Characterization of the Methionine Sulfoxide Reductase Activities of PILB, a Probable Virulence Factor from *Neisseria meningitidis*

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PILB has been described as being involved in the virulence of bacteria of *Neisseria genus*. The PILB protein is composed of three subdomains. In the present study, the central subdomain (PILB-MsrA), the C terminus subdomain (PILB-MsrB), and the fused subdomain (PILB-MsrA/MsrB) of *N. meningitidis* were produced as folded entities. The central subdomain shows a methionine sulfoxide reductase A (MsrA) activity, whereas PILB-MsrB displays a methionine sulfoxide reductase B (MsrB) activity. The catalytic mechanism of PILB-MsrB can be divided into two steps: 1) an attack of the Cys-494 on the sulfur atom of the sulfoxide substrate, leading to formation of a sulfenic acid intermediate and release of 1 mol of methionine/mol of enzyme and 2) a regeneration of Cys-494 via formation of an intradisulfide bond with Cys-439 followed by reduction with thioredoxin. The study also shows that 1) MsrA and MsrB display opposite stereoselectivities toward the sulfoxide function; 2) the active sites of both Msrs, particularly MsrB, are rather adapted for binding protein-bound MetSO more efficiently than free MetSO; 3) the carbon Cα is not a determining factor for efficient binding to both Msrs; and 4) the presence of the sulfoxide function is a prerequisite for binding to Msrs. The fact that the two Msrs exhibit opposite stereoselectivities argues for a structure of the active site of Msrs different from that of MsrAs. This is further supported by the absence of sequence homology between the two Msrs in particular around the cysteine that is involved in formation of the sulfenic acid derivative. The fact that the catalytic mechanism takes place through formation of a sulfenic acid intermediate for both Msrs supports the idea that sulfenic acid chemistry is a general feature in the reduction of sulfoxides by thios.

Peptide methionine sulfoxide reductase (MsrA) activity is described as being involved in the virulence of the pathogens

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‡ The abbreviations used are: Msr, MsrA, and MsrB, methionine sulfoxide reductase, methionine sulfoxide reductase A, and methionine sulfoxide reductase B, respectively; dinedone, 5,5-dimethyl-1,3-cyclohexanedione; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzo-

Escherichia coli, Streptococcus pneumoniae, Erwinia chrysanthemi, Mycoplasma genitalium, and Neisseria gonorrhoeae (1–4). Inspection of the alignment of the corresponding protein sequences shows that all possess in common a sequence that displays an MsrA activity. This MsrA activity has now been well characterized at the structural level (5, 6) and the enzymatic level (7). In particular, a sulfenic acid intermediate has been shown to be formed on Cys-51 of *E. coli* MsrA during the reduction of the sulfoxide function of methionine sulfoxide (MetSO). The active site can be represented as an open basin in which Cys-51, located at the N terminus of an α-helix, is accessible. Compared with the *E. coli* MsrA, the MsrAs from *S. pneumoniae* and from *N. meningitidis* or *N. gonorrhoeae* (called PILB) contain, in addition, an extension at the C terminus and at the C and N termini, respectively. This raised the question of the role of these extensions, in particular of the C-terminal extension. Sequence comparisons of the C-extension of PILB show amino acid identities with open reading frames of which no function has been assigned until recently. These sequences are detected in all kingdoms. Recently, the functions of the *E. coli* ortholog YeaA and an open reading frame downstream from the *msrA* gene from *Staphylococcus aureus*, which both have at least 50% amino acid identities with the C-subdomain of PILB, has been determined and shown to display a new Msr activity, called MsrB (8, 9).

The fact that the MsrB activity of YeaA is thioredoxin-dependent (8) indicates that at least a Cys residue is involved in the catalytic mechanism. Inspection of the amino acid sequences shows that two Cys are often conserved in putative MsrBs (see Fig. 1). One Cys, Cys-439, which is located in a CGWP(S/A)/F motif is at least 50% conserved. The second one, Cys-494, which is included in an RYCV/V/M/N motif is almost conserved.

In the present study, we show that in addition to an MsrA activity that is displayed by the central subdomain, called PILB-MsrA, the C terminus of PILB, called PILB-MsrB, possesses a thioredoxin-dependent MsrB activity. The catalytic mechanism of PILB-MsrB is shown to proceed via the sulfenic acid chemistry. The role of Cys-439 and Cys-494 has been demonstrated. The stereoselectivity in the reduction of the sulfoxide function and the catalytic parameters of the two subdomains have also been determined. The results are in favor of a structure of the active site of MsrBs different from that of the MsrAs.

