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Structural Studies of MoeA Point Mutants

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Beamline(s): X26C

Introduction: The molybdenum cofactor (Moco) is an essential component of a diverse group of enzymes catalyzing important redox transformations in the global carbon, nitrogen and sulfur cycles. The Moco consists of a mononuclear molybdenum coordinated by the dithiolene moiety of a family of tricyclic pyranopterin structures, the simplest of which is commonly referred to as molybdopterin. Genes involved in Moco biosynthesis have been identified in eubacteria, archaea and eukarya. Although some details of the biosynthetic pathway leading to Moco formation are still unclear, the pathway can be divided into three phases. (i) Early steps in which a guanosine derivative, most likely GTP, is converted into precursor Z. This reaction is different from other pterin biosynthetic pathways, since C8 of the purine is not eliminated, but is incorporated into the pyran ring of the tricyclic pyranopterin. (ii) Transformation of precursor Z into molybdopterin, generating the dithiolene group responsible for Mo-coordination. This step has interesting parallels to the activation of ubiquitin, the first step of ubiquitin-dependent protein degradation. (iii) Metal incorporation into the apo-cofactor. This step appears to be catalyzed by MoeA and MogA. Whereas MogA and the G-domains of the eukaryotic fusion proteins bind molybdopterin with high affinity, MoeA and the E-domains display moderate affinity for molybdopterin in cooperative binding process.

Methods and Materials: Two MoeA point mutant structures (e188q, s371w) have been solved by molecular replacement method, at resolutions between 2 Å and 3 Å. Both belong to space group P212121, and contain one copy of MoeA dimer in the asymmetric unit. All diffraction data were collected on beam line X26C at the National Synchrotron Light Source at Brookhaven National Laboratory at a wavelength of 1.1 Å on a Quantum IV ADSC CCD detector.

Results: The MoeA monomer is a highly elongated club-shaped molecule and is composed of four clearly independent domains. In the crystal, MoeA is present as a dimer which is formed by interactions involving domains I, III and IV from each monomer. Although the dimer is also quite elongated, its main chain dimensions are 44 Å by 44 Å by 115 Å, it definitively has a more globular shape than the monomer. The inactivating mutations cause no major changes in the overall structure, the core regions formed by domains I, III and IV are essentially identical to the native structure. Domain II, however, is rather mobile, it undergoes rotational motions around a hinge generated by residues 49 and 153. A putative activity site of MoeA was proposed to be located in the cleft formed by domain II of monomer A and domain III/IV of monomer B, based on this point mutations of conserved residues in this putative activity site have been made. Biochemical and structural studies of these MoeA mutants (nine of which have their structure solved) provided clues of the metal incorporation mechanism accomplished by MoeA and MogA.

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References: S. Xiang, J. Nicolas, K.V. Rajagopalan and H. Schindelin, "The Crystal Structure of Escherichia coli MoeA and Its Relationship to the Multifunctional Protein Gephyrin" Structure, 9, 2001