Structure of the Molybdate/Tungstate Binding Protein Mop from Sporomusa ovata

Ulrike G. Wagner,* Erhard Stupperich,†
and Christoph Kratky‡
*Institut für Chemie
Strukturbiologie
Karl-Franzens-Universität
A-8010 Graz
Austria
†Abteilung Angewandte Mikrobiologie
Universität Ulm
D-89081 Ulm
Germany

Summary

Background: Transport of molybdenum into bacteria involves a high-affinity ABC transporter system whose expression is controlled by a repressor protein called ModE. While molybdate transport is tightly coupled to utilization in some bacteria, other organisms have molybdenum storage proteins. One class of putative molybdate storage proteins is characterized by a sequence consisting of about 70 amino acids (Mop). A tandem repeat of Mop sequences also constitutes the molybdate binding domain of ModE.

Results: We have determined the crystal structure of the 7 kDa Mop protein from the methanol-utilizing anaerobic eubacterium Sporomusa ovata grown in the presence of molybdate and tungstate. The protein occurs as highly symmetric hexamers binding eight oxyanions. Each peptide assumes a so-called OB fold, which has previously also been observed in ModE. There are two types of oxyanion binding sites in Mo at the interface between two or three peptides. All oxyanion binding sites were found to be occupied by WO₄²⁻ rather than MoO₄²⁻.

Conclusions: The biological function of proteins containing only Mop sequences is unknown, but they have been implicated in molybdate homeostasis and molybdopterin cofactor biosynthesis. While there are few indications that the S. ovata Mop binds pterin, the structure suggests that only the type-1 oxyanion binding sites would be sufficiently accessible to bind a cofactor. The observed occupation of the oxyanion binding sites by WO₄²⁻ indicates that Mop might also be involved in controlling intracellular tungstate levels.

Introduction

The biological role of molybdenum is due to its involvement in two types of cofactor for a number of enzymes [1]. Molybdenum nitrogenase has a unique molybdenum-iron-sulfur cofactor called FeMoco; all other molybdoenzymes are oxidoreductases with a molybdopterin cofactor called Moco. Tungsten is chemically similar to molybdenum, which it can replace in the cofactors of several enzymes, such as the tungstopterin enzymes of hyperthermophilic archaea, which appear to be obligately tungsten dependent [2, 3]. Both molybdenum and tungsten occur in the environment (typically at low submicromolar concentrations) as the soluble oxyanions molybdate (MoO₄²⁻) and tungstate (WO₄²⁻), which have similar sizes, shapes, and H bonding properties to those of sulfate, phosphate, and vanadate.

Bacteria have a high-affinity molybdenum uptake system belonging to the ABC superfamily of transporters [4, 5]. Components of this system include a periplasmic binding protein (ModA), an integral membrane protein (ModB), and an energizer protein (ModC). The ModA, ModB, and ModC proteins from various organisms (e.g., Escherichia coli, Haemophilus influenzae, Azotobacter vinelandii, and Rhodobacter capsulatus) are very similar [5]. Molybdate transport is tightly coupled to utilization in E. coli and Klebsiella pneumoniae, while other organisms appear to have a molybdenum storage protein. In all organisms studied so far, molybdate transport genes are regulated by a repressor protein, ModE [5].

Earlier studies on the accumulation of molybdenum in cells of Clostridium pasteurianum demonstrated that after entry into the cell, molybdenum binds to an abundant Mo binding protein of approximately 30 kDa [6, 7]. After treatment of the cell lysate with ammonium sulfate, a 7 kDa long protein (68 amino acids long) was obtained that bound approximately 0.7 mol of molybdenum per mol of protein [8]. Subsequent genetic analysis of this Mop protein [9] showed that there are three variants in C. pasteurianum whose nucleotide sequences show greater than 90% homology [10]. Spectroscopic evidence suggested that Mop binds an inactive form of molybdopterin, but the putative Mo-pterin species as isolated from Mop failed to yield a molybdopterin that had detectable molybdenum cofactor activity [8].

