Crystal Structure of the Gephyrin-related Molybdenum Cofactor Biosynthesis Protein MogA from *Escherichia coli*  

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Molybdenum cofactor (Moco) biosynthesis is an evolutionarily conserved pathway in archaea, eubacteria, and eukaryotes, including humans. Genetic deficiencies of enzymes involved in this biosynthetic pathway trigger an autosomal recessive disease with severe neurological symptoms, which usually leads to death in early childhood. The MogA protein exhibits affinity for molybdopterin, the organic component of Moco, and has been proposed to act as a molybdochelatase incorporating molybdenum into Moco. MogA is related to the protein gephyrin, which, in addition to its role in Moco biosynthesis, is also responsible for anchoring glycine receptors to the cytoskeleton at inhibitory synapses. The high resolution crystal structure of the *Escherichia coli* MogA protein has been determined, and it reveals a trimeric arrangement in which each monomer contains a central, mostly parallel β-sheet surrounded by α-helices on either side. Based on structural and biochemical data, a putative active site was identified, including two residues that are essential for the catalytic mechanism.

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. In the past few years, several crystal structures of enzymes containing this cofactor have been determined (1–4). These initial structures each define one of the four currently recognized families containing Moco (5). Moco deficiency is a severe disease in humans that usually leads to premature death in early childhood and is inherited as an autosomal recessive trait. The affected patients show neurological abnormalities such as attenuated growth of the brain, untreated seizures and often, dislocatedocular lenses. Recently, the first mutations in a number of genes encoding Moco biosynthetic proteins have been identified (6–8).  

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 at present, the pathway can be divided into three phases (9, 10). (i) In early steps, a guanosine derivative, most likely GTP, is converted into precursor Z. This reaction is different from other perin biosynthetic pathways, since C8 of the purine is not eliminated but is incorporated into the pyran ring of the tricyclic pyranopterin (11, 12). (ii) Precursor Z is transformed into molybdopterin, generating the dithiolene group responsible for molybdenum coordination. This reaction is catalyzed by molybdopterin synthase, a two-subunit enzyme (13). In the activated form of the synthase, the C terminus of the small subunit is converted to a glycine thio-carboxylate (14) that appears to be a sulfur donor for the conversion of precursor Z to molybdopterin. In turn, molybdopterin synthase is resulfurated by MoeB (10). (iii) Finally, the metal is incorporated into the apo-cofactor. Based on the observation that high concentrations of molybdate in the growth medium can partially rescue a *mogA* mutant, MogA has been proposed to act as a molybdochelatase incorporating molybdenum into molybdopterin (15). Evidence of tight binding of molybdopterin to a domain of the Cnx1 protein from *Arabidopsis thaliana* that is homologous to MogA supports this possible function of the protein (16).

In the central nervous system, gephyrin (17) is responsible for the postsynaptic anchoring of inhibitory glycine receptors to the cytoskeleton, linking the β-subunit of the receptor and tubulin (18, 19). Gephyrin also appears to be involved in the postsynaptic localization of major GABA<sub>R</sub> receptor subtypes (20). Recently, gephyrin was shown to interact with RAFT1, a DNA-activating protein kinase. Through this binding, gephyrin is also involved in rapamycin-sensitive signaling (21). Sequence analysis of gephyrin indicates that it originated from a fusion of two genes: one related to MogA (Fig. 1) and the other to MoeA, another protein involved in Moco biosynthesis. In gephyrin, these two entities are linked by an additional 160-residue domain. Although the different activities of gephyrin have not been mapped onto its primary sequence, direct participation of gephyrin in molybdenum cofactor biosynthesis in mammalian cells and plants has been demonstrated (22, 23).

We present here the purification, characterization, and high resolution crystal structure of *Escherichia coli* MogA. The 195-residue protein is folded into a compact molecule with an/a architecture and forms a trimer in both crystal forms studied. Based on the location of conserved residues, results from site-directed mutagenesis studies, and residual electron density

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‡ The atomic coordinates and structure factors (codes 1DI6 and 1DI7) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

¶ K.V. Rajagopalan et al., unpublished data.
possibly representing trace amounts of molybdopterin, we have
assigned one region of the enzyme as the putative active site.
The structure of MogA provides a framework for the inter-
pretation of amino acid substitutions leading to Moco deficiency
in humans and also represents a starting point for an understand-
ing of how the multiple activities of gephyrin are organized in
the context of its three-dimensional structure.

