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Enhanced intracellular survival protein (Eis), a secreted protein encoded by the Rv2416c gene of *M. tuberculosis*, was shown to enhance intracellular survival of *M. smegmatis* in macrophages. It modulates the host immune responses by suppressing macrophage autophagy, inflammation, and cell death through the inhibition of reactive oxygen species (ROS) generation. Its GCN5-related N-acetyltransferase (GNAT) domain at the N-terminus was found to be essential for the regulation of ROS generation and proinflammatory responses. Eis is also capable of acetylating kanamycin to confer resistance. To provide insights into its role in pathogenesis by *M. tuberculosis*, we have determined the crystal structures of *M. tuberculosis* Eis in both the ligand-free and ligand-bound states. It is comprised three domains. Domain 1 possesses the GNAT fold, as predicted by sequence analysis. Unexpectedly, domain 2 is also folded into the GNAT structure, while domain 3 structurally resembles the sterol carrier protein-2 domains with a hydrophobic cavity. In addition, we have also determined the crystal structure of Eis protein from *M. smegmatis*, as a complex with CoA, which is bound to domain 1 only.

Keywords: enhanced intracellular survival, immune response, mycobacterium tuberculosis

MS36.P13

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Crystal structure of the TNF-α inducing protein (Tipα) from *Helicobacter pylori*: DNA docking study

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Helicobacter pylori infection is one of the highest risk factors for gastroduodenal diseases including gastric cancer. TNF-α is one of the essential cytokines for tumor promotion and thus a *H. pylori* protein which induces TNF-α is believed to play a significant role in gastric cancer development in humans. The *HP0596* gene product of *H. pylori* strain 26695 was identified as the TNF-α inducing protein (Tipα). Tipα is secreted from *H. pylori* as dimers and enters the gastric cells. It was shown to have a DNA binding activity. Here we have determined the crystal structure of Tipα from *H. pylori.* Its monomer consists of two structural domains ("mixed domain" and "helical domain"). Tipα exists as a dimer in the crystal and the dimeric structure represents a novel scaffold for DNA binding. The DNA-combined structures obtained from Haddock 2.1, high ambiguity driver docking, suggest possible binding mode and its biological role.

[1] Jang *et al., J Mol Biol* **2009**, *392*, 191-197.

Keywords: tipα, helicobacter pylori, gastric cancer

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LipL32, a virulence factor from pathogenic *Leptospira*,

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Leptospirosis caused by *Leptospira* is one widespread zoonotic disease. The major target of *Leptospires* in kidney is the renal proximal tubular cells. *Leptospira* outer membrane proteins would lead to tubulointerstitial nephritis and acute renal malfunction. LipL32 is a virulence factor and the major lipoprotein of outer membrane proteins from pathogenic *Leptospira*. The crystal structure of LipL32 was determined by multiwavelength anomalous dispersion at 2.3 Å. LipL32 contains a novel polyD sequence with a cluster of seven aspartate residues, which form an acidic surface patch for Ca^{2+} binding. The calcium binding to LipL32 was determined by ITC. A significant conformational change was induced when Ca^{2+} bound to LipL32. LipL32 can recognize extracellular matrix components and adheres to the host cell to evade an immune response. The binding of fibronectin to LipL32 was observed by Stains-all circular dichroism and ELISA experiments. The interaction between fibronectin F30 and LipL32 is associated with Ca^{2+} binding. The Ca^{2+} binding to LipL32 might be important for extracellular matrix interaction with the host cell in *Leptospira*.

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Keywords: Ca2+ binding protein, fibronectin, leptospira

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Crystal structure of Ia-Actin complex with novel ligand

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ADP-ribosylation is one of the important enzyme modification after the protein translation. ADP-ribosylating toxin (ADPRT) adds ADP-ribosyl group of NAD to target and lead to disorganization of the cell. It is thought that some pathogenic bacteria use the ADPRT to infect into the host cell. ADPRT can be classified into four groups as the target difference. Actin specific ADPRT, such as iota toxin from *C.perfringens* ADP-ribosylates Arg-177 of α-Actin, inhibits actin polymerization and induces cell rounding. It finally causes diarrhea against human and domestic animals. Up to now, many actin ADPRT's structures are available including Ia (catalytic subunit of iota toxin) by us [1], however, there was no information how toxin binds to actin and how proceeds the ADP-ribosylation reaction. Recently, we reported the first crystal structure of Ia-Actin complex with β-TAD, which is nonhydrolyzable NAD analog, as its ligand [2]. It provided the information of the interaction between ADPRT and actin.

Here we report a new complex structure of Ia, α-Actin and ADP instead of β-TAD. The crystal showed maximum X-ray diffraction to 2.6Å resolution, space group = *P*212121, and unit cell parameters, α = β = γ = 90°, a = 56.8, b = 126.5, c =138.1. We solved the structure with Molecular Replacement using Ia-Actin-β-TAD complex and refined with R factor = 25.8%. Currently, we are doing the refinement. Additionally, we also show that ADP inhibits ADP-ribosylation of α-Actin by Ia using the enzyme activity assay for the first time.

Under the same crystallization condition, we found no crystals of complex of Ia-α-Actin without β-TAD or ADP. Together with these fact and complex structure analysis, we conclude the next binding scheme; Upon the binding of small ligand, such as β-TAD or ADP, it induces the small conformational change of Ia and it triggers further binding with actin.

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