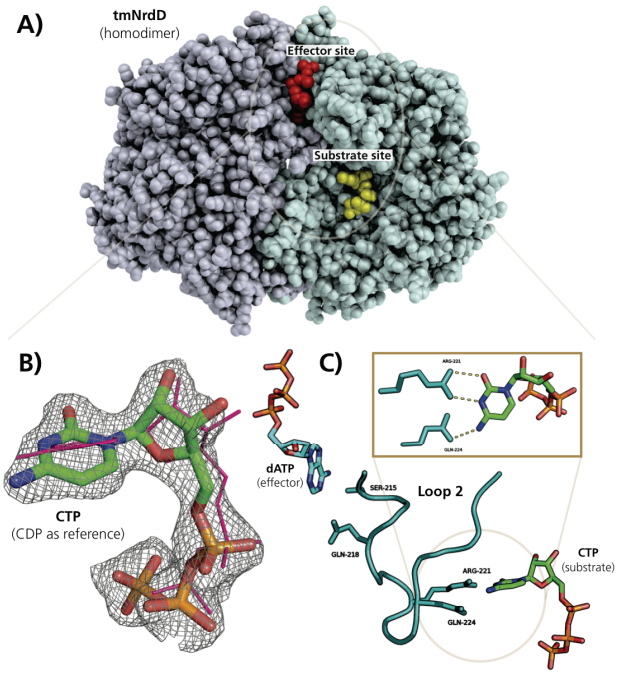

Figure 1. A) The homodimer of tmNrdD in complex with dATP (effector) and CTP (substrate) from X-ray crystallography. B) CTP shown with a 2F$_o$-F$_c$ mesh contoured at 1.2 σ (CDP when in complex with tmNrdJ shown in magenta as a reference). C) Overview of key amino acids in loop 2 for nucleotide recognition.

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.

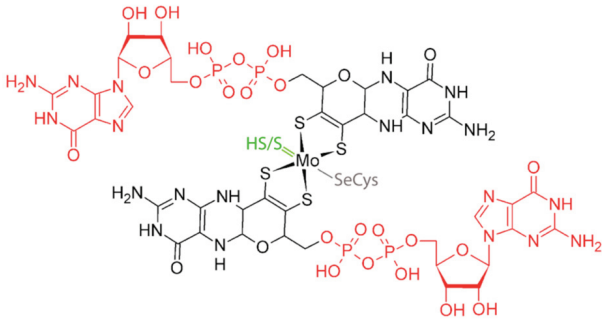

Figure 1. Structure of the Mo-*bis*PGD substrate of FdhD, or how to transfer a sulfur to the molybdenum atom at the center of the substrate. The GDP moiety of Mo-*bis*PGD is highlighted in red.

Keywords: Enzyme, symmetry, sulfur transfer

MS5-P57 Crystal structure of Csd3 from *Helicobacter pylori*, a cell-shape determining metallopeptidase

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Helicobacter pylori is associated with various gastrointestinal diseases such as gastritis, ulcer, and gastric cancer. Its colonization of the human gastric mucosa requires high motility, which depends on the helical cell shape. Seven cell shape-determining genes (*csd1*, *csd2*, *csd3*/*hdpA*, *ccmA*, *csd4*, *csd5*, and *csd6*) have been identified in *H. pylori*. These proteins play key roles in determining the cell shape through modifications of the cell-wall peptidoglycan by alteration of crosslinking or by trimming of peptidoglycan muropeptides. Among them, Csd3 (also known as HdpA) is a bi-functional enzyme. Its d,d-endopeptidase activity cleaves the d-*Ala*^{4}-*mDAP*^{3} *peptide bond between crosslinked* muramyl tetra- and penta-peptides. It is also a d,d-carboxypeptidase that cleaves off the terminal d-Ala^5 from the muramyl *pentapeptide*. Here we have determined the crystal structure for this protein, revealing the organization of its three domains in a latent and inactive state. The N-terminal domain 1 and the core of domain 2 share the same fold despite a very low level of sequence identity and their surface charge distributions are different. The C-terminal LytM domain contains the catalytic site with a Zn^{2+} ion, like similar domains of other M23 metallopeptidases. Domain 1 occludes the active site of the LytM domain. The core of domain 2 is held against the LytM domain by the C-terminal tail region that protrudes from the LytM domain. This work could serve as the foundation in discovery of novel inhibitors that would prove helpful in fighting infections by the major human pathogen *H. pylori*.

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Keywords: Helicobacter pylori, csd3, cell shape determinant, M23B metallopeptidase