EXPERIMENTAL PROCEDURES
Cloning, Expression, and Purification of MogA
The E. coli mogA gene was cloned from genomic DH5α DNA with
the aid of the polymerase chain reaction. Using the published gene se-
quence (24), primers were designed to allow cloning into the
NcoI sites in the multiple cloning region of the pET-15b expression
vector (Novagen) to yield pMWgA15. In the course of cloning, the second
sequence (24), primers were designed to allow cloning into the
context of its three-dimensional structure.

FIG. 1. Sequence alignment of eight MogA proteins from different species. Gephyrin, cinnamon, and Cnx1 are considerably longer than
MogA, and only those regions corresponding to MogA are shown. Strictly conserved and similar residues are highlighted in
black respectively. Residues 72–77 (E. coli numbering) comprise the TXGAGT motif, where X is almost always a Thr. This alignment was
generated with the program ALSCRIPT (40). Secondary structure elements as determined for the NatH1 structure with the program PROMOTIF
(41) are indicated.

Nucleic acid sequences were verified by automated sequencing of both
strands. Mutant MogA proteins were purified by the same procedure as
the wild type protein with the exception that the RK5251(DE3) cell line
described below was used for expression.

Activity of MogA Proteins
Complementation of mogA E. coli Mutants—The λ DE3 lysogeniza-
tion kit from Novagen was used to integrate the gene for T7 RNA
polymerase into the chromosomes of the RK5206 and RK5231 mogA−
strains. The resulting strains, RK5206(DE3) and RK5231(DE3), were
then transformed with pWM15gA expressing either wild type or one of
the seven mutant MogA proteins. The eight RK5206(DE3) expression
strains were streaked onto Luria Broth plates and grown overnight at
30 °C. The presence of nitrate reductase activity in the cells was deter-
mined by an overlay assay (25).

Inhibition of the Reconstitution of Apo-nitrate Reductase Activity—
Wild type, D49A, and D82A MogA proteins were purified as described
above. Aliquots of xanthine oxidase (Sigma) in 25 mM potassium phos-
tate, pH 7.4, were denatured anaerobically for 1 min at 80 °C and then
used as the source of molybdopterin. Equal volumes of MogA protein (79 µM)
and molybdopterin (0.2 µM) were incubated aerobically for 5 min. Subsequently, 20-µl aliquots of this solution were mixed with 10 µl
of 0.5 M sodium molybdate and 25 µl of an extract of the nit-1 mutant of
Neurospora crassa in a total volume of 100 µl. The remainder of
the assay for nitrate reductase activity was performed as described previously (26).

Crystallization and Structure Determination
Hexagonal rods (Space group P6, with a = 65.7 Å and c = 65.1 Å)
containing 1 monomer per asymmetric unit were obtained by vapor
diffusion against a reservoir containing 1.0–1.1 M sodium citrate in 0.1
M Hepes, pH 7.5, within a few days. A second crystal form (P212121)
with a = 45.9 Å, b = 74.2 Å, and c = 166.8 Å) was grown from 16–20% PEG
4000 and 0.1 M Tris, pH 8.5. These crystals were difficult to reproduce
and were not used for structure solution. The hexagonal crystal form
was solved by multiple isomorphous replacement using a mercury
derivative (1 mM EMTS) and a platinum derivative (1 mM PIP). Initial
native (NatL) and derivative data sets were collected to resolutions of
2.5 (NatL and EMTS) and 3.3 Å (PIP) at room temperature on a Rigaku
RU 200 rotating anode x-ray generator equipped with double focusing
mirror optics and an R-axis II imaging plate detector. Data were in-
dexed, integrated and scaled with the HKL software (27). For sub-
sequent calculations, the CCP4 suite was used with exceptions as indi-
cated (28). The EMDS derivative was solved by Patterson methods
and direct methods using SHELX (29) and the PIP derivative was solved by
difference Fourier calculations. Phase refinement was performed with
SHARP (30) to a resolution of 2.5 Å followed by solvent flattening with
Crystal Structure of the Escherichia coli MogA Protein