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1 The abbreviations used are: Msr, MsrA, and MsrB, methionine sulfoxide reductase, methionine sulfoxide reductase A, and methionine sulfoxide reductase B, respectively; dinedone, 5,5-dimethyl-1,3-cyclohexanedione; DTT, dithiothreitol; DTNB, 5,5′-dithiobis(2-nitrobenzo-

ate; MetSO, methionine sulfoxide; Pht, phthalyl; TNB, thionitrobenzoate (3-carboxy-4-nitrobenzenethiol); HPLC, high pressure liquid chromatography.

12016 This paper is available on line at http://www.jbc.org
EXPERIMENTAL PROCEDURES

Plasmid Constructions, Site-directed Mutageneses, Production, and Purification of Wild-type and Mutant N. meningitidis PILB—Plasmids pSKPILbMsrA, pSKPILbMsrB, and pSKPILbMsrA/MsrB, designed for PILB-MsrA, PILB-MsrB, and PILB-MsrA/MsrB production, respectively, were obtained by cloning internal fragments of the PILB open reading frame synthesized by PCR (sequences of oligonucleotides not shown) into the cloning system Zz4911 (genomic DNA kindly provided by Dr. M. K. Taha), into the plasmid pDB125KSN” between the NdeI and SacI sites. Site-directed mutageneses were performed using the QuikChange site-directed mutagenesis kit (Stratagene).

For PILB-Msrs purification, cells were harvested by centrifugation, resuspended in a minimal volume of buffer A (50 mM Tris-HCl, 2 mM EDTA, pH 8) containing 20 mM dithiothreitol (DTT) and sonicated. The Msrs were then precipitated at 40, 50, and 60% ammonium sulfate (NH₄)₂SO₄) saturation for PILB-MsrA/MsrB, PILB-MsrA, and PILB-MsrB, respectively. The contaminating proteins were removed by applying the enzymatic-mediated solution onto exclusion size chromatography on ACA 54 resin at pH 8 (buffer A). Purified fractions were then pooled and applied onto a Q-Sepharose column equilibrated with buffer A, followed by a linear gradient of KCl (0–0.4 M) using a fast protein liquid chromatography system (Amersham Biosciences). The PILB-MsrA/MsrB was eluted at 100 mM KCl, whereas PILB-MsrA and PILB-MsrB passed through. PILB-MsrA and PILB-MsrB were further purified on phenyl-Sepharose (Amersham Biosciences) equilibrated with buffer A, containing 1% (NH₄)₂SO₄. PILB-Msrs were eluted with a linear gradient from 1 to 0 M in buffer A.

At this stage, wild-type PILB-MsrA/MsrB and wild-type and mutant PILB-Msrs and PILB-Msrs were pure as checked by electrophoresis of the isomer obtained by crystallization from methanol. The absolute configuration of the L-Met-R,S-SO and Ac-L-Met-R,S-SO enantiomers were pooled and concentrated in order to eliminate acetoin and trifluoroacetic acid.

Msr Activities of PILB from N. meningitidis

Initial rate measurements were carried out at 25 °C by following the appearance of free methionine measured by HPLC. To do so, aliquots of the reaction mixture were removed at different times of incubation up to 2.5 min, and the reaction was stopped by the addition of trifluoroacetic acid to a final concentration of 1%. In each aliquot, the amount of Met formed was measured as previously described by Boschi-Muller et al. (7).

Determination of Msr Activity in the Presence of DTT—Msr activities were determined with D,L-Met-R,S-SO as a substrate at a concentration of 150 mM. The reaction mixture also contained 10 mM DTT and 5 µM wild-type PILB-MsrA/MsrB or wild-type or mutant PILB-Msrs in buffer A.

Initial rate measurements were carried out at 25 °C on a Kontron Umax spectrophotometer by following the decrease of the absorbance at 340 nm. The initial rate data were fitted to the Michaelis-Menten equation using a least squares analysis to determine kcat and Km values were determined at saturating concentrations of the other substrate.

Stoichiometry of Met Formation in the Absence of a Regenerating System—The reaction mixture, containing 150 mM D,L-Met-R,S-SO and a 100–500 µM concentration of wild-type or mutant PILB-MsrA/MsrB or wild-type or mutant PILB-Msrs, was incubated at 25 °C for 10 min in buffer A. Then the Met formed was quantified as previously described by Boschi-Muller et al. (7).