Mop-like sequences were since then detected in a number of genes believed to code for components of the Mo transport system. Thus, H. influenza has a single Mop-like protein encoded by a gene located outside the molybdenum transport mod locus [11]. Similarly, upstream from the modEABC operon of A. vinelandii, an open reading frame (modG) encoding a hypothetical 14 kDa protein was detected. It consists of a tandem repeat of a sequence that is homologous to C. pasteurianum Mop. The Mop protein from H. influenza and ModG from A. vinelandii are Mo binding proteins of unknown function that are believed to be involved in Mo storage and homeostasis [12]. A tandem Mop-like repeat also constitutes the C-terminal Mop binding domain of ModE, the molybdate-dependent transcriptional regulator [13–16]. ModC, the peripheral membrane protein of the molybdate transporter, also contains a Mop-like sequence.

Structural data are available for a number of proteins of the Mo transport system. These proteins include the molybdate-dependent transcriptional regulator ModE from Escherichia coli [15] and the periplasmic molybdate binding protein ModA from E. coli [17] and A. vinelandii [18, 19]. For ModG, the 14 kDa molybdate binding protein from A. vinelandii, the crystallization was reported [20]. X-ray absorption spectroscopy of Mo has been performed for Mop from H. influenzae and also for ModG and ModA from A. vinelandii and the E. coli ModE. This X-ray absorption spectroscopy has indicated that all these proteins bind tetrahedral molybdate with a Mo–O distance of 1.76 Å [21].

Key words: molybdate; molybdopterin cofactors; tungstate; molybdate homeostasis
In 1994 we reported the crystallization and preliminary crystallographic characterization [22] of a 40 kDa protein that had been isolated from the methanol-utilizing anaerob Sporomusa ovata and for which there was evidence that it carries a corrinoid cofactor [23]. Straightforward solution of its crystal structure by multiple isomorphous derivatization was prevented by our inability to prepare heavy-atom derivatives with standard soaking protocols. The project was also hampered by the fact that expression of the protein by S. ovata was found to be unstable and unpredictably irreproducible. Partial sequence data were only available from ladder sequencing of peptides obtained from tryptic digests. From a preparation of S. ovata grown in the presence of subtoxic concentrations of selenomethionine, we were able to isolate a “derivative” protein, whose crystals eventually allowed us to solve the crystal structure of the native protein. During the interpretation of the experimental electron density, it became obvious that the structure is incompatible with a corrinoid protein and that we had in fact determined the crystal structure of a metal-oxyanion binding protein. In retrospect, it appears that our original protein preparation had contained a mixture of two proteins, of which one was a corrinoid protein and the other was a metal-oxyanion binding protein. This also would explain the above protein sequence data from tryptic digests, which yielded only one peptide whose sequence is found in the crystal structure, but which also yielded several others that are not.

Here, we report the crystal structure for the metal-oxyanion binding protein, which turned out to be a hexamer of peptides with 67 amino acids binding 8 oxyanions. Analysis of the sequence as deduced from the observed electron density indicated that this protein belongs to the class of Mop proteins [8, 9].

### Results and Discussion

#### Structure Determination and Validation

The structure of the putative molybdopterin binding protein Mop from Sporomusa ovata was determined by a combination of single-isomorphous replacement (SIR) and single-anomalous dispersion (SAD) techniques. Besides a data set from a crystal of “native” protein, structure solution also relied on anomalous data collected from a crystal of “derivative” protein isolated from an S. ovata incubation in the presence of selenomethionine. While the selenomethionine had not been incorporated into the peptide, the derivative protein had the metal-oxyanion binding sites occupied by something lighter than the WO₄ observed in the native structure (see below). This was most probably SeO₄ or MoO₄. The anomalous signal from the derivative crystals plus the differences between derivative and native data permitted the extraction of phases to yield an interpretable density map, as described in the Experimental Procedures.

Eventually, the model was refined against native data extending to a resolution of 1.5 Å to yield a residual of R = 18.5% (Rmerge = 19.80%). The structure is very well defined throughout, and there is no need to describe the experimental electron density in terms of multiple conformations. The “most favored” regions of a Ramachandran plot, as defined by the program PROCHECK [24], contain 95.1% of the non-glycin and non-proline residues, with the remaining residues located in “additional allowed” regions. Mean bond length and bond angle deviations from ideal values are given in Table 1.