**RESULTS**

Structure Determination—The gene encoding MogA was cloned from genomic E. coli DNA using the polymerase chain reaction. For homologous expression, the gene was inserted into the pET15b vector, and the resulting plasmid was transferred into host cells containing an isopropyl-thiogalactoside-inducible expression system. Ammonium sulfate precipitation followed by ion exchange, hydrophobic interaction, and size exclusion chromatography yielded 0.5 M (NH₄)₂SO₄ and 0.1 M Bicine, pH 9.0. Purification of the expressed wild type protein to greater than 98% homogeneity employed fractionated ammonium sulfate precipitation followed by ion exchange, hydrophobic interaction, and size exclusion chromatography.

While attempting to collect high resolution data of the hexagonal crystal form, all newly grown crystals showed merohedric twinning with twinning ratios between 0.3 and 0.5. Twinning was not present in the crystals used for structure solution. Refinement against a twinned 1.6 Å data set was attempted using SHELXL (35). However, some regions in the structure that were well defined in SIGMAA weighted 2\( F_o \) – \( F_c \) electron density maps at 2.5 Å resolution appeared fragmented in the twinned data set. A search for new crystallization conditions led to the discovery that the hexagonal crystals could also be obtained from solutions containing 2.0–2.4 M (NH₄)₂SO₄ and 0.1 M Bicine, pH 9.0. These crystals diffract x-rays to 1.4 Å resolution at beamline X26C at the National Synchrotron Light Source. Two data sets (NatH1 and NatH2) were collected to resolutions of 1.45 and 1.6 Å, respectively, with a MAR Research imaging plate detector. Refinement against both data sets was performed using a combination of X-PLOR and REFMAC (36). All data between 20 Å and the respective high resolution limits were included, and partial structure factors for the bulk solvent contribution were calculated in X-PLOR.

SOLOMON (31). The resulting electron density map was of reasonable quality and a polyalanine model of two stretches with a total of 165 out of 195 residues was built with O (32). After torsion angle dynamics refinement with X-PLOR (33) at 2.5 Å resolution, model and experimental phases were combined, and the sequence was assigned using the single Trp at position 31 as a marker. Further refinement using X-PLOR yielded a preliminary model (\( R_{	ext{cryst}} = 0.206 \) and \( R_{	ext{free}} = 0.280 \)) comprising residues 4–13 and 22–186. With this model, the orthonborhmic crystal form was solved by molecular replacement using AMORE (34).

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RESULTS

**Structure Determination**—The gene encoding MogA was cloned from genomic E. coli DNA using the polymerase chain reaction. For homologous expression, the gene was inserted into the pET15b vector, and the resulting plasmid was transferred into host cells containing an isopropyl-β-D-1-thiogalactopyranoside-inducible expression system of the T7 RNA polymerase gene. Purification of the expressed wild type protein to greater than 98% homogeneity employed fractionated ammonium sulfate precipitation followed by ion exchange, hydrophobic interaction, and size exclusion chromatography.

The crystal structure of MogA was solved by multiple isomorphous replacement using the hexagonal crystal form (Tables I and II). The structure has been refined against two different native data sets at 1.45 Å (NatH1) and 1.6 Å (NatH2) resolution to crystallographic R-factors of 0.187 (\( R_{	ext{free}} = 0.213 \)) and 0.205 (\( R_{	ext{free}} = 0.227 \)), respectively (Table II). The overall quality of both models is very good as judged by the low free R-factors, the low deviations from stereochemical ideality and the appearance of the Ramachandran diagram. For the two models, 95.4 and 91.0% of the residues were found in the most favored regions of the Ramachandran diagram as defined by PROCHECK. The overall dimensions of the slightly ellipsoidal molecule are 47 × 32 × 37 Å. An interesting region in the structure is located between residues 135 and 169. Residues 135–144 form a 6-stranded \( \alpha \)-helix on one side and four \( \alpha \)-helices and a 310 helix on the opposite side (Fig. 2, A and B). The single anti-parallel strand (\( \beta \)) is located near one edge of the \( \beta \)-sheet. The overall secondary structure of the MogA molecule is a trimer (Fig. 2 C). In the hexagonal crystal form, the trimer is formed by a crystallographic 3-fold axis, whereas the trimer constitutes the content of the asymmetric unit in the orthonborhmic crystal form. Analytical ultra-

