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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

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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*.

[1] J-Y Tung, C.-W. Yang, S.-W. Chou, C.-C. Lin, Y.-J. Sun, *J. Biol. Chem*. **2010,** *285(5)*, 3245-52.

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 2.6 Å resolution, space group = *P*212121, and unit cell parameters, $\alpha = \beta = \gamma = 90^\circ$, 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.