

Crystal Structure of *Escherichia coli* MoeA

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Beamline(s): X26C, X12B

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. MoeA has been implicated in converting molybdate into a thio-molybdate derivative and MogA has been proposed to act as a molybdochelatase incorporating molybdenum into molybdopterin. 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: The structure of MoeA has been determined by multiple isomorphous replacement and has been refined at 1.95 Å resolution to an R-factor of 0.221 with an R_{free} of 0.283. The crystals belong to space group $P2_12_12_1$ with $a=88.3$ Å, $b=97.4$ Å and $c=98.7$ Å and contain two monomers in the asymmetric unit. The structure was solved by multiple isomorphous replacement using a Hg-derivative (1mM EMTS), an Ir-derivative (10 mM $\text{Na}_3[\text{IrCl}_6]$), a Sm-derivative (10 mM Sm-acetate) and seleno methionine (SeMet) substituted protein. With the exception of the SeMet derivative, 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. The SeMet data set was collected at beam line X12B at 0.98 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. The linear arrangement of domains I to III creates the extended stalk of the molecule which has an overall length of about 95 Å. A structural homology search with DALI against a non-redundant set of protein structures reveals that MogA is the closest structural relative indicating that domain III of MoeA and MogA have arisen from a gene duplication event. MoeA forms a dimer in solution as demonstrated by sedimentation equilibrium centrifugation and dynamic light scattering studies. In the crystal, MoeA is also present as a dimer which is formed by interactions involving domains I, III and IV from each monomer.

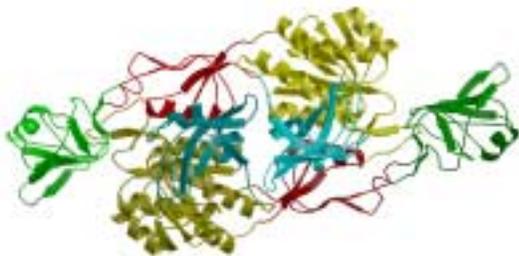


Figure 1. Structure of the MoeA dimer viewed along the twofold axis. Corresponding domains are shown in different shades of the same color: domain I in red, domain II in green, domain III in yellow and domain IV in blue.

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 two monomers have closely related structures, with an rms deviation of 0.28 Å for the Ca atoms of residues 7 to 48 and 145 to 409. Domains II, which are located on opposite ends of the dimer, however, differ in their orientation relative to the core of the dimer. Domain II undergoes a 12° rotation around a hinge generated by residues 49 and 153. A multiple sequence alignment of MoeA from different organisms including the E-domain of the eukaryotic orthologs reveals that conserved residues, which might be relevant for the function of the protein are mainly located in domains II and III.

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