FA1-MS13-P04

Structure and Assembly of a PLP Dependent Dodecameric L-aspartate β-decarboxylase. Hui-Ju Chen[a,b], Tzu-Ping Ko[a], Chia-Yin Lee[c], Nai-Chen Wang[a], Andrew H.-J. Wang[a,b]. [a]*Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan.* [b]*Institute of Biochemical Science , National Taiwan University, Taipei 10617, Taiwan.* [c]*Department of Agricultural Chemistry, National Taiwan University, Taipei 10617, Taiwan.*
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The type-I PLP enzyme L-aspartate β-decarboxylase (ASD) converts L-aspartate to L-alanine and CO_2, which also has minor aminotransferase (AT) activity. Similar to the homodimeric AT, the protein subunit of ASD comprises a large and a small domain, of 410 and 120 residues, respectively. The crystal structure reveals a dodecamer made of six identical dimers arranged in a truncated tetrahedron whose assembly involves tetramer and hexamer as intermediates. Helical motifs I and II, which is not seen in AT, participate in the oligomer formation of ASD. Triple mutations of S67R/Y68R/M69R or S67E/Y68E/M69E in motif I produced an inactive dimer, attesting that the dodecameric structure is essential to the enzyme's function. The cofactor PLP is bound covalently to Lys315 in the active site, while its phosphate group interacts with a neighboring Tyr134. Removal of the bulky side chain of Arg37, which overhangs the PLP group, improved the substrate affinity. Mutations in flexible regions produced the more active K17A and the completely inactive R487A. Lys17 is located in a flexible N-terminal region, where conformation changes are likely to facilitate substrate entrance to the active site. The role played by Arg487 and is not entirely clear, although the structure suggests that substrate binding may trigger essential conformational changes of its associated loop for catalysis. ASD has been implemented in facilitating separation of D-aspartate, which is highly demanded in manufacturing of many antibiotics, from the D,L-mixture produced by reacting fumaric acid with ammonia. The second product L-alanine is also useful, for example, in food industry.

Chen HJ, Ko TP, Lee CY, Wang NC, Wang AHJ., **2009**, *Structure*, 17, 1-13.

Keywords : aminotransferase; tetrahedron; oligomer

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Crystal Structure of *Staphylococcus Aureus* Phosphopantetheine Adenylyltransferase in Complex with 3'-phosphoadenosine 5' -phosphosulfate (PAPS) Reveals a New Ligand Binding Mode. Hye-Jin Yoon[a], Hyung Ho Lee[a], Ji Hyeon Park[a], Se Won Suh[a]. [a]*Department of Chemistry, Seoul National University, Korea.*
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Coenzyme A (CoA) is an essential cofactor in numerous biosynthetic, degradative, and energy-yielding metabolic pathways and is required in several key reactions in intermediary metabolism. It is synthesized in five steps from pantothenate (vitamin B5), cysteine, and ATP. Phosphopantetheine adenylyltransferase (PPAT), a member of the nucleotidyltransferase superfamily, catalyzes the penultimate step in this biosynthetic pathway. That is, it catalyzes reversible transfer of an adenylyl group from ATP to 4'-phosphopantetheine (Ppant), yielding dephospho-CoA (dPCoA) and pyrophosphate. Bacterial PPAT and mammalian PPAT are highly dissimilar, thus making the bacterial enzyme an attractive target for antibacterial discovery. Previous structural studies of PPAT revealed how the PPAT enzyme recognizes several ligands. ATP, ADP, Ppant, and dPCoA bind to the same binding site in highly similar manners, while the mode and site of CoA binding is somewhat different. To aid structure-based discovery of new antibacterial compounds against major human pathogens such as *S. aureus*, detailed structural information on the binding modes of different ligands to the PPAT active site is desirable. In this study, we have solved the crystal structure of *S. aureus* PPAT as a binary complex with 3'-phosphoadenosine 5'-phosphosulfate (PAPS), representing the first such complex of any PPAT with PAPS. The PAPS binding site overlaps with that of ATP but their binding modes are distinct from each other. Therefore, this study provides additional structural information for structure-based design of PPAT inhibitors as a potential antibacterial agent.

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Keywords: PPAT; crystallization; structure

FA1-MS13-P06

Single-stranded DNA-binding Protein Complex from *Helicobacter Pylori.* Yuh-Ju Sun[a], Kun-Wei Chan[a]. [a]*Institute of Bioinformatics and Structural Biology, Tsing Hua University, Hsinchu 300, Taiwan.*
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Single-stranded DNA-binding protein (SSB) plays an important role in DNA replication, recombination, and repair. SSB consists of an N-terminal single-stranded DNA-binding domain with an oligonucleotide/oligosaccharide binding fold and a flexible C-terminal tail involved in protein-protein interactions. SSB from *Helicobacter pylori* (*Hp*SSB) was isolated, and the ssDNA-binding characteristics of *Hp*SSB were analyzed by fluorescence titration and electrophoretic mobility shift assay. The crystal structure of the C-terminally truncated protein (*Hp*SSBc) in complex with 35-mer single-stranded DNA [*Hp*SSBc-$(dT)_{35}$] was determined at a resolution of 2.3 Å. The *Hp*SSBc monomer folds as an OB-fold with a Y-shaped conformation. The ssDNA wrapped around the *Hp*SSBc tetramer through a continuous binding path comprising five essential aromatic residues and a positively charged surface formed by numerous basic residues.

