Passive Acquisition of Ligand by the MopII Molbindin from Clostridium pasteurianum

STRUCTURES OF APO AND OXYANION-BOUND FORMS*

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MopII from Clostridium pasteurianum is a molbindin family member. These proteins may serve as intracellular storage facilities for molybdate, which they bind with high specificity. High resolution structures of MopII in a number of states, including the first structure of an apo-molbindin, together with calorimetric data, allow us to describe ligand binding and provide support for the proposed storage function of the protein. MopII assembles as a trimer of dimers and binds eight oxyanions at two types of binding sites located at intersubunit interfaces. Two type 1 sites are on the molecular 3-fold axis and three pairs of type 2 sites occur on the molecular 2-fold axes. The hexamer is largely unaffected by the binding of ligand. Molybdate is admitted to the otherwise inaccessible type 2 binding sites by the movement of the N-terminal residues of each protein chain. This contrasts with the structurally related molybdate-dependent transcriptional regulator ModE, which undergoes extensive conformational rearrangements on ligand binding. Despite similarities between the binding sites of ModE and the type 2 sites of MopII the molbindin has a significantly reduced ligand affinity, due in part, to the high density of negative charges at the center of the hexamer. In the absence of ligand this effects the movement of an important lysine side chain, thereby partially inactivating the binding sites. The differences are consistent with a biological role in molybdate storage/buffering.

MopI domains, which occur in a variety of bacterial and archael proteins, specifically bind molybdate. The simplest mop-containing proteins are the so-called molbindins (1, 2), consisting entirely of either one or two mop domains. The physiological role of these proteins is unclear, although they have been implicated in molybdate storage and homeostasis (3). Other mop-containing proteins are ModC, a component of the high affinity ABC-type molybdate transporter (4) and ModE, the molybdate-dependent transcriptional regulator (5, 6).

ModE-like proteins occur in a number of organisms such as Escherichia coli, where this protein mediates both the molybdate-dependent transcriptional repression of the modABCD operon (MoO$_4^{2-}$ transport (7–9)) and the molybdate-dependent activation of the moaABCDE operon (molybdopterin biosynthesis (10)). ModE also influences the expression of molybdoenzymes such as dimethyl sulfoxide reductase (6) and nitrate reductase A (11). Functional ModE is a homodimeric protein, which folds into two distinct domains, an N-terminal DNA-binding domain and a C-terminal molybdate-binding domain. The latter consists of four mop domains (two from each chain).

The structure of the mono-mop molbindin from Sporomusa ovata in complex with tungstate (12) shows Mop subunits assembled into a trimer of dimers forming eight oxyanion binding sites. A similar arrangement was found for ModG from Azotobacter vinelandii, a di-mop molbindin. The crystal structure of this protein shows a trimeric arrangement where one ModG molecule takes the place of each Mop dimer (13). The structures of complete Escherichia coli ModE in its apo form (14) as well as its ligand-bound C-terminal domain (2) have been determined. The protein binds two molybdate anions per dimer. The quaternary structure adopted by the four mop domains of the dimer superimposes on two Mop dimers or two di-mops from the molbindins.

The nitrogen-fixing bacterium Clostridium pasteurianum possesses three distinct genes encoding molbindins (15); the three proteins, Mop I through III, are ~7 kDa in size and show a high degree of amino acid sequence conservation (>86% identity). They consist of a single mop domain and thus belong to the mono-mop molbindin family.

We report a number of high resolution crystal structures for C. pasteurianum MopII: two different structures of the apoprotein (Apod1 and Apod2), one structure of the tungstate complex, and two structures of Mop-molybdate complexes (Moo1 and Moo2, named after the Protein Data Bank name for molybdate). The apoprotein and the ligand-bound protein crystallized in different monoclinic crystal forms, both with six Mop molecules per asymmetric unit. In the case of the apoprotein they form a complete hexamer, whereas the ligand-bound structures contain two separate Mop trimers, which are complemented to hexamers by crystallographic symmetry. The structures provide the first view of an apo-molbindin and allow us to characterize the mechanism by which Mop acquires ligands and to compare this mechanism to that described for ModE (2). Calorimetric studies complement the crystallographic analysis, further characterizing molybdate binding to Mop and enabling us to compare ligand affinities with those of ModE.

