MS5-P54 Crystal structure of PhoU from *Pseudomonas aeruginosa*, a negative regulator of the Pho regulon

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In *Escherichia coli*, seven genes (*pstS*, *pstC*, *pstA*, *pstB*, *phoU*, *phoR*, and *phoB*) are involved in sensing environmental phosphate (Pi) and controlling the expression of the Pho regulon. PhoU is a negative regulator of the Pi-signaling pathway and modulates Pi transport through P_i transporter proteins (PstS, PstC, PstA, and PstB) through the two-component system PhoR and PhoB. Inactivation of PhoY2, one of the two PhoU homologs in *Mycobacterium tuberculosis*, causes defects in persistence phenotypes and increased susceptibility to antibiotics and stresses. Despite the important biological role, the mechanism of PhoU function is still unknown. Here we have determined the crystal structure of PhoU enzyme from *Pseudomonas aeruginosa.* It exists as a dimer in both the crystal and solution, with each monomer consisting of two structurally similar three-helix bundles. The overall structure of *P. aeruginosa* PhoU dimer resembles those of *Aquifex aeolicus* PhoU and *Thermotoga maritima* PhoU2. However, it shows distinct structural features in some loops and the dimerization pattern.

Keywords: PhoU, PA5365, Pho regulon, phosphate homeostasis, drug tolerance

MS5-P55 What can we learn about nucleotide metabolism from a thermophilic anaerobic ribonucleotide reductase?

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The first time that radical species were characterised in enzyme mechanisms was upon the discovery of ribonucleotide reductases (RNRs) more than 50 years ago. Radical chemistry in enzymology has later been found to take place in a wide range of enzymes in which challenging chemistry is carried out. RNRs catalyse the reduction of ribonucleotides to deoxyribonucleotides. This is a key step in the *de novo* synthesis of building blocks for DNA and is found in the vast majority of known organisms.

Here we present the anaerobic RNR from the thermophile *Thermotoga maritima* studied by X-ray crystallography, small-angle X-ray scattering, enzyme assays and complementary biophysical methods. In many regards the *T. maritima* system appears to be a typical anaerobic RNR. However, its (catalytic subunit, tmNrdD) active site stands out greatly by lacking a pre-positioned cysteine residue, expected to take part in radical delivery to the substrate. This has not been seen in any other structurally studied RNR of any type. In contrast, it still maintains a glycyl radical site in a conserved position for Gly* radical enzymes. This radical site can be activated by introducing the reduced radical SAM activase, in presence of its cosubstrate S-adenosyl methionine. The highly unexpected structural arrangement found in the active site and its implications upon the biochemistry and structural biology of RNRs will be discussed.

RNRs are not only fascinating enzyme systems to study because of the above mentioned chemistry or their significance in nucleotide metabolism, but also because of their intricate allosteric regulation to maintain balanced dNTP pools. RNRs contain allosteric sites to regulate substrate specificity and in some cases separate sites for overall activity regulation. By the use of X-ray crystallography the structural changes induced by different combinations of effectors and effector/substrate complexes has been studied in the *T. maritima* anaerobic RNR. These changes in hydrogen bonding networks between the specificity and active site, upon nucleotide binding, allows for a better understanding of the cooperativity between the sites.

Many things can be learned about RNRs from the anaerobic *T. maritima* system and an overview highlighting its biochemistry, active site, allosteric regulation and further puzzle pieces from complementary methods will be presented.

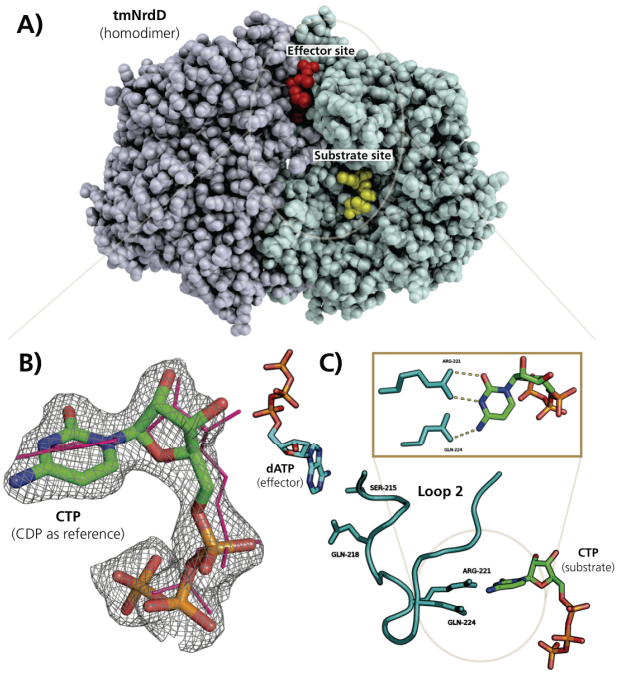

Figure 1. A) The homodimer of tmNrdD in complex with dATP (effector) and CTP (substrate) from X-ray crystallography. B) CTP shown with a 2F$_o$-F$_c$ mesh contoured at 1.2 σ (CDP when in complex with tmNrdJ shown in magenta as a reference). C) Overview of key amino acids in loop 2 for nucleotide recognition.

Keywords: Glycyl radical enzymes, radical SAM, metalloproteins, anaerobic enzymology, allosteric regulation, nucleotide metabolism, X-ray macromolecular crystallography, small-angle X-ray scattering

MS5-P56 Sulfur shuttling across a chaperone during molybdenum cofactor maturation

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Formate dehydrogenases (FDHs) are of great interest for being natural catalysts able to sequester atmospheric CO_2 used for generating reduced carbon compounds with possible uses as fuel. Most FDHs are metalloenzymes harboring a molybdenum or tungsten cofactor in their active site. Activity of FDHs in *Escherichia coli* strictly requires the sulfurtransferase *Ec*FdhD which likely transfers sulfur from IscS (a general sulfur transfer platform) to the molybdenum cofactor (Mo-*bis*PGD, see Figure 1) of FDHs. Here we show that *Ec*FdhD binds the molybdenum cofactor *in vivo*. Additionally, *Ec*FdhD has sub-micromolar affinity for GDP used as a surrogate of the molybdenum cofactor's nucleotide moieties. The *Ec*FdhD crystal structure was solved in complex with GDP showing the symmetrical binding of two GDPs on the same protein dimer face, a dynamic loop harboring two functionally important cysteine residues on the opposite face and a tunnel connecting these two faces at the center of the dimer. Strikingly, the distance between the two GDP molecules is similar to the distance between the two GDP moieties of the Mo-*bis*PGD present at the active site of FDHs, allowing us to propose a model for the sulfuration mechanism of Mo-*bis*PGD where the sulfur atom is shuttled across the chaperone's dimer. This model is supported by structure-guided mutagenesis and functional studies with distinct variants either affected on catalysis/sulfur transfer or GDP binding. Overall, our results provide a first molecular basis for sulfuration of Mo-*bis*PGD prior to its insertion into FDHs. Additionally, it provides a nice example of how the symmetry of an enzyme can mirror the symmetry of its substrate.