Keywords: *helicobacter pylori*; oligonucleotide binding fold; single-stranded DNA binding protein

FA1-MS13-P07

Structural Basis for Novel Interactions Between Human TLS Polymerases and PCNA. Asami Hishiki[a], Hiroshi Hashimoto[a], Tomo Hanafusa[b], Keijiro Kamei[b], Eiji Ohashi[b], Toshiyuki Shimizu[a], Haruo Ohmori[b], Mamoru Sato[a]. [a]*Graduate School of Nanobioscience/Yokohama City University/ Yokohama-Japan.* [b]*Institute for Virus Research/Kyoto University/Kyoto-Japan.*
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Genomic DNA carrying genetic information is constantly damaged by various internal and external agents. Most types of DNA damage are removed by multiple DNA repair mechanisms, but some of them, especially those generating relatively small distortion of the DNA double helix structure, may escape DNA repair and persist in S-phase. When a replicative DNA polymerase encounters such a persisting lesion, it often stalls there. Translesion synthesis (TLS) is a DNA damage tolerance mechanism that allows continued DNA synthesis, even in the presence of damaged DNA templates. Mammals have multiple DNA polymerases specialized for TLS (TLS polymerases), including Pol-eta, Pol-iota, and Pol-kappa. These enzymes show preferential bypass for different lesions. Proliferating cell nuclear antigen (PCNA), which is a ring-shaped homo-trimeric protein and functions as a sliding clamp for the replicative polymerase Pol-delta, also interacts with the three TLS polymerases. Although many PCNA-binding proteins have a highly conserved sequence termed the PCNA-interacting protein box (PIP-box), Pol-eta, Pol-iota, and Pol-kappa have a non-canonical PIP-box sequence. In response to DNA damage, Lys164 of PCNA undergoes mono-ubiquitination by the RAD6-RAD18 complex, and the ubiquitination is considered to facilitate TLS. Consistent with this, these three TLS polymerases have one or two ubiquitin binding domains (UBDs) and are recruited to replication forks via interactions with ubiquitinated PCNA involving the non-canonical PIP-box and UBD. However, it is unclear how these TLS polymerases interact with PCNA. To address the structural basis for interactions between different TLS polymerases and PCNA, we determined the first crystal structures of PCNA bound to peptides containing the non-canonical PIP-box of these polymerases. Crystal structures reveal that the non-canonical PIP-boxes of Pol-eta, Pol-iota and Pol-kappa interact with PCNA differently from one another, explaining why Pol-eta, Pol-iota, and Pol-kappa have a lower affinity for PCNA than replicative polymerase with a canonical PIP-box. Our results also provide that the PIP-box of Pol-kappa has much lower affinity for PCNA than those of Pol-eta and Pol-iota. Furthermore, mutational and structural analyses reveal that the PIP-box sequence of Pol-iota differs from that previously assigned by one residue and that it has a very novel structure with multiple intra-molecular interactions. The revised alignment based on our structures indicates that acidic residues are conserved in the non-canonical PIP-boxes of Pol-eta, Pol-iota, and Pol-kappa, and those form ion-pairs with His44 of PCNA. These structures enable us to speculate how these TLS polymerases interact with Lys164-mono-ubiquitinated PCNA. We discuss how the different interactions between PCNA and the non-canonical PIP-boxes of the three TLS polymerases correlate with interactions between Lys164-mono-ubiquitinated PCNA and the UBDs of these polymerases.

Keywords: crystal structure analysis; protein complex structure; structural biology of DNA replication

FA1-MS13-P08

Structural Characterization for the Nucleotide Binding Ability of Subunit A of the A1AO ATP Synthase. Malathy Sony Subramanian Manimekalai[a], Anil Kumar[a], Asha Manikkoth Balakrishna[a], Gerhard Grüber[a]. [a]*Nanyang Technological University, School of Biological Sciences, 60 Nanyang Drive, Singapore 637551, Republic of Singapore.*
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Archaeal ATP synthases are energy providing machines and this multi-subunit complex is composed of two domains: a water soluble A_1 domain containing the catalytic site contributed by subunits A and B as well as the central stalk subunits and the membrane bound A_O domain involved in ion translocation. The stoichiometry of the entire complex is proposed to be A_3:B_3C:D:E_2:F:H_2:a:c_x [1]. The ATP synthesis is carried out in the A_3:B_3 hexamer of the A_1 domain. Subunit A has been regarded as having catalytic function while subunit B has nuclotide binding and/or regulatory function [2,3]. In our attempt to understand the nucleotide binding ability of A-subunit, we have co-crystallized the catalytic A subunit from *Pyrococcus horikoshii* OT3 with various nucleotides and its analogue Mg-AMPPNP (5'-adenylyl-β,γ-imidodiphosphate) with defined parameters. The structures of nucleotide bound subunit A were determined to 2.6 Å for Phosphate and 2.4 Å resolutions for AMPPNP and ADP (Adenosine diphosphate). All the nucleotides are found to bind to the P-loop region ($_{234}$GPFGSGKT$_{241}$) with notable conformational difference in the side chains of residues S238, K240 and T241 upon nucleotide binding. Comparison of the P-loop sequence with the catalytic β-subunit (GGAGVGKT) of the related F_1F_0 ATP synthases reveled a significant replacement, the polar Ser238 to the non-polar Val residue. In order to understand this significance, a S238A mutant was created and its structure was determined to 2.4 Å resolutions which showed a vast conformational difference in the backbone of the P-loop. This structural variation in light with the functional diversity of F-ATP synthases and A-ATP synthases will be discussed.

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Keywords: archaeal ATP synthase; A1AO ATP synthase crystal structure