EXPERIMENTAL PROCEDURES

Crystalization, Data Collection, Structure Solution, and Refinement—The cloning, expression, and purification of MopII as well as crystallization conditions and data collection procedures for the tungstate, Moo1, and Apo1 complexes are as previously described (16). After...
it was determined that the Moo1 structure contained only partially loaded binding sites, more crystals were grown, but this time with the addition of 1 μl of 8 mM Na2MoO4 directly to the drop. Data from these crystals were collected in-house using a Rigaku RU-200 rotating anode x-ray source (Cu Kα, λ = 1.5418 Å) and an R-Axis IV image plate detector. The resulting data set (Moo2) was processed and scaled to 2.4 Å using DENZO/SCALEPACK (17).

In the hope of improving the resolution limit of the apo-structure (Apo1: 1.8 Å) a second data set was collected in-house from a crystal grown under the same conditions as described before (16). The experimental setup, scaling, and processing were as for the Moo2 data; the high resolution limit for the new data set (Apo2) is 1.5 Å.

Phases were obtained by single isomorphous replacement with anomalous scattering (SIRAS) using the tungsten anomalous signal from the tungstate data and the isomorphous differences between the anomalous scattering (SIRAS) using the tungsten anomalous signal from the tungstate data and the isomorphous differences between the tungstate and the Moo1 data. We note that the partial occupancy of the tungstate Moo1 Moo2 Apo1 Apo2

### Table I

**Data collection, processing, refinement, and model statistics**

| Description | Moo1 | Moo2 | Apo1 | Apo2 |
|-------------|------|------|------|------|
| Unit cell, a, b, c (Å); β (°) | 56.37, 58.51, 94.84; | 56.81, 78.38, 95.24; | 55.14, 77.76, 93.49; | 79.08, 82.40, 56.82; |
| Resolution range (Å) | 20.00–1.60 | 20.00–1.83 | 30.00–2.40 | 25.00–1.80 |
| No. of measurements | 405,754 | 206,409 | 173,881 | 124,432 |
| No. of unique reflections | 53,579 | 36,043 | 10,097 | 33,112 |
| Redundancy | 1.9 (1.7) | 2.8 (2.8) | 4.0 (2.9) | 1.9 (1.7) |
| Completeness (%) | 93.6 (90.4) | 97.4 (98.8) | 98.3 (89.2) | 97.6 (93.9) |
| Rmerge (%) | 8.8 (26.4) | 5.5 (33.6) | 5.0 (25.9) | 4.1 (28.0) |
| R and Rfree (%) | 15.4/18.3 | 21.5/24.8 | 21.0/25.7 | 17.9/21.7 |
| Wilson B (Å²) | 10.0 | 21.8 | 59.1 | 19.5 |
| Average isotropic B (Å²) | Overall | 19.3 | 27.6 | 52.0 | 21.9 | 19.1 |
| Protein backbone | 18.6 | 27.3 | 52.2 | 20.7 | 17.8 |
| Protein side chains | 17.5 | 25.6 | 51.3 | 18.8 | 16.3 |
| Solvent | 20.0 | 29.3 | 53.4 | 23.0 | 19.5 |
| Lipid | 28.7 | 33.6 | 44.3 | 33.8 | 29.2 |
| Phases | 11.1 | 25.3 | 40.8 | n/a | n/a |
| Cruickshank’s DPI (Å²) | 0.09 | 0.18 | 0.35 | 0.15 | 0.09 |
| r.m.s.d. bond lengths (Å) | 0.013 | 0.014 | 0.011 | 0.019 | 0.011 |
| r.m.s.d. bond angles (°) | 1.3 | 1.9 | 1.5 | 2.0 | 1.5 |
| r.m.s.d. dihedral angles (°) | 8.2 | 9.2 | 9.7 | 8.4 | 6.3 |
| Ramachandran plot: Residues in most favored region (%) | 93.7 | 93.7 | 91.3 | 96.8 | 96.6 |
| in additionally allowed region (%) | 6.3 | 6.3 | 8.7 | 3.2 | 3.4 |
| PDBe accession codes | 1GUG | 1GUN | 1GUO | 1GUS | 1GUT |

