Structure of Mycobacterium tuberculosis Methionine Sulfoxide Reductase A in Complex with Protein-Bound Methionine

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Peptide methionine sulfoxide reductase (MsrA) repairs oxidative damage to methionine residues arising from reactive oxygen species and reactive nitrogen intermediates. MsrA activity is found in a wide variety of organisms, and it is implicated as one of the primary defenses against oxidative stress. Disruption of the gene encoding MsrA in several pathogenic bacteria responsible for infections in humans results in the loss of their ability to colonize host cells. Here, we present the X-ray crystal structure of MsrA from the pathogenic bacterium Mycobacterium tuberculosis refined to 1.5 Å resolution. In contrast to the three catalytic cysteine residues found in previously characterized MsrA structures, M. tuberculosis MsrA represents a class containing only two functional cysteine residues. The structure reveals a methionine residue of one MsrA molecule bound at the active site of a neighboring molecule in the crystal lattice and thus serves as an excellent model for protein-bound methionine sulfoxide recognition and repair.

Both free and protein-bound methionine (Met) residues can be oxidized to methionine sulfoxide (Met-O) by reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) produced by aerobic metabolism. The formation of two distinct forms of Met-O, methionine-(S)-sulfoxide (Met-S-O) and methionine-(R)-sulfoxide (Met-R-O), has been observed in vitro (27, 49). Peptide methionine sulfoxide reductase A (MsrA; EC 1.8.4.6) reduces Met-S-O to Met (32, 41), while MsrB (16, 37, 42), which demonstrates no sequence identity to MsrA (21), uses Met-R-O as a substrate (27, 49). Both MsrA and MsrB proteins reduce Met-O in a manner independent of protein-bound metals or cofactors and appear to be highly conserved across living organisms ranging from bacteria to plants to mammals.

It has been proposed that Met residues in proteins can protect cells against ROS and RNI damage by acting as an ROS sink (23). Surface-exposed Met residues in proteins can scavenge ROS and RNI to generate Met-O followed by reduction of these Met residues by MsrA. The net result is a decrease in the cellular concentration of these reactive intermediates at the expense of thiol-containing reducing agents (see below). In addition, the oxidation of Met can affect the biological activity of many proteins (reviewed in reference 5), and it has been suggested that an alternative or additional role of MsrA is to act as a regulator of such proteins in a manner analogous to the removal of a phosphoryl group by phosphatases (43). For example, the rate of closing of a voltage-dependent K\(^+\) channel is modulated by the oxidation of a single Met residue. This effect is reversed when MsrA is introduced, suggesting that Met oxidation followed by reduction by MsrA could serve as an on/off mechanism to regulate the biological activity of the channel (11). Similarly, Met oxidation has been shown to affect the ability of calmodulin to bind calcium, an effect that is reversed when MsrA is added, which in turn suggests that the oxidation and reduction of Met residues may play a role in the regulation of calcium homeostasis (51).

Because Met oxidation can impair the function of proteins critical to viability, the absence of MsrA can lead to various abnormalities in different organisms. For example, Streptococcus pneumoniae, Neisseria gonorrhoeae, Escherichia coli, and Mycoplasma genitalium, which are deficient in MsrA, demonstrate altered cytoadherence patterns (50). Mice lacking MsrA develop an atypical walking pattern (tip-toe) and have a reduced life span (30). While msrA mutant mice show increased sensitivity to oxygen (30), other organisms deficient in MsrA also exhibit increased sensitivity to exogenous oxidants. For example, E. coli (33), Erwinia chrysanthemi (17), M. genitalium (12), and Staphylococcus aureus (42), which lack the msrA gene, demonstrate increased sensitivity to H\(_2\)O\(_2\). Similarly, yeast strains lacking MsrA are more sensitive to oxidative stress (31). It has also been demonstrated that an msrA mutant strain of E. coli is hypersensitive to nitric oxide and that introduction of a plasmid containing msrA from Mycobacterium tuberculosis can restore this defect (45). Furthermore, MsrA has been shown to be important for the survival of E. chrysanthemi (17) and M. genitalium (12) once they have gained entry into host cells. MsrA plays a significant role in the intracellular survival of Mycobacterium smegmatis (T. Douglas, D. S. Daniel, C. Jagannath, and S. Dhandayuthapani, unpublished data). M. smegmatis has the ability to survive inside mononuclear phagocytes for several days (1), and this species has been used as a surrogate to identify genes of M. tuberculosis that are important for intracellular survival (28, 29, 34, 48). We hypothesize that by analogy, MsrA in M. tuberculosis plays a similar role.
Finally, there is growing evidence that Met oxidation is likely to be important in the aging process (44) as well as in the etiology of neurodegenerative diseases (15).

