This structure and the result of inhibition by ADP will be available for design of new drugs which inhibit ADPRTs such as S. enterica SpvB, which is required for human macrophage infection.

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Keywords : ADPRT, iota toxin, complex structure

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Structural basis for the helicobacter pylori-carcinogenic TNF⟨-inducing protein

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Stomach cancer is strongly associated with infection by Helicobacter pylori. In 2005, we identified a new H. pylori gene encoding a TNF-⟨ inducing protein (Tip⟨) that acts as a carcinogenic factor. Tip⟨ is secreted from H. pylori as a homodimer whose subunits are linked by disulfide bonds. Using blast search, there is no similar sequence with Tip⟨, thus it is unique carcinogenic factor protein. We also characterized a Tip⟨ deletion mutant (del-Tip⟨) that lacks the N-terminal six amino acid residues (LQACTC), including two cysteines (C5 and C7) that form disulfide bonds, but nonetheless shows a weak ability to induce TNF-a expression.

Here we report the crystal structure of del-Tip⟨ at 2.47Å resolution. As expected, the structure of del-Tip⟨ is novel; It has a novel elongated structure containing a 40 Å-long a helix, and forms a heart-shaped homodimer via non-covalent bonds. Moreover, their circular dichroism spectra strongly suggest that the structures of the del-Tip⟨ and Tip⟨ homodimers are very similar. We conclude that the TNF-⟨ inducing activity is correlated with the dimer formation. It means that strong dimer via covalent bond shows the strong activity for Tip⟨ and weak activity for del-Tip⟨ with weak interaction. Tip⟨'s unique mode of dimer formation provides important insight into protein-protein interactions and into the mechanism underlying the carcinogenicity of H. pylori infection.

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Keywords: TNF-⟨ inducing protein, helicobacter pylori, carcinogenic factor

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A Model of action for peripheral Membrane-Associated GT-B Glycosyltransferases

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Peripheral membrane-associated GT-B glycosyltransferases (GTs) are a ubiquitous family of enzymes that play essential roles in a variety of important biological processes in all living organisms. They transfer a sugar moiety from nucleotide- or lipid-phospho-sugar donors to a wide range of membrane-associated acceptors. Here we focus in PimA, an essential enzyme involved in the biosynthesis of phosphatidyl-myo-inositol mannosides (PIMs), which are key glycolipids of the mycobacterial cell envelope[1]. PimA is a paradigm of this family of GTs, which the molecular mechanism of substrate/membrane recognition and catalysis is still unknown. We have solved the crystal structure of PimA from *M. smegmatis* in complex with its donor substrate GDP-Man[2]. The notion of a membrane-associated protein via electrostatic interactions is consistent with the finding of an amphipathic α-helix in the N-terminal domain of PimA. Based on structural, biophysics and biochemical studies, we proposed a model of interfacial catalysis in which PimA recognizes the fully acylated acceptor substrate, phosphatidyl-myo-inositol (PI), with its polar head within the catalytic cleft and the fatty acid moieties only partially sequestered from the bulk solvent. In addition, we provided strong evidence showing that PimA undergoes significant conformational changes upon substrate binding[3]. Altogether, our experimental data support a model wherein the flexibility and conformational transitions confer adaptability of PimA to the substrates, which seems to be of importance during catalysis. The proposed mechanism has fundamental implications for the comprehension of the peripheral membrane-associated GTs at the molecular level.

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Keywords: membrane glycosyltransferase, tuberculosis, catalysis

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Structural studies on FlhBc from *Salmonella typhimurium* and *Aquifex aeolicus*

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Many bacteria swim in liquid environment by means of flagella. The bacterial flagellum is a huge complex structure made from more than 30 different proteins. All flagellar axial proteins are transported across cytoplasmic membrane by the flagellum-specific secretion apparatus, which shares similarity to the type III secretion system of the bacterial needle utilized by some bacteria in pathogenesis. The protein transport is highly regulated. Membrane protein FlhB has been found to play an active role in this regulation. The protein consists of two domains: a hydrophobic N-terminal part, which is predicted to have four transmembrane helices, and a C-terminal cytoplasmic

domain (FlhBc). Homologues of FlhB were found in all bacterial type III secretion systems. The sequence of the protein is highly conserved suggesting that the protein function is also similar.

