The crystal structure of *Escherichia coli* MoaB was determined by multiwavelength anomalous diffraction phasing and refined at 1.6-Å resolution. The molecule displayed a modified Rossman fold. MoaB is assembled into a hexamer composed of two trimers. The monomers have high structural similarity with two proteins, MogA and MoeA, from the molybdenum cofactor synthesis pathway in *E. coli*, as well as with domains of mammalian gephyrin and plant Cnx1, which are also involved in molybdopterin synthesis. Structural comparison between these proteins and the amino acid conservation patterns revealed a putative active site in MoaB. The structural analysis of this site allowed to advance several hypothesis that can be tested in further studies.

Molybdoenzymes are ubiquitous mediators of many essential redox reactions (1, 2). All molybdoenzymes except nitrogenase contain a molybdalenium cofactor (Moco) that consists of a mononuclear molybdalenium covalently coordinated by molybdopterin (3, 4). In eubacteria, Moco is often modified by various nucleotides. In *Escherichia coli*, some of the molybdoenzymes contain a modified form of Moco known as molybdopterin guanine dinucleotide (MGD) (5). In some others, the metal ion is often coordinated by two MGDs (6, 7).

The biosynthesis of MGD (see Fig. 1) is catalyzed by the products of the *moa*, *mob*, and *moe* operons, as well as the product of the *moga* (8) and *mobA* (9–11) genes. In eukaryotes, the homologs of Moga and MoeA are fused to form a single multifunctional multidomain protein (12), called gephyrin in mammals (13), cinnamon in *Drosophila melanogaster* (14), and Cnx1 in *Arabidopsis thaliana* (15).

Mutants in the *E. coli* MGD biosynthetic pathway are viable under experimental conditions, albeit with compromised phenotypes (16, 17). In humans, mutations in the enzymes of Moco biosynthesis result in a Moco deficiency, leading to several neurological complications and subsequent death at a young age (18).

The structures of members of the *E. coli* Moco biosynthesis pathway, *MoaC* (17), *MogA* (19), *MobA* (10, 11), *MobB* (20), *ModE* (13), *MoeA* (21, 22), and molybdopterin synthase (*MoaD*-*MoaE*) (23), have been determined to clarify their roles in the pathway (Fig. 1). However, the exact enzymatic activities of these proteins are not well understood. Similarly, very little is known about the biochemical and physiological roles of the MoaB protein, although it is believed that MoaB may be involved in the synthesis of precursor Z (8). BLAST data base (24) searches also indicate the involvement of MoaB in the biosynthesis of Moco. Here we report the crystal structure of *E. coli* MoaB at a 1.6-Å resolution. Amino acid sequence and structural similarities with other members of the Moco pathway strengthen the possibility of a role for MoaB.

**EXPERIMENTAL PROCEDURES**

**Cloning and Protein Purification—** *E. coli* MoaB was cloned, expressed, and purified using standard procedures (25). The hexahistidine tag was removed before crystallization. The purified MoaB protein was dialyzed against 10 mM HEPES, pH 7.5, 500 mM NaCl, and concentrated using a BioMax concentrator (Millipore). Before crystallization, any particulate matter was removed from the sample by passage through a 0.2-μm Ultrafree-MC centrifugal filtration device (Millipore).

**Crystallization and X-ray Data Collection—** Two microcrystals of the dialyzed MoaB protein were mixed with an equal volume of precipitant containing 2.0 M ammonium sulfate and 20% (v/v) ethylene glycol. Droplets were equilibrated against the precipitant with hanging drop vapor diffusion, and crystals grew after 2–5 days at 21 °C. For diffraction studies, the crystals were flash-frozen in liquid nitrogen with 25% (v/v) ethylene glycol added to the crystallization buffer as the cryoprotectant. The crystals belonged to the hexagonal space group P321 with the cell parameters at 100 K being *a* = *b* = 69.11 Å, *c* = 126.22 Å, *α* = *β* = 90°, *γ* = 120°, and contained two molecules of protein/asymmetric unit. The Matthews coefficient (26) was 2.33, which corresponds to the solvent content of ~46% in crystals.