#### Molecular Architecture

In the crystal, the 67 amino acid long peptides occur as highly symmetric hexamers with noncrystallographic internal 32 symmetry. The asymmetric unit contains two such hexamers related via a noncrystallographic 2-fold axis. Deviations from the noncrystallographic symmetry between the 12 peptides are very small: root-mean-square deviations (rmsd) are less than 0.3 Å between the Cα atoms of any two peptides, and they are less than 0.85 Å for all atoms. Within each hexamer, the peptides show a folding motif that was originally described for a number of oligonucleotide or oligosaccharide binding proteins [25]. This OB (oligonucleotide/oligosaccharide binding) fold has...
subsequently also been observed in the structure of ModE, the molybdate-dependent transcriptional regulator from *E. coli* [15]. The fold consists of a 5 strand Greek-key β sheet coiled to form a closed β barrel that is capped by a short α helix. In the case of Mop from *S. ovata*, the barrel consists of four strands of one peptide plus a fifth strand from an adjacent peptide, as shown in Figure 1. The capping α helix is located between strands β3 and β4.

The six polypeptides (A–F and G–L, respectively, for the two molecules of the asymmetric unit) are assembled to form a trimer of dimers: two peptides (A–B, C–D, and E–F, respectively) dimerize to form long C2-symmetric barrels consisting of two OB motifs (Figures 1 and 2a). The three dimers are then assembled around a noncrystallographic 3-fold axis to yield a hexamer of 32 symmetry.

**Metal Oxyanion Binding Sites**

Each hexameric Mop molecule contains eight metal binding sites of two different types; all of them are only formed upon oligomer assembly, i.e., each binding site is located on the interface between two or three dimers. Two of the binding sites (henceforth denoted as “type-1” sites) are located on the noncrystallographic 3-fold axis (Figure 3a) in a cavity that is open to the surface (Figure 4). Oxyanion binding involves three
pairs of hydrogen bonds from three oxyanion oxygen atoms to the backbone amide nitrogen atoms of residues 21 and 22 from three symmetry-equivalent peptides (Figures 5a and 6a). Thus, polypeptides A, C, and E form one type-1 binding site, and chains B, D, and F form the other. The experimental density indicates that the oxyanions populating the type-1 sites are rotationally disordered, which is also plausible from an energetic point of view; tilting the oxyanion by about 30° from the fully symmetric orientation (i.e., with one metal-oxygen bond coinciding with the noncrystallographic 3-fold axis) brings the “apical” oxygen within hydrogen bonding distance of the residue-20 amide group without significant elongation of the other NH...O bonds (Figure 6a).

There are six other metal oxyanion binding sites (“type-2” sites), which are not positioned on a noncrystallographic symmetry element. They are located at the interface between two dimers and involve hydrogen bonding to two peptides related by a noncrystallographic diad (Figure 3b); two such binding sites are thus formed by peptides A and D, two by C and F, and two by E and B (Figure 7). The oxyanion binding involves
Figure 4. Surface Representation of the Mop Hexamer

Surface representations of the Mop hexamer colored according to electrostatic potential (red, negative potential; blue, positive potential). The figure shows a section through the molecule parallel to the 3-fold axis. Two anion binding sites are shown; the upper one is located on the 3-fold axis, which runs vertically. The figure illustrates that the type-2 anion binding site (upper right of figure) is not accessible from the outside. Other inner surfaces surround solvent-filled cavities.

In the course of structure refinement, it became apparent that the two types of binding site are occupied differently, as judged from the experimental electron density. The 12 type-2 sites refine well when populated with WO$_4$, and this leads to acceptable atomic displacement parameters (average B factor of the 12 tungsten atoms is 8.8 Å$^2$). In fact, inductive coupled plasma mass spectroscopy (ICP-MS) with a “native” protein sample indicated the predominant presence of tungsten and yielded no detectable amount of molybdenum. Since the experimental electron density is about half as high for the type-1 sites compared to the type-2 sites, it is clear that the former have to be occupied by “something lighter” than WO$_4$. Originally, we had assumed MoO$_4$ to occupy these sites, which refined acceptably (average B factor of the four Mo atoms was 10.8 Å$^2$). However, the result of the ICP-MS analysis (which failed to detect molybdenum in the sample) as well as analysis of experimental anomalous differences (as described in the Experimental Procedures) strongly suggested that all sites are in fact occupied by tungstate, with partial (about 50%) occupancy for the type-1 sites.

In the crystal, the electron density observed at the type-1 sites is thus the superposition of about 50% empty sites with 50% sites occupied by (rotationally disordered [see above]) tungstate ions. Lack of observation of crystallographic disorder in this region indicates that little conformational change occurs upon oxyanion binding to these sites, i.e., the type-1 oxyanion binding sites are preformed even in the absence of oxyanions.
Figure 5. Hydrogen Bonds Involving the Oxyanions
(a) Coordination around the anions on the noncrystallographic triads (type-1), which were refined as half-occupied tungstates.
(b) Coordination and averaged distances of the "buried" anion (type-2).

