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*.
E-mail: franck.borel@ibs.fr

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

Chun-Jung Chen[a], Shyh-Yu Shaw[b], [a]*Life Science Group, National Synchrotron Radiation Research Center, Hsinchu.* [b]*Department of Chemistry, National Cheng-Kung University, Tainan, Taiwan.*
E-mail: cjchen@nsrrc.org.tw

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