### Table I

| Data collection statistics |
|---------------------------|
|                           |
| \( R_{	ext{cryst}} = \frac{\sum |I - \langle I \rangle|}{\sum I} \) |
| \( R_{	ext{free}} = \frac{\sum |I - \langle I \rangle|}{\sum I} \) |
|                           |
| NatH1                    |
| NatH2                    |
| NatL                     |
| EMTS                     |
| PIP                      |
| Maximum resolution (Å)   | 1.45 | 1.6 | 2.5 | 2.5 | 3.3 |
| Completeness             | 0.998 (1.00) | 0.915 (0.977) | 0.984 (0.936) | 0.954 (0.821) | 0.981 (0.978) |
| Mean redundancy           | 7.4 | 4.4 | 4.2 | 2.4 | 2.9 |
| \( R_{	ext{cryst}} \) (\( \langle I \rangle \)) | 0.058 (0.477) | 0.052 (0.289) | 0.054 (0.326) | 0.087 (0.205) | 0.127 (0.465) |
| Mean figure of merit (Å) | 19.4 (3.1) | 18.0 (4.0) | 12.3 (2.5) | 13.1 (5.1) | 9.9 (2.9) |

### Table II

| Multiple isomorphous replacement and refinement statistics |
|----------------------------------------------------------|
|                                                         |
| Phasing power                                           | 2.31 | 1.26 |
| Number of reflections                                    | 28063 | 18448 |
| Number of protein/solvent atoms                          | 1406/143 | 1403/160 |
| \( R_{	ext{cryst}} \) (\( R_{	ext{free}} \)) | 0.187 (0.213) | 0.205 (0.227) |
| Deviations from ideal values in Bond distances            | 0.012 | 0.012 |
| Angle distances                                          | 0.028 | 0.027 |
| Ramachandran statistics                                  | 0.954/0.039/0.0/0.007 | 0.910/0.084/0.0/0.006 |
FIG. 2. Ribbon representations of the MogA structure. A, the MogA monomer viewed perpendicular to the central \( \beta \)-sheet. \( \beta \)-Strands are shown as curved arrows in green, and \( \alpha \)-helices and the \( 3_{10} \) helix are shown as ribbons in red and blue, respectively. Secondary structure elements, N and C termini, and the residues adjacent to the disordered loop are labeled. The sulfate molecule bound near the TXGGTG motif is indicated. B, the MogA monomer viewed along the \( \beta \)-sheet and superimposed with a transparent surface representation of the protein. Note the pocket in the molecular surface located between \( \alpha 5 \) and the \( 3_{10} \) helix. C, structure of the MogA trimer viewed along the 3-fold axis. Each color represents a different monomer. In addition to the sulfate, the side chains of the strictly conserved residues Asp-49 and Asp-82 are shown. Figs. 2, 3B, and 5B were produced with Molscript (42) and Raster3D (43).
centrifugation studies of MogA yield a molecular mass of 59,675 Da, consistent with a trimeric arrangement of a protein with a monomer molecular mass of 21,048 Da. The trimer is formed by interactions involving residues primarily located in β-strands 4 and 5, α4 and the 310 helix (residues 94–124), the loop comprising residues 77–83, and residues 156–169, including helix α6. Approximately 1400 Å² of accessible surface area is buried upon trimerization, which corresponds to 16% of the molecular surface of each monomer. The interface between two monomers has polar interactions with a total of 7 hydrogen bonds in each monomer-monomer interface, as well as a hydrophobic core including Pro-78, Met-96, Pro-97, Phe-99, Pro-112, Ile-115, Leu-116, His-156, Tyr-164, Cys-165, Leu-168, and Leu-169. Several of these residues are type-conserved (Fig. 1), suggesting that MogA from other organisms might also form a trimer.