This is not surprising in view of the fact that a large fraction of the hydrogen bonding interactions between oxyanion and protein involves backbone NH groups of both types of binding sites (see above).

Comparison to the Structure of the Transcriptional Regulator ModE
In all organisms studied so far, molybdate transport genes are regulated by a repressor protein, ModE [14], whose C-terminal molybdenum binding domain consists of a tandem repeat of a Mop-like sequence (Figure 8b). In solution, ModE occurs as a homodimer binding two molecules of Mo with a K_d of 0.8 µM [26, 27]. The three-dimensional structure of the ModE protein from E. coli in the absence of molybdate was recently reported [15].

The fold of the Mop peptide as observed in the present crystal structure superimposes very well on the C-terminal subdomain of ModE (Figure 2b, 25% sequence identity, 41% homology for ModE residues 123–254). In fact, the three-dimensional structure comparison program DALI [28] identified the second Mop sequence in ModE as the most closely related protein structure to the S. ovata Mop. The mean C_i deviation for these residues (Figure 2b) is 0.83 Å; superposition of the S. ovata peptide on the first Mop sequence of ModE (according...
As in solution, crystals of *E. coli* ModE contain homodimers of two peptides, whose C-terminal domains are related by an approximate noncrystallographic 2-fold axis. Although no molybdate sites were observed experimentally, a number of residues possibly involved in binding the oxyanion were identified on the basis of sequence comparison with other Mop proteins [15]. Superposition of the two C-terminal domains of the ModE crystal structure on two adjacent dimers of the *S. ovata* Mop structure (Figure 2c) shows excellent agreement. This indicates which residues are likely to be involved in oxyanion binding by ModE: sidechain atoms of Ser-126, Thr-163, Ser-166, and Lys-183 and mainchain NH of residues Arg-128, Thr-163, and Ala-184. Some but not all of these residues have previously been suggested from sequence comparisons [15].

Circular dichroism and fluorescence spectroscopy data had suggested that binding of molybdate or tungstate to ModE should cause a conformational change of the protein [26, 29]. In view of this evidence, the good agreement between *S. ovata* Mop (in the presence of oxyanions) and *E. coli* ModE (in the absence of oxyanions) shown in Figure 2c is somewhat surprising. Thus, one C-terminal domain of ModE superimposes on one *S. ovata* dimer with an rmsd of 1.56 Å, whereas two adjacent *S. ovata* dimers superimpose with 1.77 Å on the corresponding ModE dimers. These results indicate only small overall conformational changes upon oxyanion binding. This is even more surprising since binding of ModE to DNA is known to be molybdate dependent, and it is hard to see how in the absence of a conformational change upon molybdate binding the N-terminal DNA binding domain could sense the presence of bound molybdate.

**Is Mop a Molybdopterin Protein?**

Early spectroscopic evidence suggested that the Mop protein from *C. pasteurianum* bound an inactive form of molybdopterin, which yielded “multiple pterin-like fluorescent compounds”...
upon denaturation and oxidation [8]. Although this has henceforth not been verified, we are also not aware of any evidence contradicting the original observation. The present crystal structure yields little evidence for a pterin-like compound attached to any of the oxyanions, but the possibility cannot be ruled out that such pterin-like residues were originally present and were lost in the course of the protein isolation, which was not performed under exclusion of oxygen. If this was the case, the structure in fact suggests that the two “apical” tungstates in the type-1 binding sites are more likely candidates for derivatization than are the oxyanions in the type-2 sites since the former are closer to the surface and sterically more accessible.

Diffuse residual density located in the entrance to the binding cavity was observed near each of the “apical” oxygen atoms of tungstates occupying the type-1 sites. The disorder observed for the oxyanions occupying the type-1 sites prevents unambiguous interpretation of this density, which could either be due to solvent or to a disordered oxyanion substituent, which might then have originated from the degradation of a tungstopterin.

**Biological Implications**

Molybdoenzymes (and tungstenzymes) are ubiquitous in biology, yet most organisms live in an environment with molybdate (and tungstate) concentrations much below 1 μM [1]. In order to reach sufficient intracellular molybdate concentrations for the synthesis of molybdenum-containing cofactors, bacteria have developed a high-affinity ABC transporter system consisting of a periplasmic binding protein (ModA), an integral membrane protein (ModB), and an ATP binding energizer protein (ModC). Expression of these proteins is regulated by the repressor protein ModE. In addition, some organisms also express molybdenum storage proteins [4, 5].

