MS03 P01

Three Structural Snapshots Of The FPPS Catalytic Cycle Revealed By X-Ray Analyses Jean-Michel Rondeau,[a] Francis Bitsch,[a] Emmanuelle Bourgier,[a] Martin Geiser,[a] René Hemmig,[a] Markus Kroemer,[a] Sylvie Lehmann,[a] Paul Ramage,[a] Sébastien Rieffel,[a] André Strauss,[a] Jonathan R. Green[b] and Wolfgang Jahnke,[a] [a]*Discovery Technologies and* [b]*Musculoskeletal Diseases, Novartis Institutes for Biomedical Research, CH-4002, Basel, Switzerland.*
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Keywords: structural enzymology, drug mechanism, activity and mechanism of enzymes

Farnesyl diphosphate synthase (FPPS) is now well established as the molecular target of nitrogen-containing bisphosphonate (N-BP) drugs [1-3]. We have determined the X-ray structures of human FPPS in its unliganded state, in complex with the N-BP drug zoledronic acid, and in the ternary complex with zoledronic acid and the substrate isopentenyl diphosphate (IPP). By revealing three structural snapshots of the enzyme catalytic cycle, each associated with a distinct conformational state, these structures provide a novel understanding of the mechanism of FPPS catalysis and inhibition. In particular, the accumulating substrate, IPP, was found to bind to and stabilize the FPPS/N-BP complex, rather than competing with and displacing the N-BP inhibitor. Among other factors such as efficient bone targeting, this particular mode of FPPS inhibition contributes to the exceptional in vivo efficacy of N-BP drugs.

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MS03 P02

Crystal Structure of α-Carbonic Anhydrase from *Chlamydomonas reinhardtii* Kaoru Suzuki[a*], Satoru Shimizu[b], Risa Ohbayashi[a], Yoshiteru Sato[b], Akio Takénaka[b] ,Takeshi Sekiguchi[a] and Shi-Yuan Yang[a] [a]*College of Science and Engineering, Iwaki Meisei University.* [b]*Grd. School Biosci. Biotech., Tokyo Institute of Tech., Japan.* E-mail: kaoru@iwakimu.ac.jp

Keywords: carbonic anhydrase, crystal structure, *Chlamydomonas*

Carbonic anhydrase (CA) plays important roles in biological processes such as photosynthesis, respiration, secretion of HCO_3^-, pH homeostasis and ion exchange. The proteins commonly contain a zinc ion in the active site for catalyzing the hydration of CO_2 and *vice versa*. It is known that there are three classes of CA, designated α-, β- and γ–CAs, depending on the amino acid sequence similarities. The α-class is different from others in the structural architecture. Furthermore, even in the α-class, the enzyme from unicellular green alga, *Chlamydomonas reinhardtii* (*ch*CA) is unique in post-translational modifications that it is glycosylated and spliced into two peptides. Such glycosylations are found in only mammalian CAs but they are not spliced. To reveal the structural details and the role of N-glycosylation, an X-ray analysis of *ch*CA has been performed.
The wild type *ch*CA was isolated and purified, and then crystallized by the hanging drop vapor diffusion method. X-Ray diffraction data were collected at 100K with synchrotron radiation. The initial structure was determined by the MAD method of zinc atoms, and the atomic parameters including water molecules were refined to R=18.3 (R_{free}=21.9) % at 1.88 Å resolution.
*ch*CA is a homodimeric protein, the two subunits being crystallographically independent. In each subunit, residues from Ser298 to Asn345 are spliced to separate into long and short peptides. The two subunits are, however, linked together by a disulfide bond. In the catalytic site, a zinc ion is bound to the three conserved His163, His165 and His182 in a tetrahedral configuration. A water molecule is trapped at the fourth position of the Zn atom.
As seen in the figures, the electron density maps indicate that N-glycosylations occur at the three sites, Asn101, Asn135 and Asn297. A sequence comparison with other organisms shows that the first and the third N-glycosylations are conserved in mammalian CAs. The present structure is the first example of CA attached to N-glycosides. The additional N-glycosylation at the second site of Asn135 is also unique in chCA.
In the crystal, *ch*CA molecules are interacted to each other with a six-fold screw symmetry to form a long column. Furthermore, these columns are fused through the lateral interactions like a beehive. Each catalytic site is exposed to the central tunnel. It suggests that *ch*CA in the crystalline state also catalyze the reaction.

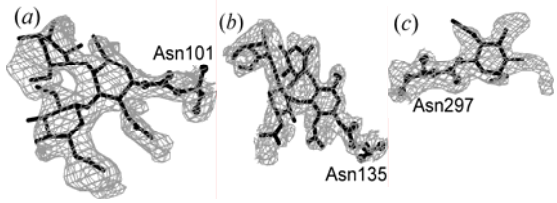

Figure. $2|F_o|-|F_c|$ maps contoured at 1□ level for N-glycosylation sites at Asn101(*a*), at Asn135(*b*) and at Asn297(*c*).