Structural Homologues—A search for structural homologues of MogA with the program DALI (38) revealed a rather large number of related structures (102 structures with a Z-score greater than 3). The best matches were the N-terminal domain of the receptor-negative regulator of the amidase operon (Protein Data Bank entry 1pea), the N-terminal domain of the leucine/isoleucine-valine-binding protein (Protein Data Bank entry 2liv), and the C-terminal domain of methylmalonyl-CoA mutase (Protein Data Bank entry 1req-A). Interestingly, residues 94–124 in MogA, which include strands β4 and β5 as well as a4 and the 310 helix, have no equivalent residues in these matches. In the context of the three-dimensional structure of MogA, this part of the sequence can be viewed as an insertion, which allows MogA to trimerize (see above). Despite a significantly lower Z-score, one additional match is worth mentioning: the periplasmic molybdate transport protein ModA, which is involved in molybdate uptake. Like the receptor-negative regulator of the amidase operon and the leucine/isoleucine-valine-binding protein, ModA is a two-domain protein, and MogA shares similarity with only one of the domains.

Residual Density Feature—An interesting aspect of the crystal structure is the presence of an unusual electron density feature shown in Fig. 3A near the highly conserved TXGGTG motif (residues 72–77 in E. coli MogA). This density can be explained by neither protein atoms nor water molecules. Due to its tetrahedral appearance and the presence of high sulfate concentrations in the mother liquor, this feature has been modeled as sulfate. However, there is additional density extending from one of the sulfate oxygens, which might indicate that substoichiometric amounts of molybdopterin or a related
compound remained bound to the enzyme during purification. The tetrahedral density feature could thus mark the position of the terminal phosphate group of molybdopterin. Given the close structural similarity between the anions sulfate, phosphate and molybdate, the question arises whether this binding site could also accommodate molybdate. Two lines of evidence argue against this possibility. (i) Atomic absorption spectroscopy of purified MogA failed to detect the presence of molybdenum both before and after equilibrium dialysis against sodium molybdate (data not shown). (ii) Refinement against a 2.5 Å resolution data set collected from a crystal grown from citrate and 50 mM Na₂MoO₄ did not show bound molybdate at any of the three sulfate binding sites. Together, these results indicate that MogA does not bind free molybdate. Failure to bind molybdate has been previously described for the MogA-homologous domain of Cnx1 (16).

Conformational Changes—A comparison of the two models derived from the 1.45 and 1.6 Å data sets reveals surprisingly large conformational changes for residues between positions 107 and 113. These residues form the C-terminal end of α4 and the N-terminal half of the 3₁₀ helix. Although the overall root mean square deviation between the main chain atoms of both models is 0.4 Å, the deviation is substantially larger (up to 3.9 Å) for the residues in this region, as shown in Fig. 3B. In the NatH1 structure, residues 111–117 form a 3₁₀ helix, which is shortened in the NatH2 form by two residues at its N terminus. Small structural changes are also present in the β-hairpin formed by residues 145–156, which could be a consequence of its close proximity to the side chains of His-109 and Phe-110. One additional structural change involves the side chain of Asp-49, which adopts different side chain conformations in the two models. The NatH1 and NatH2 data sets were collected from crystals grown from two different MogA protein preparations under slightly different crystallization conditions. The NatH1 crystals were grown from 2.0–2.1M (NH₄)₂SO₄, compared with 2.3–2.4 M (NH₄)₂SO₄ for the NatH2 crystals. Either difference or a combination of both might explain the structural differences between the two models.