A two-wavelength anomalous dispersion experiment with crystals of Se-Met-derivatized protein was carried out on the 18ID beamline of the Structural Biology Center Collaborative Access Team of the Advanced Photon Source (Argonne National Laboratory, Argonne, IL). The absorption peak and the rising inflection point were determined by calculating and plotting I and F* values against energy (27) from the fluorescence spectrum measured for a test crystal. Later, higher resolution data were collected from the unexposed part of the same crystal, which had been stored in liquid nitrogen. All crystallographic data were measured with the custom built 3 × 3 tilted charge-coupled device detector (28) with a 210 × 210-μm² active area and fast duty cycle (~1.7 a). The experiment, data collection, and visualization were controlled with d*TREK* (29), and all data were integrated and scaled with the program package HKL2000 (30). Some of the basic statistics of data collection and processing are provided in Table I.

Guanosine Triphosphate Binding—Purified recombinant MoaB pro...
tein was subjected to binding assays using reaction conditions consisting of 0.7 mg/ml protein, 50 mM KCl, 100 nCi [α-32P]GTP (3000 Ci/ mmol, PerkinElmer Life Sciences), and 50 mM pH buffer (sodium acetate, pH 5.0, MES, pH 6.5, Tris, pH 7.5, Tris, pH 8.0) in a 50-μl reaction volume. Divalent cations were added as MgCl2, CaCl2, or MnCl2 to a concentration of 5 mM. After 15 min at 30 °C, reactions were stopped, and the protein was precipitated by the addition of an equal volume of saturated ammonium sulfate, and dried. The protein was then resuspended in 10 μl of binding buffer, and the precipitated GTPase activity was essentially as previously described (31). Briefly, pelletized proteins were resuspended in binding buffer and hydrolyzed by adding perchloric acid to 6% concentration for 15 min. The pH was adjusted to 3.5 with potassium phosphate, the reactions were clarified by centrifugation, and aliquots were spotted on polyethyleneimine-cellulose TLC plates (Selecto Scientific Inc.). A GDP standard was enzymatically derived by the incubation of [γ-32P]GTP substrate with thermosensitive alkaline phosphatase (Invitrogen) in the manufacturer’s supplied reaction buffer. Separation of phosphorylated reaction products was achieved by TLC using 0.65 M KH2PO4 as running buffer. Plates were dried and exposed to Fuji RX film.

RESULTS

Structure Solution and Refinement—Initially, only two Se sites, one from each of the molecules in the asymmetric unit, were found with the CNS (32) program, and phases were calculated to a 2.5-Å resolution. An inspection of the log-likelihood gradient maps revealed the non-isotropic nature of these sites. After phase refinement and accounting for this anisotropy, two other Se sites were also located. However, they were disordered to the extent that the phases did not improve significantly (Table II). Experimental phases were extended from 2.5- to 2.0-Å resolution with density modification, using data collected at the inflection point of the absorption edge.

The initial model for the most of both molecules was built with the program ARP/wARP (33). The remainder of the model was built and all side chains were corrected manually. This model was then refined against the 1.6-Å resolution data with density modification, using data collected to the extent that the phases did not improve significantly (Table II). Experimental phases were extended from 2.5- to 2.0-Å resolution with density modification, using data collected at the inflection point of the absorption edge.

The structural coordinates have been deposited in the Protein Data Bank (36) with the access code 1MKZ.

Spatial Model of MoaB—The current model of MoaB consists of two crystallographically independent molecules, A and B. Molecule A contains 170 amino acid residues and has Met3 and Ser54 absent. Molecule B has 171 residues, with Met1 absent. Gly171 and Ser172, adducts of cloning, were clearly visible in the electron density maps of both molecules. The Arg22 and Glu100 side chains in molecule A were incomplete because of poor

**Table I**

Basic data collection and processing statistics for MoaB crystals

| Infection point | Peak | Low resolution | High resolution |
|-----------------|------|----------------|-----------------|
| Energy (keV)    | 12.62934 | 12.66075 | 12.000 | 12.000 |
| Wavelength (Å)  | 0.97954 | 0.97929 | 1.03231 | 1.03231 |
| Resolution (Å)  | 50–1.87 | 50–1.87 | 50–2.7 | 3.9–1.54 |
| No. of observations | 311,387 | 311,440 | 60,562 | 251,553 |
| Unique reflections | 55,534 | 55,494 | 8,840 | 45,003 |
| Completeness (%) | 99.9 (99.0) | 99.2 (98.0) | 86.8 (90.2) | 99.5 (95.8) |
| I/σ(I) | 26.5 (2.5) | 26.2 (2.3) | 28.9 (14.5) | 22.4 (2.6) |
| Rsym | 0.057 (0.45) | 0.06 (0.52) | 0.056 (0.137) | 0.087 (0.41) |

* Bijvoet pairs for scaling the multiwavelength anomalous dispersion data sets were kept separately.