*DPI, Data Precision Indicator (20).*

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**RESULTS AND DISCUSSION**

**Overall Structure**—The 68-amino acid Mop polypeptide consists predominantly of β-strands (52% of the residues in six strands), most of which (β2–β5) form a twisted antiparallel β-sheet. There is also a short α-helix (10% of residues) between β4 and β5 and two 3_10-helical segments (β1 and β2; 8% of residues) between β3 and β4 and between β5 and β6, respectively (Fig. 1, A and B). In all crystal structures six Mop subunits form a compact hexamer with 32-point group symmetry and a diameter of about 60 Å (Fig. 2, A and B). An area of approximately 17,400 Å² (~55% of the accessible monomer surface) is buried upon oligomerization. The previous structures of *S. ovata* Mop and ModG show a similar quaternary structure.

The Mop hexamer is observed by mass spectrometry and by native polyacrylamide gel electrophoresis (data not shown). Moreover, considering that all ligand binding sites are formed through the interaction of two or more subunits, it is likely that...
the hexamer represents the functional unit. We are furthermore more confident that the reported hexameric apo-structures are physiologically meaningful, because the mass spectra show the hexamer peak even in the absence of ligand.

For the following discussion the six chains have been assigned chain identifiers A through F as shown in Fig. 2C. We use these chain names to differentiate between the two trimers and more generally the two poles of the molecule (ABC and DEF). In the case of the two apo-structures, the chain names used are identical to those in the deposited coordinate sets. The crystallographic symmetry in the ligand-bound structures means that the chain identifiers A-F as used here are actually chains A, B, C, A', B', C' or D', E', F', D, E, F (where X* is symmetry-related to X).

The hexamer possesses three unique intersubunit interfaces (Fig. 2C), one between subunits from the same trimer (yellow edges in Fig. 2C) and two interfaces between subunits from opposite trimers (both with internal 2-fold symmetry; red and green edges). The most extensive interaction occurs at the “red” interface, which contributes $-3 \times 2950 \, \text{Å}^2$ of the buried accessible surface and involves 29 residues on each side. The main feature of this interface is the interaction between β5 from either subunit and β6 from the other subunit. This results in the extension of the central β-sheets to two Greek-key β-barrels, which display structural similarity to the oligonucleotide/oligosaccharide binding fold (31). The interaction between β5 and β6 (primes are used to denote a different chain) accounts for eight of the sixteen direct hydrogen bonds formed across the red interface, the remaining hydrogen bonds are formed between strand β4 and side chains from β2 as well as backbone atoms from β2*. In addition to these direct interactions, this interface also includes 16 water-bridged hydrogen-bonding contacts.

Contacts across the green interface (about $3 \times 1180 \, \text{Å}^2$ of buried surface) are formed by 13 residues from helix a1, strands β1 and β4, and the $3\perp$-helix η2 on either side; interactions include a salt bridge between Arg60 and Glu48 and 10 direct hydrogen bonds. Six of the eight oxyanion binding sites are formed at this interface (see below).

The yellow interface buries $6 \times 1040 \, \text{Å}^2$ of solvent-accessible surface area with about 70% of this surface being hydrophobic in nature. Its principal feature is the antiparallel alignment of β6 with β1', which is stabilized by three direct hydrogen bonds and two bridging waters and further extends the central β-sheet. Strands β3 and β4 as well as η2 from both sides participate in further contacts, providing three more direct hydrogen bonds.

