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Structural and Functional Insights of Virulence Proteins and Associated Factors from *Pseudomonas aeruginosa*

ABSTRACT

Pseudomonas aeruginosa is an opportunistic multi-drug resistance pathogen which secretes some virulence factors or proteins either extracellularly or via some specialized secreting nanomachines called injectisomes into the host cells. These virulence proteins also referred to as effectors molecules subvert the host immune response by targeting some essential proteins or some immunomodulatory substances in the host cells. Therefore, it is essential to comprehend these pathogenic variables which are necessary for the development and maintenance of bacterial pathogenicity. Atomistic level insights into these virulence factors or proteins with or without their targeting partners decipher the molecular level information in pathogenesis.

To overcome the infection caused by these virulent organisms, natural killer cells like macrophages secrete itaconate, an immunometabolite substance which specifically inhibits isocitrate lyase of bacterial glyoxylate cycle. To overcome the inhibitory effect of itaconate, *P. aeruginosa* release three enzymes: succinyl-CoA:itaconate CoA transferase (Ict), (*R*)-specific itaconyl-CoA hydratase (Ich) and (*S*)-citramalyl-CoA lyase (Ccl) which alter itaconate to pyruvate and acetyl-CoA. Here, we report the first crystal structure of itaconyl-CoA hydratase from *P. aeruginosai* (*Pa*Ich) at 1.98 Å resolution. The overall structure of *Pa*Ich resembles the structure of MaoC family (*R*)-specific enoyl-CoA hydratase consisting of two domains, N-terminal domain and C-terminal domain connected by a long intervening loop. Each domain is comprised of a 'hotdog fold' where a central α-helix is represented as sausage wrapped by a β-sheet scaffold represented as a bun. Crystal structure analysis of *Pa*Ich showed that a unique N-terminal hotdog fold containing a 4-residue short helical segment 'α3', named as an 'eaten sausage', followed by a flexible loop region slipped away from the conserved β-sheet scaffold, whereas the C-terminal hotdog fold is similar to all MaoC family hydratases.

PvrA was discovered to be an upregulated regulatory gene during infection, which increases the virulence of the bacteria in the host. Here, we report the crystal structure of PvrA from *P. aeruginosa* at 2.30 Å resolution. Structural analysis of PvrA showed that it present as a dimer which is very similar to other TetR family transcriptional regulators such as AmtR and AcrR. It consists of a common N-terminal H-T-H DNA binding domain similar to other TetR family

regulators. We have found that region consists of ~5 residues (Val_8-Arg_{12}) of H-T-H motif of chain B is slightly bent downwards compared to chain A.

Signature of Candidate

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