sites of this enzyme. The crystalline complexes were prepared by soaking hexagonal crystals of unliganded enzyme in solutions of substrate peptides under different conditions of pH. We have earlier reported structure of HIV-1 protease complexed with the oligopeptide HKARVL*nFEAnLS ('*' is the site of cleavage, nF-nitrophenylalanine and nL is nor-Leucine), which was found to be trapped as a tetrahedral intermediate. The present study has been carried out on a complex with the same oligopeptide, but at a pH value of 6.2 as against the value of 2.0 used in the earlier study. X-ray diffraction data to 1.8Å resolution has been collected on the FIP beamline at ESRF. The crystal structure, solved using difference Fourier methods, has been refined to crystallographic *Rw/Rf* values of 21.3% / 25.5%, to 1.8Å resolution. In the refined structure, the substrate peptide is found cleaved, and the product peptides are not yet fully dissociated from the enzyme active site. On the basis of comparison of structures of the present product complex with those of Michaelis complex and tetrahedral intermediate complex, a mechanism is proposed for the action of HIV-1 protease. An important and novel feature of this mechanism is invocation of bifurcated hydrogen bonds as a means to influence proton migration rates during different steps of the cleavage reaction.

Keywords: HIV-1 protease, X-ray crystallography, reaction mechanism

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Crystal structure of *Helicobacter pylori* spermidine synthase suggests a distinct active site

Yuh-Ju Sun[1], Po-Kai Lu[1], Jia-Yin Tsai[1], Chen-Hsi Chu[1], Hsiang-Yi Chien[1], Haimei Huang[2]
[1]Institute of Bioinformatics and Structural Biology, Department of Life Science, 101, Section 2 Kuang Fu Road, Hsinchu, Taiwan 30013, Republic of China, Hsinchu, Taiwan, 30013, Taiwan, [2]Institute of Biotechenology, 101, Section 2 Kuang Fu Road, Hsinchu, Taiwan 30013, Republic of China, Hsinchu, Taiwan, 30013, Taiwan, E-mail : yjsun@life.nthu.edu.tw

Spermidine synthase (putrescine aminopropyltransferase, PAPT) catalyzes the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine during spermidine biosynthesis. *Helicobacter pylori* PAPT (HpPAPT) has a low sequence identity with other PAPTs and lacks the signature sequence found in other PAPTs. The crystal structure of HpPAPT, determined by multiwavelength anomalous dispersion, revealed an N-terminal β-stranded domain and a C-terminal Rossmann-like domain. Structural comparison with other PAPTs showed that HpPAPT has a unique binding pocket between two domains, numerous non-conserved residues, a less acidic electrostatic surface potential, and a large buried space within the structure. HpPAPT lacks the gatekeeping loop which facilitates substrate binding in other PAPTs. PAPTs are essential for bacterial cell viability; thus, HpPAPT may be a potential antimicrobial drug target for *H. pylori* due to its characteristic PAPT sequence and distinct conformation.

Keywords: *Helicobacter pylori*, spermidine synthase,

rossmann-like domain

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Crystallographic studies of ferredoxin-NAD(P)+ reductase from *Chlorobium tepidum*

Tomoo Shiba[1], Norifumi Muraki[1], Daisuke Seo[2], Takeshi Sakurai[2], Genji Kurisu[1]
[1]The University of Tokyo, Department of Life Sciences, Komaba 3-8-1, Meguro-ku, Tokyo, 153-8902, Japan, [2]Kanazawa University, Kakuma, Kanazawa 920-1192, Japan, E-mail：tshiba@xtal.c.u-tokyo.ac.jp

Ferredoxin-NAD(P)$^+$ reductase (FNR) is a key enzyme that catalyzes the photoreduction of NAD(P)$^+$ to generate NAD(P)H during the final step of the photosynthetic electron-transport chain. FNR from the green sulfur bacterium *Chlorobium tepidum* is a homodimeric enzyme with a molecular weight of 90 kDa; it shares a high level of amino-acid sequence identity to thioredoxin reductase rather than to conventional plant-type FNRs. In order to understand the structural basis of the ferredoxin-dependency of this unique photosynthetic FNR, *C. tepidum* FNR has been heterologously expressed, purified and crystallized. The *C. tepidum* FNR was crystallized in two forms, I and II, from screening conditions consisting of 20% PEG 3350 containing 200 mM ammonium sulfate or diammonium tartrate as precipitant. Form I crystals belong to the orthorhombic space group $C222_1$, with unit cell parameters a = 100.5, b = 128.0, c = 128.4 Å. Assuming the presence of one dimer in the asymmetric unit, the Matthews coefficient (V_M) is 2.6 Å^3/Da corresponding to a solvent content of 53.2%. Form II crystals belong to the tetragonal space group $P4_122$ or $P4_322$, with unit-cell parameters a = b = 82.0, c = 162.7 Å. The V_M value of 3.5 Å^3/Da indicates that the form II crystal contains one protein molecule per asymmetric unit. Diffraction data were collected from a form I crystal to 2.4 Å resolution on the synchrotron-radiation beamline NW12 at the Photon Factory.