With the exception of Apo2 (see below) the backbone conformations are remarkably similar between subunits both within any structure (root mean square deviation (r.m.s.d.) values for Cα atoms after pairwise alignment less than 0.4 Å) and between different structures (r.m.s.d. values for Cα atoms less than 0.5 Å). In the case of the two apo-structures, an even higher degree of similarity is observed between subunits from
the same trimer (r.m.s.d. less than 0.08 Å). The observed similarity is not an artifact of refinement, because only weak restraints on non-crystallographic symmetry were applied or, in the case of Apo2, none at all.

The Ligand Binding Sites—C. pasteurianum MopII possesses eight oxyanion binding sites per hexamer, all of which make use of the oligomeric assembly: two sites lie on the molecular 3-fold axis at the centers of the two trimers, whereas the remaining six are located at the green interfaces (Fig. 2, A and B). In accordance with Wagner et al. (12) these anion binding positions are referred to as type 1 and type 2 sites, respectively.

Type 1 sites are formed by the bend between β2 and β3 from three Mop chains related by the molecular 3-fold axis (Figs. 1A, 2, and 3A). The symmetry of the binding site is mirrored by the ligand binding mode: The central metal atom and one of the oxygen atoms lie on the 3-fold axis. This axial oxygen points into the hexamer, whereas the other three oxygens face outwards and each one toward a different subunit (cf. Fig. 2B). The bound oxyanion forms twelve hydrogen bonds with the protein, three of them at the axial oxygen, which is contacted by the side-chain hydroxyl of Thr<sup>22</sup> from all three Mop chains. The other oxygen atoms each accept three hydrogen bonds from the backbone amides of Val<sup>20</sup>, Val<sup>21</sup>, and Thr<sup>22</sup> of one protein molecule, although the latter is relatively weak at an average distance of 3.3 Å. It is noteworthy that no positively charged groups are involved in binding of the oxyanion at this site, in fact no such groups exist within a radius of 9 Å from the oxyanion. With the exception of a hydrogen bond from the Thr<sup>22</sup> side chain, interactions with the ligand are limited to the protein backbone. The lack of sequence conservation for the residues involved (Fig. 1B) is consistent with this observation.

Type 2 sites occur in pairs at the three green interfaces and are created by β1 as well as β2 from one subunit and the α<sup>1</sup> helix from the other subunit. The protein donates eight hydrogen bonds to the ligand, five from β1/β2, and three from α<sup>1</sup>. Two of the ligand oxygens accept only one hydrogen bond (from the Ser<sup>4</sup> hydroxyl and the Lys<sup>60</sup> amine, respectively), the third oxygen forms the remaining three hydrogen bonds with the same subunit (with the Arg<sup>6</sup> backbone amide and both the amide and the side chain hydroxyl from Ser<sup>61</sup>). The fourth oxygen accepts all three hydrogen bonds from the other subunit (amide and hydroxyl of Ser<sup>40</sup> and hydroxyl of Ser<sup>43</sup> from consecutive turns of the helix, Fig. 3B). In this case partial compensation for the negative charge of the ligand is afforded by the side chain of Lys<sup>60</sup>. Most of the residues participating in the formation of type 2 sites are at least functionally conserved, as should be expected given that most of them contact the oxyanion with their side chains. Only Ser<sup>43</sup> and Ser<sup>61</sup> are replaced by alanine or glycine in some mohbindins (Fig. 1B). Arg<sup>6</sup> is conserved in almost all mop proteins, despite the fact that its side chain does not contribute to molybdate binding, instead forming an important salt bridge with Glu<sup>46</sup> as described above.

Binding Sites in the Apo-structures—Aside from a few exceptions that will be described, the similarity in backbone conformation between the various MopII structures can be extended to the side-chain conformations, with subunit-subunit alignments typically resulting in r.m.s.d. values below 0.7 Å for ~480 atoms. Most of the ligand binding sites in the apo-structures are also similar to the corresponding ligand-filled sites. In the absence of ligand, the positions of its oxygen atoms are populated by solvent atoms.