Biochemical data suggest a catalytic mechanism for MsrA that involves the formation of a sulfenic acid intermediate followed by thiol-disulfide exchange between three conserved cysteine residues and thioridoxin to regenerate the active site (3, 4, 25, 32). Here we present the crystal structure of \( M. tuberculosis \) MsrA (MsrA\(_{Mtb}\)), an MsrA that contains only two cysteine residues, in complex with protein-bound Met. The structure reveals differences relative to the Bos taurus (MsrA\(_{Bt}\)) and \( E. coli \) (MsrA\(_{Ecoli}\)) structures that may assist in the understanding of observed differences in the ability of thioridoxin to efficiently regenerate the active site in these two classes of proteins.

**MATERIALS AND METHODS**

**Preparation and crystallization of MsrA.** The gene \( r v0137c \) (marA) was amplified by PCR using primers TBMSRAEX1 (5'-GGGAGTCCGCGCATATGACGAGCAGAATCAG-3'); \( NdeI \) site underlined) and TBMSRAEX2 (5'-CGGGGATCCGGCCGCCGCCTG-3'); BamHI site underlined) and \( M. tuberculosis \) H37Rv chromosomal DNA, prepared according to published procedures (13). The gene was inserted into the \( NdeI \) and BamHI sites of the pET16b vector (Novagen) to create recombinant MsrA\(_{Mtb}\) protein with a factor Xa-cleavable N-terminal His\(_{10}\) tag. Recombinant protein was overexpressed in \( E. coli \) BL21(DE3) and purified from cell lysates using nickel affinity chromatography. The purified protein was dialyzed overnight into 25 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 1 mM tris(2-carboxyethyl)phosphine hydrochloride, a nonthiol-containing reducing agent, followed by concentration to 30 mg/ml for crystallization trials. The N-terminal His\(_{10}\) tag was not removed prior to crystallization screening experiments. Polycrystalline clusters of MsrA\(_{Mtb}\) were obtained initially from Hampton Research Crystal Screen I condition 34 using the sitting drop vapor diffusion method. Optimization of these crystallization conditions using hanging drop methods produced suitable single crystals in 2.0 M sodium formate-0.1 M sodium citrate, pH 6.0, after 1 week of incubation at 4°C. Prior to data collection, crystals were soaked in 6.3 M sodium formate-0.1 M sodium citrate, pH 6.0, for 2 min and flash-cooled in liquid nitrogen.

**Data collection.** Data were collected using a Rigaku FR-D rotating anode X-ray generator (Rigaku Corporation) equipped with an R-AXIS IV image plate system (Molecular Structure Corporation). Crystals were mounted on a single phi-axis goniostat and cooled during data collection using an X-STREAM low-temperature system (Molecular Structure Corporation). One-degree oscillation images were collected over a total sweep of 150° with a 4-min exposure time per image. The crystal-to-detector distance was 70 mm. The data were processed with HKL2000 (38) using all data in the 50.0 to 1.5 Å resolution range. The MsrA crystals belong to space group \( P2_12_12_1 \) with unit cell dimensions \( a = 40.55, b = 64.11, c = 73.56 \) Å.

**Structure determination.** The structure of MsrA\(_{Mtb}\) was determined by the molecular replacement method (40) using the program AMORE (36). MsrA\(_{Ecoli}\) (Protein Data Bank accession code 1FVA) was used as the search model because the similarity of their primary sequences with 38 and 44% identity, respectively, (Fig. 1) the electron density of the MsrA\(_{Mtb}\) structure is generally very well defined (Fig. 2). Two residues at the N terminus that are part of the linker region between the His\(_{10}\) tag and the MsrA\(_{Mtb}\) protein sequence are clearly observed in addition to 166 residues of the MsrA\(_{Mtb}\) protein. Sixteen residues at the C terminus were not modeled due to disorder in this region. Cys-154 near the active site has extra electron density extending from the sulfur position, which creates the appearance of a modified cysteine residue. However, calculation of an anomalous difference Fourier map shows the same extension at the sulfur position of Cys-154, while the peaks for Cys-13 and Met-1 are roughly spherical (Fig. 3).

