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Keywords: enoyl-ACP_reductase, dimer, tetramer

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Crystal Structure of *Actinobacillus pleuropneumoniae* HMW1C glycosyltransferase

Fumihiro Kawai,[a] Susan Grass,[b] Youngchang Kim,[c] Kyoung-Jae Choi,[a] Joseph W. St. Geme, III,[b] Hye-Jeong Yeo,[a] [a]*Department of Biology and Biochemistry, University of Houston, Houston, TX 77204.* [b]*Departments of Pediatrics and Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC 27710.* [c]*Structural Biology Center, Argonne National Laboratory, Argonne, IL 60439.* E-mail: fkawai@uh.edu

The *Haemophilus influenzae* HMW1 adhesin is an N-linked glycoprotein that mediates adherence to respiratory epithelium, an essential early step in the pathogenesis of *H. influenzae* disease. HMW1 is glycosylated by HMW1C, a novel glycosyltransferase in the GT41 family that creates N-glycosidic linkages with glucose and galactose at asparagine residues and di-glucose linkages at sites of glucose modification. Here we report the crystal structure of *Actinobacillus pleuropneumoniae* HMW1C (ApHMW1C), a functional homolog of HMW1C. The structure of ApHMW1C contains an N-terminal all α-domain (AAD) fold and a C-terminal GT-B fold with two Rossmann-like domains and lacks the tetratricopeptide repeat fold characteristic of the GT41 family. The GT-B fold harbors the binding site for UDP-hexose, and the interface of the AAD fold and the GT-B fold forms a unique groove with potential to accommodate the acceptor protein. Structure-based functional analyses demonstrated that the HMW1C protein shares the same structure as ApHMW1C and provided insights into the mechanism of HMW1C glycosylation of HMW1.

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Keywords: HMW1C, glycosyltransferase, two-partner secretion

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Structural characterization of the fructose 1,6-bisphosphatase (II) from *M. tuberculosis*

HitenJ.Gutka,[a,b,c] ScottG.Franzblau,[a,b] FarahnazMovahedzadeh,[a,b] Cele Abad-Zapatero,[b,c] [a]*Institute for Tuberculosis Research, University of Illinois at Chicago (UIC), Chicago, IL, (USA).* [b]*Department of Medicinal Chemistry and Pharmacognosy, UIC, Chicago, IL, (USA).* [c]*Center for Pharmaceutical Biotechnology, UIC, Chicago, IL, (USA).* E-mail: caz@uic.edu

In spite of the availability of effective chemotherapy and Bacille-Calmette-Guerin (BCG) vaccine, tuberculosis remains a widespread fatal infection world-wide. Many factors such as, human immunodeficiency virus (HIV) co-infection, drug resistance, lack of patient compliance with chemotherapy, delay in diagnosis, variable efficacy of BCG vaccine among others contribute to the mortality due to tuberculosis.

New advances in understanding the biology of *Mycobacterium tuberculosis (Mtb)* and availability of functional genomic tools, such as microarray and proteomics, in combination with modern approaches have not resulted in new drug in the past 30 yr. The problem is compounded by the appearance and persistence of resistance strains. Therefore, there is an urgent need to identify new drug targets in *Mycobacteria* leading to new drugs. In general, gene products involved in mycobacterial metabolism, persistence, transcription, cell wall synthesis and virulence could be possible and atractive targets for the development of new drugs.

The completion of the *Mtb* genome sequence allowed the identification of genes that were predicted to encode enzymes for most central metabolic pathways, however no fructose 1,6 -bisphosphatase was assigned. In a previous study, we had identified *Rv1099c* to encode this missing link [1], showing that *Rv1099c* encodes a major FBPase in *M. tuberculosis.* The corresponding gene has been shown to be attenuated *in vivo* in a Transposon Site Hybridization screen (TraSH) [2] which makes *Mtb* FBPase an attractive drug target.