The best correspondence between apo- and ligand-bound structures is observed for type 1 sites (Fig. 4A), alignment of Apo1 with the tungstate complex puts solvent molecules within 0.7 Å of all tungstate oxygens. However, a noticeable difference lies in the position of Val<sup>20</sup>: Its side chains, which limit access to the site from the bulk solvent, move slightly outwards in the apo forms compared with ligand-bound structures, thereby widening the access bore from an average diameter of 5.6 Å (ligand-bound) to ~7.5 Å (apo) and thus facilitating ligand access. The slightly increased space requirements of the solvent structure compared with the oxyanion may account for this.

The three type 2 sites located closer toward the DEF pole of Apo1 are also structurally similar to their liganded counterparts (Fig. 4B). One minor difference concerns the side chain of Ser<sup>61</sup>, which appears in two alternate conformations in Apo1. Again ligand oxygen equivalent positions are occupied by solvent molecules. Only the solvent molecule hydrogen-bonding to the Lys<sup>60</sup> side chain is about 1.1 Å from the ideal position, which allows it to form an additional hydrogen bond to the hydroxyl of Ser<sup>61</sup> in its alternate conformation (asterisk in Fig. 4B). A more prominent difference is observed for the type 2 sites closer to the ABC poles of both apo-structures (Fig. 4B). The side chain of Lys<sup>60</sup> adopts a completely different conformation pointing away from the binding site. Subsequently, the
solvent molecule that accepted a hydrogen bond from Lys$^{60}$ in the other sites has moved even further (up to 2.0 Å from the closest aligned oxyanion oxo position, not shown).

The largest difference from the ligand-bound structures can be observed in the DEF half of Apo2 (Fig. 5): The N-terminal six residues of chains A, B, and C (chain B in Fig. 5) move up to 6 Å outwards, thereby partly exposing the type 2 site underneath. Concomitantly minor movements of surrounding residues, including the C terminus of a neighboring chain (chain A in Fig. 5) and residues 46–48 from another chain (chain D in Fig. 5), can be observed. This more open conformation demonstrates how the otherwise solvent-inaccessible type 2 sites may acquire ligand molecules.

**Ligand Binding in Moo1**—Initial refinement of the Moo1 complex resulted in the eight molybdenum atoms having unexpectedly high temperature factors compared with their oxo ligands. Given the solvent arrangement in the apo-structures, coupled with the absence of lower-mass oxyanions from the crystallization conditions, we interpret the observed electron density as resulting from partially molybdate-occupied sites. The superposition of actual oxyanion oxygens and solvent molecules (both with partial occupancy) mimics fully occupied oxo ligands. The occupancies for the molybdenum atoms were reduced to 0.5 and 0.25 for type 1 and type 2 sites, respectively, which brought the temperature factors more in line with those of the surrounding protein atoms. A further indication of the partial ligand occupancy in this structure is the observation that the side chain of Lys$^{60}$ exhibits a dual conformation in two of the protein chains, pointing both toward the nearby type 2 site (as in the fully loaded structures) and toward the center of the oligomer as in the apo-subunits. The partially occupied binding sites in Moo1 also underline the lack of structural differences between apo- and ligand-bound Mop.

In addition, the oxyanions in the two type 1 sites of Moo1 adopt a different orientation from the one seen in the other loaded structures: The ligand is again oriented along the 3-fold axis, only now the axial oxygen points outwards. The three...
The association constants $K_a$ show that type 1 sites have a more than one order of magnitude higher affinity for molybdate than type 2 sites. This corresponds to the relative binding affinities established independently by the different site occupancies in the Moo1 structure.

The small changes between unbound and bound state, in terms of van der Waals and hydrogen bonding interactions, and the lack of strong electrostatic interactions in type 1 sites, suggest that binding enthalpy should be small ($\Delta H \approx 0$). Thus the main driving force for binding is the entropic gain ($\Delta S > 0$) from the displacement of four ordered waters by one ligand molecule; this is corroborated by the experimental data. For type 2 sites the situation is similar, but as ligand binding localizes both the side chains of Lys$^{60}$ and Ser$^{61}$ and the N-terminal flap, $\Delta S$ is reduced. At the same time $\Delta H$ becomes more negative due to the electrostatic interaction between the ligand and the side chain of Lys$^{60}$, resulting in a slightly larger enthalpic contribution to ligand binding.