**RESULTS AND DISCUSSION**

**MsrA\(_{Mtb}\) structure.** The MsrA fold has been previously characterized as a member of the \( \alpha/\beta \) class in MsrA\(_{Bi}\) (Protein Data Bank accession code 1FFA) (26) and MsrA\(_{Ecoli}\) (Protein Data Bank accession code 1FF3) crystal structures (46). The MsrA\(_{Mtb}\) fold resemble these structures as expected based on the similarity of their primary sequences with 38 and 44% identity, respectively (Fig. 1). The electron density of the MsrA\(_{Mtb}\) structure is generally very well defined (Fig. 2). Two residues at the N terminus that are part of the linker region between the His\(_{10}\) tag and the MsrA\(_{Mtb}\) protein sequence are clearly observed in addition to 166 residues of the MsrA\(_{Mtb}\) protein. Sixteen residues at the C terminus were not modeled due to disorder in this region. Cys-154 near the active site has extra electron density extending from the sulfur position, which creates the appearance of a modified cysteine residue. However, calculation of an anomalous difference Fourier map shows the same extension at the sulfur position of Cys-154, while the peaks for Cys-13 and Met-1 are roughly spherical (Fig. 3).

**TABLE 1.** Statistics for MsrA\(_{Mtb}\) X-ray diffraction data and model refinement

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Value(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit cell dimensions (Å)</td>
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</tr>
<tr>
<td>Space group</td>
<td>( P2_12_12_1 )</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
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</tr>
<tr>
<td>No. of reflections</td>
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<tr>
<td>Completeness (%)</td>
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<td>Redundancy (×)</td>
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<tr>
<td>( R_{	ext{sym}} )</td>
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</tr>
<tr>
<td>( R_{	ext{free}} )</td>
<td>0.177</td>
</tr>
<tr>
<td>No. of amino acids</td>
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<tr>
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<tr>
<td>No. of water atoms</td>
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<tr>
<td>Mean B factor of: Protein atoms (Å(^2))</td>
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<tr>
<td>Water atoms (Å(^2))</td>
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</tr>
<tr>
<td>B factor from Wilson plot (Å(^2))</td>
<td>17.3</td>
</tr>
</tbody>
</table>

\(^a\) Statistics in the highest-resolution shell (1.55 to 1.50 Å) are in parentheses.

\(^b\) \( R_{	ext{sym}} = \Sigma |F_{\text{calc}}| - |F_{\text{obs}}| |/\Sigma |F_{\text{calc}}| \), where \( |F_{\text{calc}}| \) is from a working set used in the structural refinement.

\(^c\) \( R_{	ext{free}} = \Sigma |F_{\text{calc}}| - |F_{\text{obs}}| |/\Sigma |F_{\text{calc}}| \), where \( |F_{\text{calc}}| \) is from a test set not used in the structural refinement.

\(^d\) RMS deviation values are deviations from ideal values.

**Protein structure accession number.** The structure factors and refined coordinates of MsrA\(_{Mtb}\) have been deposited in the Protein Data Bank with accession code 1NWA.
that it possesses a significantly truncated N-terminal region and in that it lacks a cysteine residue conserved in the C-terminal region (Cys-227 in MsrA<sub>Bt</sub> and Cys-206 in MsrA<sub>Ecoli</sub>). MsrA<sub>Mtb</sub> has only 11 residues preceding the conserved GCFWG active-site motif, while the bovine and E. coli forms have 70 and 49 residues, respectively. The N-terminal extension in MsrA<sub>Ecoli</sub> has been proposed to have a role in folding efficiency in vivo (3), but its absence in MsrA<sub>Mtb</sub> suggests that such an extension is unnecessary for proper folding and catalysis in MsrA<sub>Mtb</sub>.

The MsrA<sub>Mtb</sub> active site and implications for substrate recognition and catalysis. The proposed catalytic mechanism for MsrA<sub>Bt</sub> and MsrA<sub>Ecoli</sub>, which involves three cysteine residues designated CysA, CysB, and CysC, is summarized as follows (3, 4): (i) CysA carries out a nucleophilic attack on the sulfenic acid moiety at CysA and a Met leaving group; (ii) a rearrangement occurs, producing a proposed sulfenic acid moiety at CysA and a Met leaving group; (iii) acid catalysis facilitates the release of the hydroxyl group of the sulfenic acid as a water molecule, followed by attack of CysB on CysA leading to the formation of a disulfide bond; and (iv) CysC similarly attacks CysB, forming a disulfide bond which in turn is ultimately reduced by the thioredoxin/thioredoxin reductase system or dithiothreitol (DTT) to recycle the active site.

