MS05 P01

Crystal Structures of Two Phosphopantetheine Adenylyltransferases Reveal an Alternative Ligand Binding Mode. Hye-Jin Yoon,[1] Hyung Ho Lee,[1] Ji Yong Kang,[1] Ji Hyeon Park,[1] Do Jin Kim,[1] Kwang-Hyun Choi,[2] Seung-Kyu Lee,[2] and Se Won Suh[1] [1]*Department of Chemistry, Seoul National University;* [2]*ProMediTech, Seoul, Korea.* E-mail: yoonhj@snu.ac.kr

Keywords: PPAT, Crystallization, Structure

Phosphopantetheine adenylyltransferase (PPAT) catalyzes the penultimate step in Coenzyme A (CoA) biosynthetic pathway. It catalyzes the reversible transfer of an adenylyl group from ATP to 4'-phosphopantetheine to form dephospho-CoA (dPCoA) and pyrophosphate. To provide insights into different modes of ligand binding, we solved six crystal structures of two PPATs from major human pathogens: Staphylococcus aureus (Sa) PPAT as binary complexes with either ATP or dPCoA (bound in two differen modes) and Enterococcus faecalis (Ef) PPAT in the apo form and as complexes with either ATP or pantetheine. The mode of ATP' binding to Sa PPAT is similar to that of dPCoA' obtained by soaking but is dissimilar from that of dPCoA obtained by co-crystallization. Unexpectedly, binding modes of ATP' and dPCoA' in Sa PPAT are distinct, as compared with the ATP- or dPCoA-bound PPAT structures that have been reported until now, while binding of dPCoA' to Sa PPAT and binding of ATP or pantetheine to Ef PPAT are similar to the previously observed binding modes. In the alternative binding mode of ATP' or dPCoA' in Sa PPAT, the adenylyl moiety is rotated by ~180° from the previously observed configuration. In addition, a large conformational change occurs in Sa PPAT only, in the loop between β4 and α4 (Leu91–Asp96) in all three structures. The alternative mode of ligand binding and this change in the loop conformation may be linked. The present structures of two PPATs should facilitate structure-based discovery of new antibacterial agents against S. aureus and E. faecalis, a major cause of hospital- and community-acquired infections.

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MS05 P02

Design of antibacterial and antimalarial drugs based on the structure of IspE F. Borel,[1] S. Richard,[2] F. Pojer,[2] L. Jacquamet,[1] T. Baiga,[2] J.A. Ramsey,[2] A. Iannello,[1] M. Bowman,[2] J.P. Noel, & J-L. Ferrer[1], [1]*IBS, CEA-CNRS-UJF, Grenoble, France*, [2]*HHMI, The Salk Institute for Biological Studies, La Jolla, USA*.
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Keywords: Xray structure, docking, drug computer-assisted design

Isoprenoids are a chemically diverse group of primary and secondary metabolites, present in all the organisms. Two five carbon containing substrates, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) constitute the basic building blocks of higher order isoprenoids. However, while all organisms share the same C5 isoprenoid building blocks, they employ two independent metabolic pathways for the biosynthesis of these metabolically essential precursors. The mevalonate pathway exists in eukaryotes, archaebacteria and a limited number of eubacteria. Conversely, plant and algae plastids, cyanobacteria and the majority of eubactera use the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway to biosynthesize IPP and DMAPP. Many pathogens and parasites, representing serious infectious disease threats to populations rely on the MEP metabolic pathway [1]. Among these are protozoa such as *P. falciparum* (malaria), *T. gondii* (toxoplasmosis), *Leishmania sp.* (leishmaniasis), or bacteria such as *B. anthracis* (anthrax), *C. diphtheriae* (diphtheria), *Brucella sp.* (brucellosis), *M. tuberculosis* (tuberculosis)... Since MEP pathway enzymes do not have human counterparts, they constitute targets of choice for the development of new antibiotic and antiprotozoa / antimalarial compounds. This pathway comprises seven enzymatic reactions and given the immense amount of success in rationally developing protein kinase inhibitors based upon competition for the ATP binding pocket, we focused attention on IspE (CDP-ME kinase) in order to develop a novel and selective inhibitor. IspE catalyzes, during the forth step of the pathway, the CDP-ME phosphorylation to produce 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate (CDP-ME2P) in an ATP- and Mg^{2+}-dependent reaction.

We based our work on the X-ray crystal structures of *Agrobacterium tumefaciens* IspE solved and refined to very high resolution (up to 1.18 Å) as apo structures and in complex with several different nucleotides including non-hydrolyzable ATP analogs. A fortuitous binding of GTP in IspE active site pocket, that does not overlap with ATP binding and not much with CDP-ME binding, provides a new route to the structure based design of possible IspE inhibitors with favorable pharmacological properties. Using our structural information, we also performed *in silico* evaluation of the binding to IspE of existing nucleotide-like compounds. This computer assisted study was validated by direct measurement of the binding affinity for some of the "*in silico* identified" compounds. From the structural informations and the computer assisted exploration of large nucleotide-like libraries, we were able to provide the guidelines for the design, synthesis and screening program for IspE inhibitors.

