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.