MsrA<sub>Mtb</sub> Cys-13 and Cys-154 are the only cysteine residues in the molecule and are equivalent to CysA and CysB, respectively. The lack of CysC in MsrA<sub>Mtb</sub> means that the mechanism of regeneration of the active site must be different from what has been proposed for the previously characterized MsrA<sub>Bt</sub> and MsrA<sub>Ecoli</sub> proteins. Mutation and truncation experiments

FIG. 1. Sequence alignment of MsrAs from <i>B. taurus</i>, <i>E. coli</i>, and <i>M. tuberculosis</i>. Residues that are identical in these proteins are boxed. CysA, CysB, and CysC (see text) are indicated above the alignment as bold letters A, B, and C, respectively. The consensus sequence found in all known MrsaAs is shown in bold lettering. The alignment was generated using the CLUSTAL W method (47).

FIG. 2. Stereo view of a 2Fo-Fc SIGMAA-weighted electron density map (39) calculated at a contour level of 1.4σ at the active-site region containing the consensus sequence GCFWG. His-0 and Met-1 from a neighboring MsrA<sub>Mtb</sub> molecule are shown in violet. Water molecules are shown in dark red, and hydrogen bonds at a distance of 3.2 Å or less are shown as dashed lines. This figure was generated using BOBSCRIPT (14), GLR (version 0.5.0; National Institutes of Health, Bethesda, Md.; http://convent.nci.nih.gov/~web/glr/glrrhome.html), and POV-Ray (version 3.5; POV-Team, Williamstown, Victoria, Australia; http://www.povray.org).
eliminating CysC performed on MsrA<sub>Bt</sub> and MsrA<sub>Ecoli</sub> produced Met-O reductase activity with little or no ability to regenerate the active site using the thioredoxin/thioredoxin reductase system (3, 4, 25). Thus, the CysA-CysB disulfide bond described above that is proposed to be formed during catalysis in MsrA<sub>Bt</sub> and MsrA<sub>Ecoli</sub> is not directly reduced by thioredoxin. In contrast, it has been shown that recombinant MsrA<sub>Mtb</sub> is active in vitro and has a comparable specific activity to unmutated MsrA<sub>Ecoli</sub> using the thioredoxin/thioredoxin reductase system (45). These results suggest that in MsrA<sub>Mtb</sub>, thioredoxin can directly reduce the CysA-CysB disulfide bond formed after product release (see below).

Examination of the three available MsrA structures indicates a structurally conserved core, including a well-conserved motif at the active site containing the consensus sequence GCFWG. However, two observations in the MsrA<sub>Mtb</sub> crystal structure suggest possible reasons for the difference in mechanisms for recycling the active-site nucleophilic cysteine residue among these MsrAs. First, structural differences are evident by visual inspection of the C-terminal region containing CysB in MsrA<sub>Mtb</sub> and that containing CysB and CysC in MsrA<sub>Bt</sub> and MsrA<sub>Ecoli</sub> (Fig. 4). In a stretch of 18 residues at the C terminus which have observable electron density in all three known MsrA structures, the RMSD for Cα atoms in MsrA<sub>Mtb</sub> residues 146 to 163 is 2.9 Å when compared to the equivalent MsrA<sub>Bt</sub> residues 211 to 228 and 3.3 Å when compared to the equivalent MsrA<sub>Ecoli</sub> residues 191 to 208. These differences are greater than the RMSD for this region comparing MsrA<sub>Bt</sub> to MsrA<sub>Ecoli</sub> (2.2 Å) and the average RMSD comparing the entire MsrA<sub>Mtb</sub> structure to the others (1.5 to 1.6 Å). Comparing the primary sequence for this 18-residue region shows only 3 identities between MsrA<sub>Mtb</sub> and MsrA<sub>Bt</sub> or MsrA<sub>Ecoli</sub>, while the latter two share 11 identities. Second, Fig. 5 shows that the position of CysB in MsrA<sub>Mtb</sub> is shifted one residue relative to the positions of CysB in the bovine or <i>E. coli</i> forms, which must lead to a different position for the CysA-CysB disulfide bond formed during the reaction cycle and in turn could lead to variation in accessibility and/or reactivity of this disulfide bond toward thioredoxin (3). Evidence for flexibility of the C terminus has been observed as a conformational change in this region in crystal structures of a free form of MsrA<sub>Mtb</sub> compared to a DTT-bound form, where a DTT molecule bridges the sulfur atoms of CysA and CysB (26). By analogy, the C-terminal region of MsrA<sub>Mtb</sub> is presumed to be flexible and, furthermore, with the shifted CysB relative to the bovine and <i>E. coli</i> forms.
forms, it may adopt a conformation that is amenable to direct reduction of the CysA-CysB disulfide bond by thioredoxin. Such a conformation might be disfavored or disallowed in MsrA \textit{Bt} and MsrA \textit{Ecoli}, to account for the low reactivity toward thioredoxin in these enzymes. Examination of distances between CysA and CysB reveals that the positions of their side chains in MsrA \textit{Mtb} can be altered such that formation of a disulfide bond is possible. The C\textsubscript{α}-C\textsubscript{α} atom distance for CysA and CysB in MsrA \textit{Mtb} is 7.8 Å. The rotamers of the cysteine side chains can be modeled such that the closest distance between sulfur atoms is 3.4 Å. In contrast, the C\textsubscript{α}-C\textsubscript{α} atom distance for CysA and CysB in MsrA \textit{Ecoli} is 11.0 Å, too long for disulfide bond formation (46). In MsrA \textit{Bt}, this distance is 7.1 Å, but the side chain rotamers of CysA and CysB in MsrA \textit{Bt} cannot be reoriented in as favorable a manner for disulfide bond formation as is observed in MsrA \textit{Mtb}. This is due to the fact that CysB in MsrA \textit{Bt} is shifted one residue toward the N terminus relative to the position of CysB in MsrA \textit{Mtb}. Thus, a more substantial conformational change to the protein backbone would be necessary to form the bond (Fig. 5).