The only other mop protein for which binding constants are published is ModE from E. coli (5), which binds molybdate with a $K_a$ of $-1.3 \times 10^6 \text{M}^{-1}$ in its two type 2 sites. The lower affinity determined for the type 2 sites of MopII ($K_a \approx 2.1 \times 10^5 \text{M}^{-1}$) is compatible with a proposed intracellular role as a passive molybdate storage facility. In the absence of a specific energy-driven ligand extraction mechanism, a storage protein must bind less strongly than the proteins that utilize the stored ligand.

The high local concentration of negative charges is especially remarkable in the context of the conformational change of the Lys$^{60}$ side chain in the apo-structures. The side chain, which otherwise forms part of a type 2 binding site, positions its amino group in the central plane between the two trimers and within 3.5 Å of two Asp$^{63}$ carboxylates. This conformation is adopted by half of the apo-Mop subunits in the Apo1 and Apo2 structures, the maximum number possible without clashes between lysines from opposite trimers. Given that this is the preferred conformation in apo-structures and that the absence of Lys$^{60}$ is likely detrimental to type 2 site binding, this observation may in part explain the reduced ligand affinity of the MopII type 2 sites compared with ModE, which, as explained above, is essential for the storage function of Mop. Additionally, the central chamber may furnish a place for storing counterions and thus provide protection from unrestricted charge separation; the presence of cations in the chamber would then partly counteract the affinity-reducing effect. Asp$^{63}$ is functionally conserved (as aspartate or glutamate) in practically all molybdate storage proteins.

**Fig. 6.** ITC titration data describing the binding of molybdate to C. pasteurianum MopII. Differential power signal recorded in a representative experiment (top) and integrated data (dots) and fitted curve (solid line) from the same experiment (bottom).

equatorial oxygens are in approximately the same position as in the other structures (Fig. 4D). The hydrogen bond donors and the number of hydrogen bonds are the same for both orientations, differing only in relative strength. While in the tungstate and Moo2 structures the interaction with the backbone amide of Thr$^{22}$ is weakest, in Moo1 the most distant interaction is with the Val$^{30}$ amide (distances about 3.5 Å). It is noteworthy that, similar to the other ligand oxygens, the new axial oxygen position is occupied by a water molecule in some of the apo-trimers, suggesting that this binding mode is not fundamentally disadvantaged, even though the temperature factors for this water is about double that of its neighbors.

**Thermodynamics of Ligand Binding**—To better establish the properties of the different binding sites and to gain insight into the possible function of molbindins, we characterized molybdate binding by isothermal titration calorimetry (ITC (32, 33)) (Fig. 6). Although we cannot exclude the possibility of cooperativity between some anion binding sites, we find a reasonable fit for our data using a model with two types of independent sites, which is in accordance with the structural evidence. The results are summarized in Table II.

The two sets of site parameters were assigned to type 1 and type 2 sites based on the refined values for $n$ (number of sites per molecule); even though at 0.61 and 1.58 both experimental values are larger than expected, their ratio ($n_1/n_2 = 0.39$) correlates well with the expected value of 0.33 (for one-third type 1 site and one-type 2 site per Mop molecule). The single largest error influencing the absolute values for $n$ is likely to stem from the estimate of protein concentration. Because this error is compensated for by $n$ it should not affect the quality of the remaining results.

**Comparison with Mop from S. ovata**—C. pasteurianum MopII and S. ovata Mop are relatively similar in amino acid sequence (37% sequence identity, see Fig. 1B). It should be noted, however, that the sequence of the S. ovata Mop has been derived from the crystal structure (12) and may harbor inaccuracies due to disordered or ambiguous electron density. The two proteins exhibit identical subunit arrangement and similar backbone conformations (r.m.s.d. about 0.7 Å for 396 superimposable C$\alpha$ atoms); the only difference of note is the deletion of Gly$^{31}$ in the S. ovata structure.