* Statistics in the last resolution shell are in parentheses.
electron densities, and in molecule B the Ser$^2$, Gln$^3$, Arg$^{21}$, Arg$^{22}$, Glu$^{81}$, and Glu$^{112}$ side chains were incomplete for the same reason.

The following side chains were modeled in alternative conformations, Molecule A, Thr$^4$, Gln$^{24}$, Gln$^{25}$, Thr$^{26}$, Gln$^{26}$, Glu$^{61}$, Arg$^{77}$, Ser$^{78}$, Gln$^{110}$, Thr$^{111}$, and Ile$^{112}$ and molecule B, Thr$^4$, Thr$^{24}$, Leu$^{25}$, Arg$^{26}$, Arg$^{61}$, Glu$^{110}$, Se-Met$^{112}$, Thr$^{111}$, Ile$^{112}$, and Arg$^{116}$. Phe$^{54}$ in the hydrophobic core of the molecule was replaced by leucine, probably as a mutation introduced by a polymerase chain reaction. Seven sulfate ions, three acetate molecules, and 238 water molecules were also revealed in the model. Four of the sulfates are bound to molecule A and three to molecule B.

The MoaB monomer is $32 \times 35 \times 50$ Å$^3$ and consists of a six-strand $\beta$ sheet, six $\alpha$ helices, and one $3_{10}$ helix packed in a modified Rossmann fold (Fig 2A). The twisted $\beta$ sheet with a $\beta_1 \parallel \beta_2 \parallel \beta_3 \parallel \beta_4 \parallel \beta_5 \parallel \beta_6$ topology is formed by five parallel $\beta$ strands, $\beta_2$ (residues 43–50), $\beta_1$ (12–18), $\beta_3$ (72–76), $\beta_6$ (131–136), $\beta_5$ (97–98), and one antiparallel $\beta_4$ (124–128) inserted between $\beta_5$ and a very short $\beta_4$. The $\beta$ sheet is sandwiched between four $\alpha$ helices, $\alpha_1$ (residues 23–40) and $\alpha_4$ (139–150) on one side and $\alpha_2$ (53–67) and $\alpha_5$ (86–92) on the other. The three remaining helices are $\alpha_3$ (99–115), a one-turn $\alpha_6$ (164–168), and a $3_{10}$ helix (116–121). Helices $\alpha_1$ and $\alpha_5$ are distorted to various degrees.

Each of the molecules in the asymmetric unit is incorporated in a compact trimer generated by the 3-fold crystallographic axis. In this formation, each monomer interacts with two other monomers (Fig 2A). The buried surface area within each pair of monomers in the trimer is $\sim 1700$ Å$^2$, whereas the total surface area of a monomer is $\sim 8400$ Å$^2$. The major contributor to trimer stability is the insertion of the $3_{10}$ helix of one monomer into the hydrophobic groove of another, which is formed between helices $\alpha_4$, $\alpha_5$, and the loop connecting $\alpha_5$ and $\alpha_6$. There is also a network of hydrogen bonds and salt bridges between each pair of monomers (Table III).

Two trimers form a hexamer, mostly through hydrophobic interactions at their interface. Size-exclusion chromatography showed that MoaB is a hexamer in solution as well (Fig 3). In crystals, hexamerization buries a total of 2680 Å$^2$ (32%) of the surface of each monomer. Strong interactions in the observed hexamer, together with the conservation of some residues involved in its formation (Table III), lead us to believe that the oligomeric state of MoaB in crystals is the same as in solution and is biologically relevant.

The shape of the hexamer can be roughly described as an $\sim 62$-Å-high cylinder with a radius of $\sim 35$ Å. It has an equatorial groove formed between the trimers and three vertical clefts each formed by four molecules of the hexamer (Fig 2B). The vertical clefts of the hexamer are lined with polar and mostly positively charged residues. The sides of the hexamer between each pair of vertical clefts are mostly neutral but with some negatively charged and a considerable number of hydrophobic residues. Three holes, $\sim 8 \times 11$ Å, on the sides of the hexamer lead to a large cave inside of it. The bases of the cylinder are rather flat, formed by three pairs of helices $\alpha_1$ and $\alpha_5$ from each monomer of the trimmer. Judging by the area of buried surface and the number and character of interactions, the trimmer appears to be a more stable formation than the hexamer.