Site-directed Mutagenesis and Molybdopterin Binding Studies—In order to characterize the functional significance of some of the strictly conserved residues in MogA (Fig. 1), site-directed mutagenesis was employed to replace the following residues with alanine: Ser-12, Asp-49, Thr-76, Arg-81, Asp-82, Ser-107, and Ser-117. The effects of these single amino acid substitutions were analyzed by functional complementation of a mutant E. coli strain, RK5206(DE3), in which the chromosomal copy of mogA has been inactivated by mu insertion. Complementation of this strain with wild type MogA results in the production of active nitrate reductase, a Moco-containing protein the activity of which is dependent on the ability of cells...
to synthesize Moco. Complementation can be easily scored on plates using an overlay assay for formate-dependent nitrate reductase activity (25). Whereas expression of the S12A, T76A, R81A, S107A, and S117A variants resulted in complementation comparable to that observed with expression of the wild type protein, expression of the D49A and D82A MogA variants resulted in no complementation, as shown in Fig. 4A. Schwarz et al. (16) have demonstrated tight binding of molybdopterin to the MogA-homologous portion of the plant protein Cnx1. Similar results were obtained upon incubation of the purified E. coli protein with molybdopterin (data not shown.) Additional evidence for binding of molybdopterin to MogA was provided by the ability of the pure protein to inhibit the Moco-mediated reconstitution of apo-nitrate reductase. This reconstitution assay is normally used to detect the presence of molybdopterin in a sample and uses a crude extract of the Neurospora crassa mutant, nit-1, as a source of apo-nitrate reductase (26). To further clarify the role of Asp-49 and Asp-82 in MogA function, their ability to inhibit this reconstitution was explored. As seen in Fig. 4B, the D49A and D82A protein variants inhibit reconstitution to a greater extent than does the wild type protein. These results suggest that the two mutant proteins may actually bind molybdopterin tighter than the native protein. Hence, the lack of complementation observed for the two Asp to Ala variants (Fig. 4A) cannot be attributed to a decreased binding of molybdopterin to MogA but rather suggests that these residues are essential for the catalytic mechanism.

DISCUSSION

An analysis of the degree of sequence conservation between 8 MogA proteins in the context of the three-dimensional structure of the MogA monomer suggests that a region at the C-terminal end of strands \( \beta_1 \), \( \beta_2 \), \( \beta_3 \), and \( \beta_5 \) is of functional importance (Fig. 5A). This area includes the density feature near the conserved TXTGTTG motif and the region undergoing the pronounced conformational changes and could, therefore, define the active site location. The view of the molecular surface also reveals the existence of a pocket in the surface near this region. Residues 107–113 form one wall of the pocket, and due to the conformational changes, the size of this pocket differs between the two models. The size and shape of the pocket appear suitable for binding the pterin moiety of molybdopterin; however, the residues forming the pocket are not as...
strongly conserved as the region directly adjacent. This could be an indication that if the pterin moiety binds to the pocket, it interacts predominantly through hydrogen-bonded interactions with main chain atoms of the protein. In the crystal structures of enzymes containing the molybdenum cofactor (4, 5), roughly half of all the hydrogen bond interactions to the pterin moiety are mediated by main chain atoms. In addition, the mobile and partially disordered loop region (residues 15–21) could contribute to molybdopterin binding.

Adjacent to the pocket is the region with the highest degree of sequence conservation, including the strictly conserved residues Ser-12, Asp-49, Thr-76, Arg-81, Asp-82, Ser-107, and Ser-117, which have been all substituted by Ala. Two of the seven substitutions (D49A and D82A) were unable to complement a mutant E. coli strain in which the chromosomal copy of MogA had been disrupted. It should be noted that more subtle changes in binding characteristics of the other protein variants might not be detected by this assay. Additional studies revealed that the two Asp to Ala variants inhibit apo-nitrate reductase reconstitution to a greater extent than the wild type protein, suggesting that these two variants bind molybdopterin more tightly. The biochemical results further support the assignment of the putative active site to the region of MogA shown in Fig. 5A, and more importantly, they suggest an important function for the two aspartic acid residues at positions 49 and 82. In the three-dimensional structure of MogA, these two Asp residues are in close proximity to each other and the conserved TXGGTG motif. As described earlier and shown in Fig. 5B, the side chain of Asp-49 adopts a different conformation in the two high resolution structures. In NatH1, Asp-49 is pointing away from Asp-82, and two well ordered water molecules are located between the side chains of the two aspartic acid residues. By contrast, in the NatH2 crystal, the two residues are in hydrogen-bonded contact at a distance of 2.8 Å.