The oxyanion binding sites are also similar; differences of...
notable that only one of the type 1 sites of S. ovata Mop is in a conformation comparable to that seen in the Mop1 structure (and was also assigned a reduced occupancy). It is possible that for Mop the Moo1-like binding mode is actually preferred, because the replacement of Thr<sup>22</sup> with a methionine may have a greater deleterious effect on the normal binding mode than on the Moo1-like mode. The central chamber is present in the S. ovata structure, but it is subdivided into several smaller cavities due to the replacement of Asp<sup>63</sup> with a glutamate.

Comparison with A. vinelandii ModG—The di-mop molybdobin ModG from A. vinelandii contains a 142-amino acid protein consisting of a tandem repeat of mop domains. A crystal structure of molybdate-loaded ModG (13) shows that ModG trimerizes to a hexa-mop arrangement similar to that of the other molydbins: The 402 C<sub>a</sub> atoms of ligand-bound C. pasteurianum Mop can be superimposed with an r.m.s.d. of below 0.85 Å. Each ModG molecule corresponds to a pair of Mop chains interacting across the red interface with the addition of a five amino acid loop between the C terminus of one Mop and the N terminus of the other. This connection introduces a polarity into the hexa-mop arrangement, producing a C-terminal trimer and an N-terminal trimer (the three ModG chains associate in a head-to-head fashion). All ligands are still bound at intersubunit interfaces.

Again the ligand binding sites are similar to those found in Mop (see Table III), and the ligand orientation is almost identical to that seen in the fully oxoanion-loaded Mop structures for both types of sites. Delarbre et al. (13) describe a hydrogen bonding network between type 1 and type 2 sites in ModG and remark on the possible implications for cooperative ligand binding. No such network is observed in the Mop II structures.

It is unlikely that the type 2 site loading mode proposed for Mop is also applicable to ModG, because half of the Mop N termini are now in the middle of the polypeptide chain. Still the loop between the two domains may be flexible enough to allow the required rearrangement. ModG is one of the few molydbins that lacks the conserved aspartate/glutamate of the central chamber. In its place there is an asparagine or a tryptophan.

Comparison with E. coli ModE—ModE, like ModG, contains a tandem repeat of mop domains, which in this case is attached to the C terminus of a winged helix-turn-helix DNA-binding domain. The main difference between ModE and the molydbins is that ModE is a dimer, not a trimer. However, its four mop domains superimpose reasonably well with appropriately chosen chains from the C. pasteurianum Mop hexamer (r.m.s.d. 1.58 Å for 258 C<sub>a</sub> atoms using the tungstate-bound ModE di-mop structure (2)). The ModE mop tetramer cannot offer any type 1 ligand binding sites and can provide only two type 2 sites between the N-terminal mop domains from both chains. The ligand binding mode is similar to that of the other mop proteins (see Table III for details).

As in the case of ModG the N termini of the individual mop domains of ModE are unable to move freely, thus ligand acquisition cannot occur by the passive mechanism proposed for Mop. Instead, the greater flexibility of ModE allows the apo protein to adopt a more open structure with binding sites that are accessible from the bulk solvent. Ligand binding results in an extensive conformational change involving both inter- and intradomain movements, with the protein chomping on the ligand (2). The more extensive rearrangements seen in ModE might be beneficial for its functioning as a molybdate-dependent transcriptional regulator, possibly transmitting the signal of molybdate/tungstate binding to the DNA-binding domain. In light of the sequence similarity among mop domains, it is remarkable how the basic theme of molybdate binding is modulated to fit the requirements of particular proteins. On the one hand there are signaling proteins like ModE with high sensitivity and low binding capacity, and on the other hand there is Mop as a storage protein with lower sensitivity but high molybdate binding capacity.
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