### Table II

| Phasing and refinement statistics for MoaB structure |
|-----------------------------------------------------|
| **Phasing in 50–2.5-Å resolution shell** |
| Inflection point | Peak | FOM** |
|------------------|------|-------|
| Number of reflections | 17,750 (1754) | 22,885 (2607) | 23,021 (2641) |

**Mean FOM after density modification in 50–2.0-Å shell was 0.92.**
Analysis of Structural Similarities and the Putative Active Site—A structural similarity search using the DALI algorithm (37) indicated that MoaB shares a common fold with several proteins also involved in Moco synthesis. MoaB had highly significant similarity with the G domains of the multifunctional mammalian protein gephyrin (12, 38), the plant protein Cnx1 (38), with E. coli MogA (19), and with the largest domain of MoA (21, 22). Z-scores were 22.0, 21.9, 19.5, and 10.9, respectively. The corresponding root mean square deviations with these homologous structures were 1.38 Å for the 150 aligned C\(^{\alpha}\) atoms, 1.47 Å for 147 C\(^{\alpha}\) atoms, 1.73 Å for 147 C\(^{\alpha}\) atoms, and 1.88 Å for 126 C\(^{\alpha}\) atoms.

In addition to the structural conservation of several proteins along the Moco pathway, oligomerization of these proteins is also remarkably well preserved (Fig. 4). Thus, the functional trimer of MogA (19) is also seen in gephyrin and Cnx1 oligomers, although the latter two are multidomain proteins. The MoaB hexamer comprises two such trimers (Fig. 4C).

The extent of structural similarity between MoaB and the gephyrin and Cnx1 proteins is not reflected in amino acid sequence similarity. The sequence identities of MoaB with the G domains of Cnx1 and gephyrin, with MogA, and with the largest domain of MoA are 33, 32, 27, and 21.5%, respectively. The structural similarity between MoaB and two other proteins from the Moco synthesis pathway, MoaC and MobA, is significantly lower, with respective root mean square deviations of 3.22 Å for 57 C\(^{\alpha}\) atoms and 3.38 Å for 72 C\(^{\alpha}\) atoms.

A number of residues are highly conserved among MoaB orthologs, Asp\(^{77}\), Gly\(^{78}\), Asp\(^{85}\), Gly\(^{89}\), Gly\(^{138}\), Ser\(^{139}\), and the motif Gly\(^{77}\)-Gly\(^{78}\)-Thr\(^{79}\)-Gly\(^{80}\) are invariant in MoaB proteins from 22 genomes. Pro\(^{137}\), which is conserved in all but two of these sequences forms another conserved motif Pro\(^{137}\)-Gly\(^{138}\)-Ser\(^{139}\). Sixteen other residues are similar, indicating conservation of mostly hydrophobic and a few polar interactions. These residues are Ile/Leu/Val\(^{15}\), Leu/Val/Ala, Ala/Thr\(^{87}\), Leu/Val/Ile\(^{94}\), Phe/Leu/Ile\(^{95}\), Arg/Lys\(^{97}\), Phe/Ile\(^{102}\), Phe/Ile\(^{106}\), Arg/His\(^{107}\), Ser/Thr\(^{121}\), Arg/Lys/Arg\(^{122}\), residues 192–134, Met/Leu/Thr\(^{136}\), and Ile/Leu/Val\(^{150}\).

When the MoaB sequence is aligned with the sequences of four other Moco synthesis proteins (referred to here as the Moco synthesis group), E. coli MogA, mammalian gephyrin, insect cinnamon, and plant Cnx1, 19 residues are invariant and a number of others show strong conservation (Fig. 5). Most of the invariant residues in the MoaB orthologs and the Moco synthesis group overlap, and only a few belong to either group exclusively. For example, the amino acids in positions (according to the MoaB sequence) 57, 90, 98, and 119 are invariant only in the Moco synthesis group, whereas the residues Gly\(^{100}\), Gly\(^{126}\), and Ser\(^{119}\) are highly conserved in MoaB orthologs only.

All of the invariant residues throughout the MoaB orthologs and the Moco synthesis group are in or near the depression on the surface of MoaB between two monomers of the MoaB trimer (Fig. 2B) where two of the bound sulfates are found (Figs. 2B and 6). A very similar depression is found in the MogA, gephyrin, and Cnx1 structures.

The polypeptide segments in the area of the MoaB surface depression are structurally the most preserved. These segments are formed by two motifs, Gly\(^{77}\)-Gly\(^{78}\)-Thr\(^{79}\)-Gly\(^{80}\) and Pro\(^{137}\)-Gly\(^{138}\)-Ser\(^{139}\). In both the MoaB and MogA structures, these motifs are responsible for binding one of the sulfates (Fig. 6B). The Ser\(^{121}\) side chain, using the MoaB nomenclature, also contributes to binding. This residue is Ser or Thr in all MoaB orthologs conserving the interacting hydroxyl group.