We have previously reported the cloning, expression and purification to homogeneity of the purified enzyme and present the initial biochemical characterization [3]. MtFBPase displayed Michaelis-Menten kinetics for the substrate fructose 1, 6-bisphosphate. Further characterization of the enzyme has shown that FBPase activity is absolutely dependent on the divalent cations Magnesium or Manganese, where replacing with other bivalent metal ions resulted in loss of activity..

Mtb FBPase has been crystallized in the apo form by hanging-drop vapour diffusion method. Crystals diffracted to a resolution of 2.7Å and belonged to the hexagonal space group $P6_122$, with unit-cell parameters a=b= 131.3, c=143.2Å. The structure has been solved by molecular replacement using *E. coli* GlpX as a probe (PDBID: *3d1r*) [4]. The structure of the GlpX-F6P complex has also been solved and both structures have been refined. Structural results can be related to the other available structures of class II FBPases.

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Keywords: mycobacterium tuberculosis, gluconeogenesis, Fructose 1,6– bisphosphatase.

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Crystal structure of SmeT bound to the biocide Triclosan

Federico M. Ruiz,[a] Alvaro Hernandez,[b] Antonio Romero,[a] José Luis Martínez,[b] [a]*Centro de Investigaciones Biológicas (CIB-CSIC), Madrid (Spain).* [b]*Centro Nacional de Biotecnología (CNB-CSIC), Madrid (Spain).* E-mail: fruiz@cib.csic.es

The wide utilization of biocides for different purposes, including toothpastes, soaps, house-hold compounds surfaces' disinfectants and even their use as additives of different materials to avoid their colonization by micro-organisms, poses a concern on the impact of these compounds on natural bacterial populations. In recent years, the possibility that widely-used biocides might co-select for antibiotic resistance has been suggested to pose a potential risk to the successful treatment of infectious diseases. I*n vitro* experiments have shown that exposure of bacterial populations to certain biocides, such as triclosan, indeed leads to selection for mutants with reduced susceptibility to antibiotics. On most occasions this resistance has been acquired as a consequence of the stable de-repression of MDR efflux pumps.

We have explored the possibility that the widely used biocide triclosan might induce antibiotic resistance using as a model the opportunistic pathogen *Stenotrophomonas maltophilia*. Biochemical,

functional and structural studies were performed, focusing on SmeDEF, the most relevant antibiotic- and triclosan-removing multidrug efflux pump of *S. maltophilia*. Expression of *smeDEF* is regulated by the repressor SmeT. Triclosan released SmeT from its operator and induces the expression of *smeDEF*, thus reducing the susceptibility of *S. maltophilia* to antibiotics in the presence of the biocide. The structure of SmeT bound to triclosan is described. Two molecules of triclosan were found to bind to one subunit of the SmeT homodimer. The binding of the biocide stabilizes the N terminal domain of both subunits in a conformation unable to bind DNA. This complex structure is the first structural evidence of the ability of triclosan to act as an effector via its binding to a transcriptional regulator (SmeT). Given that SmeT mediate the susceptibility of *Stenotrophomonas maltophilia* to antibiotics by repressing SmeDEF expression, the present results provide information that aids our understanding of the molecular basis of biocide-induced antibiotic resistance.

Keywords: complex, ligand, antibacterial

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Interaction of the type III secretion chaperone SycD with YscY

Madeleine Schreiner,, Hartmut Niemann, *Department of Chemistry, Bielefeld University, Bielefeld (Germany).* E-mail: madeleine.schreiner@uni-bielefeld.de

The type III secretion system (T3SS) is used by several Gram-negative pathogenic bacteria to inject cytotoxins, so called effector proteins, into the host cell to manipulate their host for their own benefit. The effector translocation occurs via a needle-like nanomachine, the injectisome, which spans the whole bacterial envelope. The effectors enter the cell through a pore within the host cell membrane formed by bacterial translocator proteins. Both the effectors and translocators need chaperones for their efficient translocation. T3S chaperones work without the need for ATP hydrolysis and are divided into three subclasses: class I chaperones interacting with effector proteins, class II chaperones interacting with translocator proteins and class III chaperones interacting with needle components.[1]