A fortuitous crystal-packing contact is observed in that Met-1 of one MsrA \textit{Mtb} molecule binds to the active-site pocket of a neighboring MsrA \textit{Mtb} molecule in the crystal lattice. The interaction between MsrA molecules at this crystal contact buries approximately 587 Å\textsuperscript{2} of solvent-accessible surface area per polypeptide. The specificity of the interaction appears to be mediated primarily through Met binding to the active site. In addition to this specific interaction, there exist a large number of water-mediated contacts (Fig. 6), consistent with the ability of MsrA to act on Met-O, found in a wide variety of protein molecules. Met-1 is positioned at the surface of the protein in a type I reverse turn formed by His-0 (part of the linker region of the His\textsubscript{10} tag), Met-1, Thr-2, and Ser-3 and binds in the active-site pocket of a neighboring MsrA molecule related by crystal symmetry. This interaction forms a crystal lattice comprised of continuous chains of MsrA molecules stacking upon one another (Fig. 7). This crystal contact at the active site does not appear to be favored per se by residues near Met-1 in the polypeptide chain. Electron density is observed for only two other residues N-terminal to Met-1, Arg-1\textsuperscript{−} and His-0, both of which come from the His\textsubscript{10} tag. The side chains of these residues do not interact with the neighboring MsrA molecule in the crystal (Fig. 6). As an exercise, we substituted Arg-1\textsuperscript{−}, His-0, and Thr-2 by using a database of the most commonly observed side chain rotamers (24). The results suggest that permissible rotamers for almost all amino acid substitutions could occur as judged by the resulting van der Waals interactions and potential hydrogen bonds that can be formed (data not shown). Bulky aromatic residues at these positions are the notable exception, however, as they appear sterically incompatible with the observed crystal packing pattern.