We have investigated complementation properties of FlhB from *Aquifex aeolicus* to FlhB from *Salmonella typhimurium*. *flhB* gene in *Salmonella* was substituted by *flhB* of *A. aeolicus* or by chimera *flhB* composed of different parts from *A. aeolicus flhB* and *S. typhimurium flhB*. Such mutants were tested for motility. All mutants showed motility although weaker than wild cells. We have found that some mutations in C-terminal part of FlhB resulted in enhanced motility. To explain results obtained we have determined FlhBc structures from both organisms: *S. typhimurium* and *A. aeolicus*. Comparison of the structures gives us new ideas about functional mechanism of FlhB.

Keywords: flagella, type III secretion system, FlhB

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High-resolution crystal structure of an outer membrane-anchored endolytic peptidoglycan lytic transglycosylase (MltE) from escherichia coli

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The crystal structure of the first endolytic peptidoglycan lytic transglycosylase MltE from *Escherichia coli* is reported herein. The degradative activity of this enzyme initiates the process of cell wall recycling, which is an integral event in the bacterial existence. The structure sheds light on how MltE recognizes its substrate, the cell wall peptidoglycan. It also explains the ability of this endolytic enzyme to cleave in the middle of the peptidoglycan chains. Furthermore, the structure reveals how the enzyme is sequestered on the inner leaflet of the outer membrane.

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Keywords: lytic transglycosylase, peptidoglycan, X-Ray diffraction.

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Structural characterization of the conjugation machinery in G+ bacteria

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Bacterial conjugation is a major mechanism of the horizontal gene transfer (HGT) in bacteria and thus an important component of bacterial evolution. It provides a route for the rapid acquisition of new genetic information and contributes to the spread of antibiotic resistance [1]. The latter is becoming a primary threat to human health, since currently 70% of all hospital acquired infections are resistant to at least one antibiotic, and antibiotic resistance to at least one drug has been reported for every major strain of pathogenic bacteria [2]. Whilst the plasmid conjugation in Gram-negative (G-) bacteria has been studied in detail, the corresponding process in Gram-positive (G+) bacteria is poorly understood at the molecular level [3]. Among them, the G+ bacterium *Streptococcus pneumoniae* is responsible for nearly 2 000 000 human deaths annually, and this figure represents only 15–20% of the people infected [4]. Thus, understanding HGT in pneumococcus and related bacteria may help in controlling the spread of medically important antibiotic resistance [5]. Progress in the expression, purification, crystallization and structural characterization of key proteins of the conjugative process in *S. pneumoniae* will be presented.

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Dimeric and Tetrameric forms of enyol-ACP reducatase

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Most bacteria synthesize fatty acids using a discrete and highly conserved group of enzymes that each carry out a single reaction. Among them is enoyl-ACP reductase (or ENR) which catalyzes the last step in each elongation cycle. It is considered as an attractive target for new antibiotics since it is an essential enzyme and the fatty acid synthesis machinery in human is quite different. Some pathogens such as *Enterococcus faecalis*, *Bacillus subtilis, Pseudomonas aeruginosa* have more than one ENR. There have been a number of crystal structures reported from various pathogens: some with an inhibitor and the cofactor (either NADP or NAD), some without either the cofactor or an inhibitor. The overall structures, including the active sites, are quite similar to one another in the case of the ternary complexes, and they are all found as a homo-tetramer in the crystal. The apo structures are also found as a homo-tetramer with the substrate and the cofactor binding domains mostly disordered. However, the recent studies include an apo ENR in a homo-dimeric form, and also in a homo-tetramer but with different arrangement from what was observed earlier. Detailed analysis and its implication will be presented.

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