SycD (specific yop chaperone D) is the class II chaperone of the translocators YopB (*Yersinia* outer protein B) and YopD from the enteropathogen *Yersinia enterocolitica*. Additionally, SycD plays an important role in diverse regulatory processes of the T3SS and interacts with several other T3S proteins like TyeA, YscM2 or YscY. Like all structurally characterised class II chaperones SycD comprises an overall α-helical fold consisting of three tetratricopeptide repeats (TPR) providing a concave hydrophobic groove for translocator binding. SycD is known to form homodimers in solution involving the residues A61 and L65 of the first TPR as binding platform and mutations within these dimerisation-mediating residues lead to a stable monomeric chaperone that is not able to rescue a *sycD* null mutant of *Y. enterocolitica*.[2] Thus the dimerisation is either essential for the chaperone function or the TPR1 provides a further binding region for other T3S proteins interacting with SycD.

In order to further characterise the interaction of SycD with its various binding partners we coexpressed and copurified SycD together with YscY, a 114 aa protein which is proposed to be the chaperone of the secreted T3S protein YscX (*Yersinia* secretion X). We confirmed that both proteins interact via the N-terminal region of SycD involving the first TPR1 forming an elongated heterodimeric 1:1 complex which is preferred over SycD dimerisation. Furthermore, the monomeric SycD variant containing mutations at the positions A61 and L65 within the first TPR is not able to bind YscY. Hence the SycD/YscY complex formation comprises the same binding region that is also involved in SycD dimerisation. One might conclude, that not the SycD dimerisation itself is essential for chaperone function within the T3SS, but the formation of heterodimeric or even larger multi-protein complexes.

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Cell wall modelling in pathogenic bacteria

Alessia Ruggiero,[a,b] Flavia Squeglia,[a,b] Luciano Pirone,[a] Stefania Correale,[a] Rita Berisio,[a] [a]*Institute of Biostructure and Bioimaging, via Mezzocannone 16, I-80134 Napoli, (Italy).* [b]*University Federico II of Naples, via Cinthia, I-80126 Naples, (Italy).* E-mail: alessia.ruggiero@unina.it

The interaction between bacterial pathogens and the human host after infection may manifest itself as a chronic disease or as a latent (or dormant) infection, a state capable to evade host responses. The probability of reactivation from dormancy is strongly affected by the type of host immune response and it is significantly enhanced in immuno-compromised patients, e.g. suffering from AIDS. Understanding and controlling the entry and exit from dormancy is important in the development of new anti-microbial therapies.

Resuscitation of dormant bacteria is promoted by a set of peptidoglycan hydrolases, which are secreted from slowly replicating bacteria in the extra-cellular milieu. By cleaving peptidoglycans which constitute the cell wall, these hydrolases are thought to alter cell wall mechanical properties and favour cell division and/or release anti-dormancy factors. We have determined the crystal structures of various cell wall modelling enzymes [1-5]. The comprehension of the structural features associated to cell wall modelling enzymes activity/inhibition will provide the bases for the identification of molecules (pro-latency molecules) able to restrict bacterial life to the latent, non-dangerous, state.

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Muropeptide-driven revival from dormancy in bacterial pathogens: a structural perspective

Flavia Squeglia,[a,b] Alessia Ruggiero,[a,b] Daniela Marasco,[b] Rita Berisio,[a] [a]*Institute of Biostructure and Bioimaging, via Mezzocannone 16, I-80134 Napoli, (Italy).* [b]*University Federico II of Naples, via Cinthia, I-80126 Naples, (Italy).*

Post-translational modifications are a ubiquitous means of rapidly and reversibly modifying the physical-chemical properties of a protein, triggering a number of possible consequences: change of