Characterization of the Sulfenic Acid Intermediate—The sulfenic acid intermediate was characterized spectrophotometrically by using thionbenzoate (TNB–) under nondenaturing conditions and by mass spectrometry analysis after modification (or no modification) with 5,5-diethyl-1,3-cyclohexanedione (dimedone).

For spectrophotometric characterization, TNB– was prepared by reducing the corresponding disulfide using the procedure of Silver (17). Progress curves of TNB– disappearance for wild-type and mutant PILB-Msrs and PILB-Msrs were recorded at 412 nm in buffer A. Enzyme concentrations were 7.35 and 14.7 µM, and the TNB– concentration was 60 µM. The amount of TNB– consumed was calculated using an extinction coefficient of 412 nm of 13,600 M–1 cm–1.

For spectrometric characterization, analyses were performed for wild-type and mutant PILB-Msrs and PILB-Msrs, either after modification or not by D,L-Met-R,S-SO and dimedone. All of the modification reactions were performed in buffer A in the presence of 20 µM enzyme. D,L-Met-R,S-SO was added at a concentration of 150 mM, and the mixture was incubated 10 min at 25 °C. Then dimedone at a concentration of 20 mM was added, and the mixture was incubated overnight in the dark at room temperature. Mass spectrometric measurements were performed on a LCT electrospray time-of-flight mass spectrometer (Micromass, Manchester, UK). For mass analysis in denaturing conditions, Mr samples were diluted to 10 µM in a 1:1 water/acetonic acid mixture (v/v) containing 1% formic acid. Samples were continuously infused into the ion source at a flow rate of 5 µl/min. Spectra were recorded in the positive ion mode in the mass range 400–4000 m/z, after calibration of the instrument with a solution of horse heart myoglobin (Sigma) diluted to 2 µM in the 1:1 water/acetonic acid (1% formic acid) mixture.

3 S. Marchal, personal communication.
4 C. Didierjean, unpublished results.
RESULTS AND DISCUSSION

Justification of the Truncations on PILB

PILB contains 522 amino acids and is composed of three subdomains. The N-subdomain is suggested to encode a disulfide oxidoreductase. The central subdomain is an ortholog to subdomains. The N-subdomain is suggested to encode a disulfide bond with Cys-348 followed by its reduction by Cys-206 and regeneration of Cys-206 via formation of an intermolecular disulfide bond with Cys-348. One mol of methionine was also formed with mutant C348S with a loss of one thiol, whereas no methionine was formed with mutant C206S. In the presence of DTNB reagent revealed four Cys for PILB-MsrA/MsrB and two Cys for both PILB-MsrA and PILB-MsrB under denaturing conditions (Table I). These results are in agreement with the PILB DNA sequence that indicates four Cys at positions 206 and 348 in PILB-MsrA and at positions 439 and 494 in PILB-MsrB. Under native conditions, all of the Cys were also reactive regardless of the subdomains. This shows that 1) both Cys of the MsrA subdomain are easily accessible, similar to what is described for E. coli MsrA; 2) both Cys of the MsrB subdomain are also accessible; and 3) the accessibility in PILB-MsrA/MsrB of each couple of Cys within each domain is not significantly altered by the presence of the second subdomain. This again supports an independent folding of each subdomain.