Mop is small protein with about 70 aminoacids [8, 9] that has been implicated in intracellular molybdate homeostasis [12]. Its sequence has also been detected in other molybdenum storage proteins, such as ModG from A. vinelandii, which consists of a tandem repeat of two Mop sequences. The molybdate binding domain of the molybdate-dependent transcriptional regulator ModE also contains two Mop sequences. The crystal structure of the 7 kDa Mop protein from *S. ovata* shows that the protein forms highly symmetric hexamers binding eight oxyanions each. There are two structurally distinct types of oxyanion binding sites, which were both found to be occupied by tungstate, however with less-than-full occupancy for the type-1 binding sites. Thus, the type-1 sites appear to have a lower affinity to tungstate than the type-2 sites. Oxyanion binding occurs at the interface between subunits, i.e., the binding sites are only formed upon hexamer aggregation. Hydrogen bonding interactions between the oxyanion and the protein involves mainly backbone amide NH groups, and there is experimental evidence for the type-1 sites that no conformational change occurs upon oxyanion binding.

The biological function of proteins containing only Mop sequences (such as Mop and ModG) is unknown, but they have been implicated in molybdate homeostasis and molybdopterin cofactor biosynthesis [12]. The structure of *S. ovata* Mop yields few indications for the protein to bind a molybdo- or tungstopterin, but the possibility cannot be ruled out that such a pterin substituent was cleaved off during protein isolation and purification. While to our knowledge the Mop protein has so far not explicitly been implicated as a tungstate binding protein, the fact that Mop from *S. ovata* was isolated with tungstate instead of molybdate is not surprising and is probably due to the presence of an excess of WO<sub>4</sub> in the growth medium. Molybdate and tungstate are structurally and chemically very similar, and it is well known that tungsten can replace molybdenum in a number of molybdoenzymes [2, 30]. The ModABC transporter system does not appear to discriminate between the two oxyanions [12, 13, 31, 32], although a small discrimination between tungstate and molybdate has been noted for ModE [29]. It is not known whether *S. ovata* physiologically expresses tungstenzymes, and to what extent tungstate can replace molybdate under appropriate growth conditions. The present evidence, however, suggests that Mop might not only be involved in storage and homeostasis of molybdate but also of tungstate.

**Experimental Procedures**

**Crystallization, Data Collection, and Processing**

The protein, originally assumed to be a cornioid protein, was extracted, purified, and crystallized as described earlier [22, 23]. Protein was obtained from *S. ovata* grown under anaerobic conditions in the presence of trace elements, including 1 μM MoO<sub>4</sub> and 1 μM WO<sub>4</sub>. Crystals grown from such preparations will be referred to as “native” crystals. In an attempt to prepare selenomethionine-labeled protein, *S. ovata* cultures were also grown in the presence of subtoxic concentrations (1 mM) of DL-selenomethionine. From these preparations, “derivative” protein was purified in the same way as the native protein, and it was crystallized with the same precipitants. However, gel crystallization in silica-hydrogel at 21°C was used instead of hanging-drop vapor diffusion to obtain suitable “derivative” crystals. Eventually, it turned out that little or no selenomethionine had been incorporated into the “derivative” protein. However, the “derivative” crystals nevertheless helped in the structure solution; since they had some of the oxyanion binding sites occupied by something “lighter” than tungstate, possibly molybdate or selenate, the latter one could have emerged as a product of selenomethionine degradation or metabolism.

Data collection for a native crystal (spacegroup *Ccc222*, *a* = 109.72 Å, *b* = 138.33 Å, *c* = 110.37 Å) was performed at the EMBL beamline X11 at DESY in Hamburg, Germany. Two data sets were collected for derivative crystals. Assuming selenium was the anomalous scatterer, we optimized wavelength for anomalous differences; we collected a first high-resolution (20–2.5 Å) data set at BM14 at the ESRF in Grenoble, France. However, this data set did not permit structure solution. Later, we collected a second data set at the EMBL-beamline BW7A at DESY in Hamburg. Due to the condition of the crystal, this data set was of much poorer quality, but it extended to lower resolution (50–4.9 Å). The two derivative data sets were merged (note that merging of two data sets collected on different beamlines explains the seemingly paradoxical fact that R<sub>merge</sub> is lower for the highest resolution shell [consisting only of reflections from the Grenoble data set] than for all data, see Table 1). Crystals were cooled to approximately 110 K for all measurement by using mother liquor plus 30% glycerol as cryoprotectant. All frames were processed with the program DENZO [33, 34]. Scaling of the native data was done with the program scalepack [33, 34]. For the derivative, all data collected in Grenoble were merged with the low resolution data collected in Hamburg by using the program Scala [35] from the CCP4 program suite [36] for scaling.