It is likely that these structurally conserved sites are occupied by phosphate groups of physiological compounds, a conjecture that is supported by the electron density map. As can be seen on Fig. 7, the electron density in this region cannot be entirely accounted for by Pho1. Remarkably, a very similar feature can be found in the structure of MogA. It has been suggested (19) that this could be a remnant of some phosphate-containing compound that was bound to the protein strongly enough so as not to be completely dissociated during purification and crystallization. Another sulfate in our structure is contained in a conserved depression (see Figs. 6 and 7). This region is generally quite flexible as indicated by high B factors. Part of this binding site is missing in the MogA model due to disorder as is the second bound ion.
Two more sulfate ions in molecule A and one in molecule B are bound at the C terminus. It is difficult to assess the physiological importance of these ions, because two of the residues at this end are adducts of cloning.

**Labeled GTP Binding in Solution**—Our modeling studies indicate that GTP may bind in the putative active site of MoaB. To establish reaction conditions for potential GTP binding by the MoaB protein, pH was modulated from 5.0 to 8.0, and [γ-32P]GTP binding was monitored by protein precipitation followed by scintillation counting. GTP binding was most efficient at pH 7.5; therefore, reactions were performed at pH 7.5. Between 5 and 20% of input radioactivity was recovered, compared with less than 1% in mock reactions containing no protein, indicating the binding of GTP. Various divalent cations were added to determine their effect on MoaB GTP binding. The results showed that Mg²⁺ had no impact on binding, Ca²⁺ was inhibitory, and Mn²⁺ was stimulatory (Fig. 8A). GTPase activity was assessed by monitoring reaction products with thin-layer chromatography (Fig. 8B). Ca²⁺ again appeared to decrease overall activity, whereas Mn²⁺ acted both to increase overall binding and to modulate the ratio of GDP to GTP bound to the MoaB protein. In the presence of all three cations (Mg²⁺, Ca²⁺, and Mn²⁺) simultaneously, the stimulatory and modulatory effects of Mn²⁺ were abated.

**DISCUSSION**

The exact role of MoaB in Moco biosynthesis is unknown. Strong structural similarity of MoaB to MogA and to G domains of mammalian gephyrin and plant Cnx1 (Fig. 4), which are also involved in the Moco biosynthesis, together with the sequence (Fig. 5) and spatial (Fig. 6) conservation of several residues, suggests a conserved function among these proteins. Although the bound molybdopterin has not been observed crystallographically, MogA, mammalian gephyrin, and plant Cnx1 bind to it with high affinity (12, 19, 39). The high degree of sequence and structural conservation in the surface depression suggests that this cavity may be a consensus binding site for molybdopterin.

Liu et al. (19) mutated several highly conserved residues in MogA to Ala to study their possible role in function. The MogA residues studied were Ser¹², Asp⁴⁹, Thr⁷⁶, Arg⁸¹, Asp⁸², Ser¹⁰⁷, and Ser¹¹⁷; the corresponding residues in MoaB are Ser¹⁹, Glu⁵², Thr⁷⁹, Arg⁸⁴, Asp⁸⁵, Ser¹⁰¹, and Ser¹²¹, many of which are also well conserved (Figs. 4–6). The only mutations that had a measurable effect on MogA activity were D49A and D82A. They were assumed to disrupt catalysis, because molybdopterin binding in the two mutant proteins was shown to be improved (19). The corresponding residues in MoaB, Glu⁸² and Asp⁸⁵, are highly conserved both in primary and in tertiary structures and could play a similar role. In MoaB, the proposed catalytic site contained electron density that was modeled as a sulfate ion but was proposed to correspond to the remnants of molybdopterin (19). In the MoaB structure, we observed very similar electron density (Fig. 7). Thus, the overall conservation of the binding site and of the interaction with the bound anion suggests that MoaB might bind MTP similarly to MogA, gephyrin, and Cnx1.