Stoichiometry of Methionine Formation and Thioredoxin Recycling Activity

The PILB-MsrA Subdomain—Stoichiometry of methionine formation was determined in the absence of reductant (Table II). One mol of methionine was formed with a loss of two thiols, i.e., in agreement with formation of a disulfide bond between Cys-206 and Cys-348. One mol of methionine was also formed with mutant C348S with a loss of one thiol, whereas no methionine was formed with mutant C206S. In the presence of thioredoxin, a recycling activity was observed with PILB-MsrA wild type but not with the mutants (Table II). Together, these results are in agreement with formation of a sulfenic acid on Cys-206 and regeneration of Cys-206 via formation of an internal disulfide bond with Cys-348 followed by its reduction by...
For the MsrB of PILB for regenerating the active Cys-494. The fact that an efficient recycling activity was observed with wild-type PILB-MsrB but not with the mutants (Table II). Altogether, these results show that 1) the C-terminal subdomain of PILB displays an Msr activity; 2) the mechanism involves formation of a sulfenic acid intermediate; 3) the essential Cys involved in reduction of MetSO and in formation of the sulfenic acid derivative is Cys-494; and 4) the regeneration of Cys-494 is done via formation of a disulfide bond with Cys-439 followed by its reduction by thioredoxin. This mechanism is reminiscent of that described for MsrA from E. coli except that only one intradisulfide bond is formed in MsrB of PILB for regenerating the active Cys-494. The fact that no thioredoxin-recycling process was observed in mutant C439S indicates that, similar to MsrA and more generally to all mechanisms where a sulfenic acid is formed except for the BCP protein and Prx1p (20, 21), the sulfenic acid intermediate formed in MsrB cannot be reduced via a double displacement mechanism involving formation of a disulfide bridge between Cys-449 and Cys-32 of thioredoxin followed by formation of a disulfide bond between Cys-439 and Cys-32 of thioredoxin and release of Cys-494. The fact that an efficient recycling activity was observed on mutant C439S with DTT as a reductant (Table II) indicates that DTT can easily attack the sulfenic acid intermediate on Cys-494 in contrast to thioredoxin. Thus, this supports a nonaccessibility of the sulfenic acid within the active site of PILB-MsrB to thioredoxin. In this context, knowledge of the three-dimensional structure of MsrB under the sulfenic acid intermediate state will be informative.

When PILB-MsrA/MsrB was tested in the absence of reductant, 2 mol of methionine per mol of subdomain were formed with a loss of four thiols (Tables I and II). These results indicate

### Table I

| Enzyme          | No. of Cys | Without 10 mM Met-R,S-SO | With 10 mM Met-R,S-SO | Decrease in free thiols* |
|-----------------|------------|--------------------------|----------------------|-------------------------|
| PILB-MsrA       | 2          | 1.9                      | 0.1                  | 1.8                     |
| C206S PILB-MsrA | 1          | 1.0                      | 1.0                  | 0                       |
| C348S PILB-MsrA | 1          | 0.9                      | 0.9                  | 1                       |
| PILB-MsrB       | 2          | 2.0                      | 0.1                  | 1.9                     |
| C439S PILB-MsrB | 1          | 1.0                      | 0.1                  | 0.9                     |
| C494S PILB-MsrB | 1          | 1.0                      | 1.0                  | 0                       |
| PILB-MsrA/MsrB  | 4          | 4.0                      | 0.4                  | 3.6                     |

* The difference in the number of free cysteine thiols upon treatment with 10 mM Met-R,S-SO versus no treatment.

### Table II

| Enzyme          | Stoichiometry | Activity with DTT | Activity with thioredoxin |
|-----------------|---------------|-------------------|---------------------------|
| PILB-MsrA       | 0.9           | 3                 | 220                       |
| C206S PILB-MsrA | 0!            | NA                | NA                        |
| C348S PILB-MsrA | 0.9           | 37                | NA                        |
| PILB-MsrB       | 1.0           | 3                 | 12                        |
| C439S PILB-MsrB | 1.0           | 36                | NA                        |
| C494S PILB-MsrB | 0            | NA                | NA                        |
| PILB-MsrA/MsrB  | 1.8           | 4.2               | 170                       |

The difference in the number of free cysteine thiols upon treatment with 10 mM Met-R,S-SO versus no treatment.