Keywords: FNR, ferredoxin, thioredoxin reductase

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The crystal structure of the staphylococcal amidase AmiE reveals the active site of a metalloenzyme

Sebastian Zoll[1], Bernhard Paetzold[1], Martin Schlag[2], Friedrich Goetz[2], Thilo Stehle[1,3]
[1]Interfaculty Institute for Biochemistry, AG Stehle, Hoppe-Seyler-Strasse 4, Tuebingen, Baden-Wuerttemberg, 72076, Germany, [2]Department of Microbial Genetics, Faculty of Biology, University of Tuebingen, Waldhaeuser Strasse 70/8, 72076 Tuebingen, Germany, [3]Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA, E-mail：sebastian.zoll@uni-tuebingen.de

Staphylococci are gram-positive bacteria that mainly colonise human skin and the upper respiratory tract. While most staphylococcal infections are averted by a working immune system, they can be life threatening in immunocompromised hosts. Among the most common diseases are endocarditis, pneumonia and the toxic shock syndrome. The ability of staphylococci to form biofilms upon attachment to polystyrene surfaces is another pathogenic factor and especially relevant in transplantation of medical prostheses. Peptidoglycan (PGN) hydrolases such as the major autolysin AtlE from Staphylococcus epidermidis play an important role in cell wall turnover, which renders them appealing targets for drug design. During cell division AtlE splits the equatorial septum, thus allowing the formation of daughter cells. Deletion mutants exhibit a severely disordered division pattern and are biofilm-negative. We have determined the crystal structure of the catalytic domain AmiE, a N-acetylmuramyl-L-alanine amidase at 1.7Å resolution. The protein adopts a globular fold, with several α-helices surrounding a central β-sheet. Alignments with homologous proteins revealed a conserved surface cleft, which is capable of incorporating a PGN-like ligand. A divalent cation is bound in the active site and likely participates in catalysis. Analysis of the architecture of the binding site and the location of key residues allow us to postulate a mechanism of function, which is likely to be that of a metalloenzyme. Mutations of amino acids directly involved in catalysis resulted in severe changes of adjacent loops and a loss of activity. The high-resolution structure of AmiE advances our understanding in terms of ligand binding and enzymatic function, thus providing an excellent base for future drug design.

Keywords: AmiE, amidase, peptidoglycan

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Structure of the inactive mutant of arabinanase complexed with oligosaccharides

Satomi Fukuoka[1], Yuri Sogabe[1], Asako Yamaguchi[1], Takayoshi Kinoshita[1], Takuo Sakai[2], Toshiji Tada[1]
[1]Osaka Prefecture University, Graduate School of Science, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, [2]IGA Bioresearch, Sakai, Osaka, Japan, E-mail：fukuoka07@b.s.osakafu-u.ac.jp

The three-dimensional structure of the thermostable endo-α-1,5-L-arabinanase, ABN-TS, from a strain of *Bacillus thermodinitrificans* TS-3, has been determined at 1.9 Å resolution. We have started the structure analysis of the inactive mutant of ABN-TS complexed with oligosaccharides to elucidate the substrate recognition and reaction mechanism of the emzyme. ABN-TS and the inactive mutant D27A were expressed in *E.coli* as His-tag fusion proteins at their C-termini. They were purified by Ni-affinity, anion-exchange, and size-exclusion chromatographic techniques. The activity of the purified WT (ABN-TS with His-tag) was assayed using debranched arabinan as a substrate by the Somogyi-Nelson method. The WT showed the same catalytic activity as the native ABN-TS, while the mutant D27A showed only very weak activity. The crystals of the inactive mutant complexed with oligosaccharides were prepared by co-crystallization and soaking methods using PEG8000 as a precipitant. Data collection and structure analysis are now in progress.

Keywords: arabinanase, glycoside hydrolase, crystal structure analysis

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Structure of endo-1,5- α -L-arabinanase from *Penicillium chrysogenum*

Mika Akiyoshi, Kyoko Ikoma, Asako Yamaguchi, Tetsuko Nakaniwa, Takayoshi Kinoshita, Tatsuji Sakamoto, Toshiji Tada
Osaka Prefecture University, Graduate School of Science, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, E-mail：akiyoshi07@b.s.osakafu-u.ac.jp