The two presumed phosphate ions in MoaB could be consistent with a binding of GTP, which was easily modeled in the putative binding site of MoaB (Fig. 7). In this model, the α and γ-phosphate groups of GTP are very close to the positions of the ions bound in the crystal structure. The putative catalytic Asp⁸² and Glu⁸² interact directly with these phosphates and are ideally poised to play a role, perhaps in the transformation of GTP into precursor Z. The base of the modeled GTP fits very well in the deep cavity, which is occupied by several water
molecules in our crystal structure. The invariant Ser110 (Figs. 5 and 6) is located in a deep cleft where the base of the modeled GTP is bound (Fig. 7) and could interact with the N-1 and/or N-2 atoms of the latter. Our results show that MoaB indeed binds GTP (Fig. 8A), but with low affinity. The protein also possessed a low GTPase activity (Fig. 8B). Both binding and enzymatic potential were affected by some divalent ions, especially by Mn²⁺. Coincidentally, when some divalent ions were added in crystallization trials of another Moco synthesis protein, MoeA, Mn²⁺ appeared to have more drastic effects than the others.¹

Based on the possibility of binding various compounds of Moco pathway by MoaB and on its structural similarity with MogA and MoeA, we suggest that MoaB might play a substrate-shuttling role in the biosynthetic process. The interme-

¹ R. Sanishvili, unpublished results.
diates of Moco synthesis and the cofactor are unstable, suggesting that the reactions, including the transfer of Moco to apomolybdoenyzymes, occur while the corresponding compounds are still bound to the enzymes or to transporter/storage proteins, avoiding their dissociation into the external solvent.

The protection of the substrates against solvent might occur through specific protein-protein interactions among the enzymes. It is becoming apparent that protein-protein interactions play crucial role in Moco biosynthesis. Magalon et al. (40) reported that numerous protein pairs from the Moco pathway do form complexes in vivo, including MobB-MoeA, MobB-MogA, MogA-MoeA-MobA, and MobB-MobA and that some compounds of this pathway influence these interactions. Divalent ions, as shown here, can also influence binding of some compounds in the Moco pathway, although the mechanism of such influence is not yet clear.

Possible involvement of MobB in such protein-protein interactions is supported indirectly by several observations. First, in Synechococcus sp., the MoaC ortholog is fused to the homolog of either MobA or MogA (41). Second, MogA and MoeA are combined to form the multifunctional protein gephyrin in mammals (13), Cnx1 in plants (15), and cinnamon in insects (14). All of the protein-protein complexes observed in vivo (40), except for MobB-Moba, contain either MogA or MoeA, the structural homologs of MobA. If MobB can indeed bind GTP, precursor Z, or molybdopterin as discussed above and if it can form complexes with several other proteins of Moco synthesis pathway, it is tempting to suggest that MobB plays a transporting and/or storage role.

The presence of six independent binding sites in the MobB hexamer would enhance the capacity of the protein in such a role. As reported by Schwarz et al. (39), the G domain of Cnx1 can be present in both hexameric and trimeric forms. Because the trimer of Cnx1 G domain is very similar to the trimer of MobB, and because the trimer of MobB seems to be much more stable than its hexamer, it is possible that MobB is also present in these two states of oligomerization. In our experiments with size-exclusion chromography as described under “Experimental Procedures.” The amount of bound [$\alpha$-32P]GTP was determined by scintillation counting and is expressed as a percentage of the total input [$\alpha$-32P]GTP.
chromatography, MoaB was not found in a trimeric state. However, equilibrium between trimeric and hexameric states could be modulated by particular compounds, such as reaction substrates and products, ions, or other proteins of the pathway, all of which were absent in our experiments. In the trimeric state MoaB could bind MobB and MoeA much the same way as does MogA (40).

On the other hand, MogA could replace one-half of the MoaB hexamer away from the trimer-trimer interface. Formation of such hetero-oligomers could be an effective way of substrate/product exchange along the Moco pathway without their dissociation into the external solvent. An inferred promiscuity of MoaB in protein-protein complex formation would be an asset for a transporter/storage protein.

**Summary**—The crystal structure of *E. coli* MoaB was determined at 1.6-Å resolution. The strong structural similarity of MoaB with other Moco synthesis proteins, such as MogA, gephyrin, and Cnx1, along with the amino acid conservation patterns, indicated that MoaB might bind some of the same compounds, namely molybdopterin. The presence of a sulfate ion in the putative binding site, the experimental verification of MoaB/GTP binding, and modeling studies suggest that GTP and possibly precursor Z could bind in the same binding site.

MoaB displayed very strong structural similarity to MogA and MoeA, two proteins that form numerous complexes with other proteins within the Moco synthesis pathway in *E. coli*, suggesting that MoaB might be part of such complexes and may play a role in transport or storage of several compounds in the Moco synthesis pathway.

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