**Phase Determination and Structure Refinement**

Interpretation of a difference Fourier map (derivative − native, program SOLVE [37]) initially yielded 11 metal positions. Five additional positions emerged from a difference Fourier synthesis, for which we used phases derived from the 11 initial positions assumed to be occupied by Se. To determine derivative phases from these 16 “heavy-atom” positions, the SAD routine from the CNS [38] package was used and yielded a partially interpretable density map with a resolution of 50–2.52 Å (from 0.442). Phase extension to 1.5 Å (native data) and density modification with CNS yielded an overall figure of merit of 0.915. A map computed with these phases showed clear density that was readily interpretable in most parts. Model building was done with the program O [39].

The complete model was refined with the programs ARP [40, 41] and REFMAC [42]. We used the phase-modified maximum likelihood target
function and geometric restraints. An exponential bulk solvent correction was applied, and individual isotropic atomic displacement parameters were refined along with atomic positions. The 12 peptides in the asymmetric unit were treated as independent, i.e., no restraints from noncrystallographic symmetry between the two hexamers or within each hexamer were applied during refinement. The four anions located on the noncrystallographic 3-fold axes (type-1) were originally refined as molybdates; the 12 other anions on “general” positions (type-2) were put in as tungstates. A Fourier synthesis computed with the experimentally observed anomalous differences yielded peaks on all 16 metal sites, and these peaks were about twice as high on the type-2 sites as on the type-1 sites. Since at a wavelength of 0.93 Å the anomalous signal of tungsten is almost an order of magnitude higher than the one of molybdenum, this evidence was taken to indicate that all sites are in fact occupied by WO₄²⁻, with full occupancy for the type-2 sites and about 50% occupancy for the type-1 sites. Likewise, calculation of anomalous occupancies (with the program M乎are from the CCP4 suite [36]) yielded occupancies that were about twice as large for the type-2 sites as compared to the type-1 sites for a model that had all metal sites occupied by tungsten. An alternative model in which the type-1 sites are occupied by molybdenum and the type-2 sites by tungsten yielded 5-fold higher occupancies for the type-1 sites. The final model thus had all sites occupied by tungstate; the type-1 sites had a fixed occupancy of 50%, and the type-2 sites had a fixed occupancy of 100%. Occupancies were not allowed to refine. Refinement converged at a residual R₀ = 18.50% (for all data with σ cutoff) for a model containing 380 water molecules. Five percent of randomly distributed reflections, which had not been included in any of the refinement steps, were used to compute R(ref) [43], which converged at 19.8%. The structure was validated with program PROCHECK [24]. Additional information on the data collection and refinement is given in table 1.

**Determination of the Amino Acid Sequence**

Since little sequence information was available from protein or nucleic acid sequencing, the peptide sequence had to be deduced from the experimental electron density. The result is shown in Figure 8a, where the map-derived sequence is given for each of the 12 crystallographically independent peptides. The resolution of the data is high, and the density is well defined for the whole main chain and most side chains. It was unnecessary to use multiple conformations to fit the experimental electron density. The only ambiguities concern the distinction between asparagine/aspartic acid and glutamine/glutamic acid, which was only possible in the case of Asn-7 on the basis of the hydrogen bonding pattern. The resulting assignment is also compatible with other Mop sequences. Other ambiguities concern the distinction between valine and threonine, which was often but not always possible on the basis of observed hydrogen bonds. There are several cases where the side chain of an amino acid showed interpretable density only in one or several out of the 12 independent peptides, as indicated in Figure 8a. Figures 2–4, 6, and 7 were generated with the programs MOLSCRIPT [44] and Raster3D [45].

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**References**


Accession Numbers
Coordinates have been deposited with the Protein data bank (PDB accession code 1FR3, RCSB011841).