**The MsrB of PILB and MsrA/MsrB Subdomains**—One mol of methionine per mol of PILB-MsrB was formed in the absence of reductant. Moreover, the loss of thiols was in agreement with formation of a disulfide bond between Cys-439 and Cys-494 (Table I). To investigate the role of Cys-439 and Cys-494, mutations of these residues into Ser were done. Substituting Ser for Cys-494 abolished any activity, whereas the mutant C439S showed a reductase activity with a stoichiometry of 1 mol of methionine in the absence of DTT (Table II). When the mutant C439S was incubated with MetSO in the absence of DTT and then treated with TNB, a decrease of the absorbance at 410 nm equivalent to that of 1 mol of TNB was observed. Moreover, when the mutant was incubated under the same conditions and then subsequently treated with dimeone, a mass increase of 154 Da was observed instead of the expected value of 138 Da. This difference of 16 Da remains to be explained. A possibility is that, under the experimental conditions used, the sulfide that is formed on MsrB can be easily oxidized to sulfoxide (Table III). In the presence of thioredoxin, a recycling activity was observed with wild-type PILB-MsrB but not with the mutants (Table II). Altogether, these results show that 1) the C-terminal subdomain of PILB displays an Msr activity; 2) the mechanism involves formation of a sulfenic acid intermediate; 3) the essential Cys involved in reduction of MetSO and in formation of the sulfenic acid derivative is Cys-494; and 4) the regeneration of Cys-494 is done via formation of a disulfide bond with Cys-439 followed by its reduction by thioredoxin. This mechanism is reminiscent of that described for MsrA from E. coli except that only one intradisulfide bond is formed in MsrB of PILB for regenerating the active Cys-494. The fact that no thioredoxin-recycling process was observed in mutant C439S indicates that, similar to MsrA and more generally to all mechanisms where a sulfenic acid is formed except for the BCP protein and Prx1p (20, 21), the sulfenic acid intermediate formed in MsrB cannot be reduced via a double displacement mechanism involving formation of a disulfide bridge between Cys-449 and Cys-32 of thioredoxin followed by formation of a disulfide bond between Cys-439 and Cys-32 of thioredoxin and release of Cys-494. The fact that an efficient recycling activity was observed on mutant C439S with DTT as a reductant (Table II) indicates that DTT can easily attack the sulfenic acid intermediate on Cys-494 in contrast to thioredoxin. Thus, this supports a nonaccessibility of the sulfenic acid within the active site of PILB-MsrB to thioredoxin. In this context, knowledge of the three-dimensional structure of MsrB under the sulfenic acid intermediate state will be informative.

When PILB-MsrA/MsrB was tested in the absence of reductant, 2 mol of methionine per mol of subdomain were formed with a loss of four thiols (Tables I and II). These results indicate
that both Msrs are active within PILB-MsrA/MsrB and thus that the presence of one subdomain does not prevent the reduce activity of the other subdomain.

**Catalytic Constants and Stereoselectivity in MetSO Reduction**

The catalytic constants of PILB-MsrA and PILB-MsrB were first determined using the mixture of four diastereomers DL-Met-R,S-SO as a substrate under conditions where thioredoxin concentrations were not limiting (Table IV). $K_m$ for MetSO and thioredoxin and $k_{cat}$ values of PILB-MsrA and PILB-MsrB were 9 mM, 75 μM, and 3.7 s⁻¹ and 56 μM, 34 μM, and 0.2 s⁻¹, respectively. The catalytic values of PILB-MsrA are in the range of those recently determined for E. coli MsrA (18), but the $K_m$ and $k_{cat}$ values for MetSO are significantly different from those recently described by Grimaud et al. (8) for E. coli MsrA and E. coli MsrB ($K_m$ values for MetSO are 170 μM and 6.7 μM, and $k_{cat}$ values are 20 and 0.6 min⁻¹, respectively). This is because the concentration of 5 μM of thioredoxin used in their experiments was not saturating. In the case of MsrB, the observed discrepancy could be due to structural differences between MsrBs from N. meningitidis and E. coli. In fact, a control experiment done on E. coli MsrB showed that a saturating concentration of thioredoxin is also only attained at 75 μM (data not shown). In this context, it is interesting to note that $K_m$ values of E. coli MsrB and of E. coli MsrA for E. coli thioredoxin are 3–5-fold better than $K_m$ for Chlamydomonas reinhardtii thioredoxin (data not shown). This therefore suggests that thioredoxin affinity is in part species-dependent. Therefore, $K_m$ values of N. meningitidis MsrA and MsrB for N. meningitidis thioredoxin are probably lower than those reported for E. coli thioredoxin.

DL-Met-R,S-SO was shown to be quantitatively reduced by PILB-MsrA/MsrB. In contrast, only 50% of the mixture of diastereomers MetSO was reduced by either PILB-MsrA or PILB-MsrB. Moreover, only l-Met-S-SO and d-Met-S-SO were quantitatively reduced by PILB-MsrA, whereas d-Met-R-SO and l-Met-R-SO were only reduced by PILB-MsrB. These results support the data reported by Grimaud et al. (8) on oxidized calmodulin, suggesting that MsrB is stereospecific for the $R$ isomer of the sulfoxide of MetSO, whereas as has already been shown, MsrA is stereospecific for the $S$ isomer (22, 23).