The functional discussion presented here focuses on the MogA monomer because the region in question is predominantly formed by residues from a single monomer and is easily accessible by compounds similar in size to molybdopterin without necessitating any conformational changes in the MogA trimer. As is evident from Fig. 2C, the C terminus (residues 184–191) of an adjacent monomer is closest to the conserved surface region shown in Fig. 5A. Because the level of sequence conservation is extremely low beyond residue 170 (see Fig. 1), it seems unlikely that the C terminus is important for MogA function or will be structurally conserved. Furthermore, depending on crystallization conditions, structural differences are observed in the C-terminal region of E. coli MogA indicating conformational flexibility. The C terminus is stabilized by one of the additional sulfate molecules (not the one bound near the TXGGTG motif) in the crystals obtained with ammonium sulfate as precipitant, whereas it is more mobile in the crystals grown from citrate. Most importantly, in both high resolution structures, all atoms within this C-terminal region are separated by at least 10 Å from any of the strictly conserved residues listed above. In addition to the C-terminal region, helix α6, located beneath the C-terminal residues, is relatively close to the conserved surface of an adjacent monomer. Within this helix, the side chain of Tyr-164, which is conserved among bacterial MogA sequences, is pointing toward the conserved region and is within about 8 Å distance from either Asp-49 or Asp-82. However, this residue is not conserved in eukaryotes, in which it has been replaced by the shorter histidine, suggesting that this interaction is not of functional significance either.

These findings strongly suggest that each monomer within the MogA trimer functions independently and that each putative active site is confined to a single monomer.

If MogA acts as a molybdochelatase as previously postulated, it would require binding of both molybdopterin and a molybdenum-containing compound. Although binding of molybdopterin has now been demonstrated for both the E. coli MogA protein and the orthologous plant protein Cnx1 (16), binding of molybdate could not be demonstrated for either protein. Molybdenum enters the cell as the stable oxyanion molybate, and it is possible that molybate has to undergo some type of modification prior to incorporation into Moco within the cell. A recent study (39) has suggested a role for the MoeA protein in generating a thio-molybdenum containing compound that might be used in Moco biosynthesis. Interestingly, the fusion of MoeA and MogA into a single polypeptide chain in plants, Drosophila, and humans suggests that a complex of the two proteins may participate in the last step of Moco biosynthesis. Spatial proximity of MogA and MoeA could be a requirement for this last step, particularly if reaction intermediates produced by either protein have a limited stability. The high degree of sequence conservation on one side of the MogA surface (Fig. 5A) could therefore be an indication that this part of the molecule is also involved in interactions with MoeA. It is of interest to note that the MoeA homologous domains of Cnx1 and gephyrin have been reported to bind molybdopterin independently, although with lower affinity than the MogA homologous domains (16, 23). On the other hand, the full-length proteins (Cnx1 and gephyrin) bind molybdopterin with high affinity but show different binding characteristics involving cooperativity compared with the isolated MogA-homologous domains (16, 23). In the context of the MogA structure, the latter data seem to suggest that the molybdopterin-binding site is near the putative MoeA binding site. Hence, the region displayed in Fig. 5A could comprise both the MogA active site, including the molybdopterin binding site, and the region interacting with MoeA.

We have described the purification, characterization, and high resolution crystal structure of the E. coli MogA protein. Although the structures of a number of enzymes containing Moco have been recently reported (1–4), the work presented here describes the first structure for any protein involved in Moco biosynthesis. The assignment of an active site location for MogA based on several lines of evidence provides a framework for further characterization of the biochemical function of this protein, as well as the role of individual amino acids during catalysis. Although the first mutations in genes involved in the early steps of Moco biosynthesis in humans have recently been described (6–8), no mutations in the gene encoding gephyrin, the fusion protein of MogA and MoeA, have been identified to date. Nevertheless, the structure of MogA provides a starting point for the analysis of possible point mutations in gephyrin among patients suffering from Moco deficiency. In addition, it serves as a first step toward understanding the additional functions of gephyrin, such as receptor anchoring and rapamyacin-sensitive signaling.

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