MS03 P03

Structural studies of Holliday junction resolvases from yeast and archaea Imre Törő[a], Carlo Carolis[a], Jérôme Basquin[a], Claude Sauter[b], Eric Ennifar[b], Dietrich Suck[a], [a]Structural *an Computational Biology Unit, EMBL, Heidelberg, Germany.* [b]*Institut de Biologie Moléculaire et Cellulaire, Institut Fédératif de Recherche du CNRS, Strasbourg, France.* E-mail: toro@embl.de

Keywords: Holliday junction resolvases, crystal structure, protein-DNA interactions

Holliday junctions (four-way junctions) are universal intermediates in repair and reorganization of DNA by homologous recombination. They are mobile links between two homologous DNA duplexes and generate new segments of heteroduplex DNA by branch-migration. A crucial step is the final resolution of the junction without loss of nucleotides. Holliday junction-resolving enzymes mediate the termination process by recognizing DNA four-way junctions and introducing symmetrical

nicks. Members of this ubiquitous family of structure-specific endonucleases function as dimers and require divalent cations for cleavage. We have solved the crystal structures of cruciform cutting enzyme 1 (CCE1) from *Candida glabrata* and Holliday junction cutting enzyme (Hjc) from the hyperthermophile archaeon *Archaeoglobus fulgidus* [1] at 3 and 1.7Å resolution, respectively. They represent two structurally distinct resolvase families with the same biological function, but exhibiting clearly different substrate specificities. Co-crystallization of these proteins with selected DNA substrates is under way.

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MS03 P04

The Protease State of the Heat-Shock Protein DegP from Escherichia coli Tobias Krojer, Justyna Sawa, Fuhrmann Jakob, Gazda Linn, Kirk Rebecca, Kurt Juliane, Nussbaumer Barbara, Schmidt Bastian and Tim Clausen, *Research Institute of Molecular Pathology (IMP), Dr. Bohrgasse 7, A-1030, Vienna, Austria.*
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Keywords: DegP, protease, chaperone

DegP (a.k.a. HtrA, protease Do) is a heat-shock protein which is localized in the periplasm of *Escherichia coli*. It is an essential, ATP-independent protein quality control factor and represents the only known protein that can alternate between the two antagonistic activities of a **protease** and a **chaperone** in a temperature-dependent manner [1]. The mature protein consist of a chymotrypsin-like serine protease domain and two consecutive, C-terminal PDZ domains. DegP is present as a hexamer in solution and belongs to the diverse family of cage-forming proteases. The active sites are shielded from the environment in an inner cavity, to prevent the unwanted degradation of functional proteins. The crystal structure of the chaperone conformation of DegP revealed that substrate degradation at low temperatures is additionally abolished by a distorted and blocked active site [2].
Here we present the crystal structure of DegP in complex with the small molecule inhibitor diisopropyl fluorophosphates (DFP). DFP did not lock the active sites in the canonical conformation of chymotrypsin-like serine proteases. The inhibitor is rather bound in an atypical conformation, which was never observed before in serine proteases, again underlining the remarkable flexibility of the active site. Furthermore, time-dependent analysis of degradation products by HPLC chromatography revealed that DegP acts as a processive protease, similar to other cage-forming proteases like the proteasome or ClpP. Analysis of degradation products of DegP by mass spectrometry confirmed that DegP has a clear preference for aliphatic residues in the P1 site. Furthermore, the product spectrum shows a normal distribution with a mean peptide length of 13 to 15 residues.
In summary, our results suggest that the proteolytic active sites of DegP exhibit an unusual degree of flexibility, which may be necessary to allow the efficient degradation of a wide variety of different, misfolded proteins before those become harmful for the cell. The observed product length distribution and the processive degradation of substrate proteins refer to yet unidentified trans-acting determinants which are essential for substrate binding and degradation.

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MS03 P05

Identity of Ions in the Second Site of Subtilisins
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Keywords: subtilisin, serine protease, calcium binding

Serine proteases classified under the EC number 3.4.21.62 – subtilisins - have been extensively studied by x-ray crystallography with focus on the relationship between metal binding and stability. These α/β hydrolases with broad specificity typically bind one or more calcium ions and in some cases other, mostly monovalent, ions [1]. Subtilisins BPN´/NOVO, Carlsberg or Savinase were studied systematically as for their stability related to metal binding since 1969. Two typical sites - the strong calcium binding site (Ca-I, formerly named site A) and the weak calcium binding site (Ca-II, formerly named site B) were identified. Stability of subtilisin BPN' depends on the presence of site Ca-I whereas the stabilization effect of site Ca-II depends on the concentration of ions in the solvent [2]. Site Ca-II is accompanied by a closely related site Na-II only 2.6 Å away, with high affinity for sodium. Our detailed analysis of the contents of sites Ca-II and Na-II as present in the published structures leads to the conclusion that in some cases their contents were misinterpreted. We have reclassified the typical metal binding sites of subtilisins and subtilisin-like proteases and proposed the most likely identities of the ions and atoms bound in sites Ca-II and Na-II in the publicly available structures.
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MS03 P06

The Enolase in the Methionine Salvage Pathway: Crystal Structure and Function. A.Fedorov, H.Imker, E.Fedorov, J.Gerlt, S. Almo. *Department of Biochemistry, AECOM, New York,USA.* E-mail:fedorov@aecom.yu.edu

Keywords:Enolase, RuBisCO, Crystal structure

D-Ribulose1,5-biphosphate carboxylase/oxygenase (RuBisCO), the most abundant enzyme, is the paradigm member of the recently recognized mechanistically diverse RuBisCO superfamily. The heterofunctional homologue of RuBisCO found in Geobacillus kaustophilus catalyze the enolization of 2,3-diketo-5-methylthiopentane 1-phosphate in the methionine salvage pathway. Because the RuBisCO and the enolase – catalyzed reactions differ we sought to