The catalytic constants were then determined for the isomers that are substrates (Table IV). The $K_m$ value of PILB-MsrA for l-Met-S-SO is 3-fold lower than for d-Met-S-SO, whereas the $K_m$ value of PILB-MsrB for l-Met-R-SO is decreased 6-fold compared with that for d-Met-R-SO. This suggests that the configuration at the carbon Cα is not a determining factor for efficient binding to both Msrs. Compared with the $k_{cat}/K_m$ value of 1,200 M⁻¹s⁻¹ for PILB-MsrA with l-Met-S-SO, the catalytic efficiency of PILB-MsrB is at least 60-fold lower with l-Met-R-SO. The low catalytic efficiency of PILB-MsrB raised the question of whether protein-bound MetSO is a better substrate than free MetSO. Therefore, the catalytic constants were determined for Ac-l-Met-R,S-SO-NHMe. In this case, the amino and carboxyl groups of MetSO are engaged in amide bonds, and thus no charge is present. As shown in Table IV, the $K_m$ value of MsrB for Ac-l-Met-R,S-SO-NHMe was decreased by a factor of 16 compared with that of l-Met-R-SO. Taking into account the fact that Ac-l-Met-S-SO-NHMe is neither a substrate nor an inhibitor (see below), the $K_m$ value is in fact 32-fold decreased. A similar effect was observed with MsrB from E. coli. For PILB-MsrA, $K_m$ was decreased by a factor of 7 compared with that for l-Met-S-SO, taking into account the fact that Ac-l-Met-R-SO-NHMe is not a substrate. On the other hand, the $K_m$ value of PILB-MsrB for Ac-l-Met-R,S-SO-NHMe was similar to that for Ac-l-Met-R,S-SO-NHMe, whereas a 3.7-fold increase in the $K_m$ value was observed for Ac-l-Met-R-SO-NHMe. Anyway, the highest $k_{cat}/K_m$ values of both Msrs are observed for Ac-l-Met-R,S-SO-NHMe (Table IV). Thus, this suggests that the active sites of both Msrs are adapted for binding protein-bound l-MetSO more efficiently than free l-MetSO. Finally, it is important to note that no inhibition of the two PILB-Msrs was observed in the presence of methionine and of the l-Met isomer, which is not a substrate, at a concentration as high as 100 mM, with N-Ac-l-Met-R,S-SO-NHMe as a substrate.

Several results presented in this study argue for a structure of the active site of PILB-MsrB different from that of PILB-MsrA, which can probably be extended to all MsrBs and MsrAs. In particular, the two Msrs exhibit opposite stereoselectivities toward the sulfoxide function, and the sulfoxide isomer, which

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4 A. Oly, S. Boschi-Muller, and G. Branlant, unpublished results.
CONCLUSION

We have shown that the MsrB subdomain of PILB reduces MetSO via a two-step catalytic mechanism involving sulfenic acid chemistry. The first step leads to reduction of MetSO into a sulfenic acid which is then efficiently reduced by MsrB. This dual mechanism is likely to enhance the metabolic stability of the pathogen by ensuring the efficient reduction of MetSO to Met, even in conditions where the catalytic efficiency of MsrA is lower than that of MsrB. This dual mechanism could be crucial for the survival of the pathogen in environments where MetSO is a key regulator of cellular metabolism and stress response.
catalytic Cys-494. The second step consists in the regeneration of Cys-494 via formation of a disulfide bridge between Cys-494 and Cys-439 followed by reduction of the disulfide bridge by thioredoxin. Cys-494 is almost conserved, whereas Cys-439 is only conserved in 50% of the MsrB putative sequences. This argues for the generality of the sulfenic acid mechanism described in the present study but at the same time raises the question of whether alternative mechanisms in Cys-494 regeneration are functioning.

PILB-MsrB recognizes only the R isomer of the sulfoxide function, whereas PILB-MsrA recognizes the S isomer. These differences in stereospecificities are of particular importance in vivo, since they allow the complete reduction of Met-R,S-SO back to methionine. It is also shown that 1) the presence of the sulfoxide function is a prerequisite for binding to both MsrAs and 2) the catalytic efficiency of PILB-MsrB is significantly lower than that of PILB-MsrA. The results also suggest that protein-bound l-Met-SO is a better substrate than free l-MetSO, in particular for MsrB. Several results from the present study argue for a structure of the active site of MsrBs different from that of MsrAs. In this context, the Cys-494 that is situated in the signature RYC(I/V/M)N and is oxidized into a sulfenic acid during the catalytic event is probably not located in an environment similar to that of Cys-51 of E. coli MsrA. Thus, knowledge of the three-dimensional structure of a MsrB combined with site-directed mutageneses will be useful for characterizing its active site, in particular the amino acids involved in the stereospecificity and catalysis.

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