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MS05 P03

Stereoselective Esterase from *Pseudomonas putida* for D-β Acetylthioisobutyric Acid Synthesis

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Keywords: Esterase, stereoselective hydrolysis, D-β Acetylthioisobutyric Acid

Esterase (EST) from *Pseudomonas putitda* IFO12996 catalyzes the stereoselective hydrolysis of methyl DL-β-acetylthioisobutyrate (DL-MATI) to produce D-β-acetylthioisobutyric acid (DAT) serving a key intermediate for the synthesis of angiotensin-converting enzyme inhibitors. The inhibitors, such as captopril and alacepril, are used to treat hypertension and congestive

heart failure. The EST gene was cloned and expressed in *E. coli*; the recombinant protein is a non-disulfide linked homotrimer with a monomer molecular weight of 33 kDa in both solution and crystalline state, indicating that these ESTs function as trimers. EST hydrolyzed DL-MATI to produce DAT with the degree of conversion of 49.5 % and an enantiometric excess value 97.2 % at optimum pH 8~10 and temperature 57~67 ^{o}C. The crystal structure of EST has been determined by X-ray diffraction to a resolution 1.6 Å, confirming that EST is a member of the α/β hydrolase fold superfamily of enzymes and includes a catalytic triad Ser97, Asp227 and His256. The active site is located approximately in the middle of the molecule at the end of a pocket ~12 Å deep. The EST-DL-MATI complex structure has also been determined and shows that the oxyanion hole can form by peptide NH groups of Thr98 and Trp31 that form hydrogen bonds with the carbonyl oxygen of the DL-MATI. The EST can hydrolyze methyl ester group without affecting the acetylthiol ester moiety in DL-MATI. The examination of substrate specificity of EST toward other linear esters revealed that the enzyme showed specific activity toward methyl esters and that it recognized the configuration at the C-2 position. The knowledge of substrate specificity, molecular recognition and structure of a substrate-binding site of EST is useful for enzymatic engineering for broader ester substrates.

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MS05 P04

How structural features of MsrBs shed lights to the catalytic mechanism. Fanomezana M. Ranaivoson[a], Fabrice Neiers[b], Sandrine Boschi-Muller[b], Guy Branlant[b], André Aubry[a] and Frédérique Favier[a]
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Keywords: catalytic mechanisms, substrate binding, methionine sulfoxide reductase

The thioether side-chain of methionine residues oxidizes easily to sulfoxide *in vivo*, especially under oxidative stress conditions. To maintain the cellular viability, organisms possess methionine sulfoxide (MetSO) reductases (Msrs), enzymes devoted to the repair of such oxidized proteins. To efficiently reduce the two possible stereoisomers of the sulfoxide function, two classes of Msrs have evolved: MsrA, specific to Met-*S*-SO, and MsrB specific to Met-*R*-SO [1]. The characterized catalytic mechanism for both MsrA and MsrB is based on the reactivity of two or three cysteine (Cys) residues and can be divided according to two major steps: a reductase step in which the substrate is reduced, and a recycling step needed by the enzyme to recover its initial active state [2]. The first step ends with the oxidation of the enzyme to a sulfenic acid intermediate. In the second step, the reacting Cys form an internal disulfide bond which necessitates the intervention of a reducing partner such as thioredoxin to terminate the recycling process. In the case of MsrBs, the known structure from *Neisseria gonorrhoeae* [3] shows a spatial disposition of the active site apparently optimized for the execution of both the first and the second steps. But the analysis of this structure combined with sequence alignments reveal that in other MsrBs where the reacting Cys are distributed differently, structural differences may appear in particular to allow the disulfide bond formation during the second step.
Here will be presented a series of crystallographic structures of MsrBs from two different organisms, retracing some steps of the catalytic mechanism. From the observation of these structures, some of the structural origins of the efficiency of the catalysis will be discussed, with regard to the deductions made with the structure from *N. gonorrhoeae*. In addition, the comparison between the two organisms of the current study will highlight the differences in the conformational behavior of the two MsrBs during the mechanism, due to the relative positioning of their Cys.

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MS05 P05

Yellow lupine pathogenesis-related protein as a reservoir for cytokinins Humberto Fernandes*[1], Anna Bujacz[2], Oliwia Pasternak[1], Grzegorz Bujacz[1,2], Michal Sikorski[1], Mariusz Jaskólski[1,3] [1]*Center for Biocrystallographic Research, Inst. of Bioorg. Chem., Pol. Acad. Sci., Poznan, Poland.* [2]*Technical Univ. of Lodz, Poland.* [3]*Faculty of Chemistry, A. Mickiewicz Univ., Poznan, Poland.* E-mail: humberto@man.poznan.pl

Keywords: plant pathogenesis-related proteins, plant hormones, cytokinins

Plants, forced to grow in harsh conditions and exposed to pathogenic activity, have developed several means of defense. Besides establishing a physical barrier by strengthening their cell wall, plants also produce antibiotic compounds called phytoalexins and accelerate cell death to suppress the spread of infection. Furthermore, the expression of a number of proteins is induced by various types of pathogens, or by chemicals such as ethylene or salicylic acid. These proteins, designated PR (pathogenesis-related), are grouped into seventeen classes according to their biological activity and sequence homology. PR proteins of class 10, which are coded by multigene families, are small (17 kDa), slightly acidic, and cytosolic. The main feature of their three-dimensional structure is a seven-stranded antiparallel β-sheet surrounding a long C-terminal α-helix, with a large cavity created between these two structural elements. Although PR-10 proteins are abundant in plants, their physiological role still remains unknown. One hypothesis implicates PR-10 proteins in cytokinin binding. Cytokinins act as versatile hormones in regulation of plant development and growth. Two groups of cytokinins are known, adenine derivatives, such as zeatin, which are of natural origin, and synthetic urea derivatives, such as CPPU or DPU. Our crystallographic data obtained for a zeatin complex of a yellow lupine PR-10 protein have demonstrated the protein's unusual ability to bind as many as three zeatin ligands in the internal cavity. The high data resolution and excellent quality of the electron density maps allowed modeling of the zeatin molecules with atomic accuracy. Recently, the same PR-10 protein has been crystallized in complex with the urea-type cytokinins, DPU and CPPU.