Crystal structures of PipY from Synechococcus elongatus, a paradigm for the intriguing COG0325 family of PLP-binding proteins.

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Cyanobacteria, key players in the nitrogen/carbon flows of the biosphere, are the focus of our joint efforts to decipher regulatory networks in these organisms. A key player, protein PipX, shuttles between protein PII and the transcriptional regulator NtcA, depending on the nitrogen abundance, effecting on transcriptional regulator PlmA when PII-bound, or on NtcA. Since the gene for PipX immediately precedes the *pipY* gene, making an operon with it in *Synechococcus elongatus*, we tried to clarify PipX-PipY functional interactions. As a first step, we produced and purified PipY, finding that it is monomeric (gel filtration) and that, judged from its absorption spectrum, hosts pyridoxal phosphate (PLP), which slowly dissociates from it. Incubation of aged PipY with PLP revealed a K_D PLP ~30 µM. Reaction with the PLP-targeting antibiotic D-cycloserine revealed very low affinity of PipY for it. PipY crystal structures (~1.9 Å resolutions) in PLP-bound and apo forms showed the modified TIM barrel that characterizes the fold type III PLP enzymes. Unlike these enzymes, PipY was single-domain and monomeric, and lacked residues that are crucial for catalysis. Thus PipY is highly unlikely to be an enzyme. The high exposure of the PLP, its modest affinity for the protein, and the conformational changes in PipY associated with PLP dissociation, support a role of PipY in vitamin B6 homeostasis, possibly as a provider of PLP to PLP-dependent enzymes. Our detailed structural data, and the comparison with the structure of the yeast orthologue reported previously and with the PDB-deposited structures of three bacterial *COG0325* products, make of PipY a structural paradigm for the universal *COG0325* gene family, traced back to LUCA. Our findings help understand the roles of the mutations found in the human orthologue as the cause of vitamin B6-dependent epilepsy. *Grants BFU 2014-58229-P and PrometeoII/2014/029 (Spanish & Valencian Governments) and ALBA and DIAMOND synchrotrons used.*