Although the binding of Met-1, the product of MsrA catalysis, may be an artifact of crystallization, the specific contacts involving Met-1 in the active site provide a plausible model for the mode of substrate binding and recognition by MsrA when modeling Met-1 as Met-S-O (Fig. 8). The sulfur atom of Met-1 is located in proximity (3.4 Å) to the sulfur atom of Cys-13 (CysA), which provides a view of Cys-13 poised for nucleophilic attack on Met-S-O. The e-methyl group of Met-1 is found in the hydrophobic pocket formed by Phe-14 and Trp-15 in the GCFWG active-site consensus sequence. A side chain
oxygen (O_{1}) of Asp-87 forms a hydrogen bond to the backbone amide of Met-1, possibly contributing stability during substrate binding. The other oxygen (O_{2}) of the Asp-87 side chain is involved in a hydrogen bond with conserved Tyr-152 and with a water molecule (Fig. 8) that forms a bridge to the backbone amide of Cys-13. This water molecule is conserved in the MsrA_{Bt} and MsrA_{Ecoli} crystal structures and is therefore likely to play a structural role in properly orienting the side chain of Asp-87 to interact with the backbone amide of incoming substrate Met-S-O in proteins. The aromatic ring of Tyr-152 also forms a stacking interaction with the peptide bond between His-0 and Met-1. Two conserved acidic residues in MsrA_{Bt} and MsrA_{Ecoli}, Glu and Asp in the vicinity of CysA, were previously identified as potential acid catalysts (26, 46). Based on the structure of MsrA_{Mtb} with Met-1 bound in the active site, Glu-52 seems a more feasible acid catalyst due to its orientation and proximity to the Met-1 and Cys-13 sulfur positions, while Asp-87 serves to stabilize substrate binding. Conserved residues Tyr-44 and Tyr-92, in addition to Glu-52, form hydrogen bonds to an additional structurally conserved water molecule common to the three known MsrA structures (Fig. 7). Assuming this water is displaced during substrate binding, one can envision how the Tyr and Glu residues might form a recognition site for the oxygen atom of the Met-S-O side chain by contributing hydrogen bonds in a similar fashion to a proposed model for an MsrA_{Bt} transition-state intermediate (26). To further account for substrate specificity, it appears that binding of the R form of Met-S-O would be disfavored due to steric clash. In fact, a crystal structure of the MsrB domain from the N. gonorrhoeae pilB protein reveals a mirrored active-site arrangement when compared to MsrA, illustrating that the architecture of the active sites has evolved to act on only one enantiomer of Met-O (27).

A dimethyl arsenate moiety is covalently bound to the sulfur atom of CysA in the crystal structure of MsrA_{Ecoli} (46) and may serve as a model for an enzymatic intermediate or transition state. This is similar to a model described for the MsrB domain of N. gonorrhoeae pilB based on the position of a cacodylate ion noncovalently bound in its active site (27). Structural alignment of MsrA_{Mtb} and MsrA_{Ecoli} proteins based on the minimization of differences between C_{\alpha} positions of residues of the GCFWG motif superimposes elements of the Met-1 side chain from MsrA_{Mtb} onto the dimethyl arsenate.

FIG. 7. Crystal packing showing three MsrA_{Mtb} molecules related by the crystallographic twofold screw axis parallel to the c-axis of the unit cell. Each molecule is presented as a C_{\alpha} backbone trace surrounded by a transparent rendering of its molecular surface. The N termini containing the Met-1 residue that interacts with the active site of their neighboring molecules are circled. The crystal lattice is composed of continuous chains of this packing pattern extending in the vertical direction of the graphic. This figure was generated using PyMOL (version 0.86; DeLano Scientific, San Carlos, Calif.; http://www.pymol.org).

FIG. 8. Stereoview of a putative model of Met-S-O binding to the active site prior to nucleophilic attack by Cys-13. Met-S-O-1, shown in violet, is modeled in the position of Met-1 of the crystal structure. Hydrogen bonds at a distance of 3.2 Å or less are shown as black dashed lines. This figure was generated using BOBSCRIPT (14), GLR (version 0.5.0; National Institutes of Health; http://convent.nci.nih.gov/~web/glr/glrhome.html), and POV-Ray (version 3.5; POV-Team; http://www.povray.org).
atoms in the active site of the MsrA \textsubscript{EcoG} structure. The Met-1 C\textsubscript{γ}, S\textsubscript{α}, and C\textsubscript{ε2} atoms occupy positions similar to the C\textsubscript{γ1}, A\textsubscript{S\textsubscript{α}}, and C\textsubscript{ε2} atoms of the dimethyl arsenate moiety, respectively, with an RMSD of 0.4 Å. Thus, the similarity of the positions of MsrA\textsubscript{Mt} Met-1 and the putative transition state or intermediate analog dimethyl arsenate in MsrA \textsubscript{EcoG} lends further support to the notion that the protein-bound Met-1 found in the active site serves as a model for protein-bound substrate binding.

Genes associated with bacterial virulence and survival in hosts are gaining increased recognition as potentially more-effective antimicrobial targets than current therapeutic targets treating the growing problem of multidrug-resistant bacteria (9, 10). Since \textit{M. tuberculosis} MsrA and MsrB are likely factors in intracellular survival in humans, it will be desirable to obtain crystal structures for \textit{M. tuberculosis} MsrB and the human forms of these enzymes to combine with the MsrA\textsubscript{Mt} data toward the prospect of producing a new class of antituberculosis drugs.

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**REFERENCES**


Molecular characterization of a chromosomal locus in *Staphylococcus aureus* that contributes to oxidative defence and is highly induced by the cell-wall-active antibiotic oxacillin. Microbiology 147:3037–3045.


