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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments for methionine sulfoxide reductase B from *Bacillus subtilis*

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Biological context

The cyclic oxidation of methionine (Met) residues in proteins to methionine sulfoxide (Met(O)) and reduction of Met(O) to Met plays an important role in protecting cells from the deleterious effects of free oxidants. The oxidation of Met at the sulfur atom leads to alternative epimers: Met-R(O) and Met-S(O). Two classes of methionine sulfoxide reductases (Msr), MsrA and MsrB catalyze the reduction of Met-S(O) and Met-R(O), respectively (Moskovitz et al., 2000; Kryukov et al., 2002). Genes of *msrA* and *msrB* have been identified in a wide range of organisms, from eubacteria and archea to higher eukaryotes (Moskovitz et al., 2000; Kryukov et al., 2002). The 3D structures of bovine MsrA (Lowther et al., 2000), *E. coli* MsrA (Tete-Favier et al., 2000) and MsrB from *Neisseria gonorrhoeae* (Lowther et al., 2002) have recently been determined by X-ray crystallography. Interestingly, despite the fact that there is extensive sequence similarity among members within each of the MsrA and MsrB families, and the catalytic mechanisms of MsrA and MsrB are similar, there is no detectable sequence or structural similarities between MsrA and MsrB.

As a step toward characterizing the global fold of MsrB and the mechanism of its enzymatic activity, we have over-expressed and purified Northeast Structural Genomics Consortium target SR10, the MsrB from *B. subtilis* (SwissProt ID P54155), and determined nearly complete ^{15}N, $^{1}H^{N}$, $^{13}C'$, $^{3}C^{\alpha}$, $^{13}C^{\beta}$ resonance assignments and ^{13}C-^{1}H resonance assignments of Val, Leu, Ile(δ) methyl groups. Although we have no plans to determine more extensive sidechain assignments, the assignments described here are sufficient for determining an accurate fold for MsrB, and are valuable for studies of enzyme mechanisms.

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Methods and experiments

The MsrB (SR10) gene was PCR-amplified from *B. subtilis* genomic DNA, cloned into expression vector pET21 with a C-terminal hexa-His purification tag, and then transformed into *E. coli* strain BL21(DE3)-pMgK for expression. Samples of ^{2}H, ^{15}N-, ^{13}C-labeled and Val, Leu and Ile(δ) methyl-protonated SR10 were prepared by addition of [^{13}C4, 3,3-D2]α-ketobutyrate (50 mg/l), [U-^{13}C5, 3-D1]α-ketoisovalerate (CIL) (100 mg/l) 1 h before induction in D_2O minimal media containing $(^{15}NH_4)SO_4$ and [U-^{13}C, ^{2}H]-glucose (Goto et al., 1999). The SR10 protein was purified using nickel-affinity column chromatography followed by gel filtration using Superdex^{TM} 75 column (Pharmacia). Sample purity (>95%) and molecular weight (17.7 kDa) were verified by SDS-PAGE and MALDI-TOF mass spectrometry, respectively. Isotope-enriched SR10 was prepared at ∼ 1.0 mM concentration in H_2O solution containing 5% D_2O, 20 mM NaH_2PO_4, 50 mM NaCl and 5 mM DTT at pH 6.50 +/− 0.05.

All spectra were acquired at 20 °C on 600 or 750 MHz Varian INOVA NMR spectrometers using external DSS as reference. Spectra were processed, peak-picked and analyzed with the programs Felix (MSI), SPARKY (Goddard and Kneller, University of California, San Francisco, 1991), and AutoAssign (Moseley et al., 2001). The input for AutoAssign included peak lists (frequencies and intensities) from

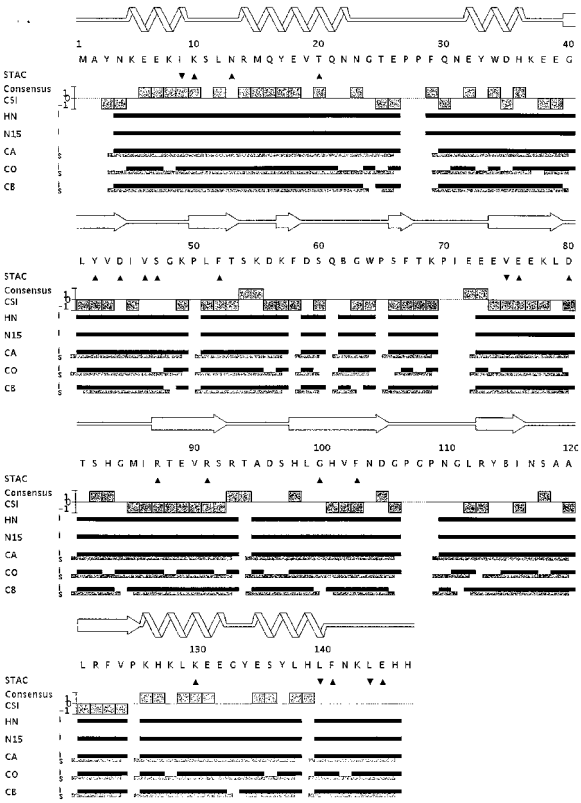

Figure 1. AutoAssign connectivity map showing sequential (grey, s) and intra-residue (black, i) connectivities used to establish backbone resonance assignments for SR10. Secondary structure based on the crystal structure of *N. gonorrhoeae* MsrB domain of pilB (Lowther et al., 2002) is plotted at the top, together with data from Chemical Shift Index (CSI) analysis. Residues with intraresidue STAC (∇) or sequential STAC (Δ) constraints derived from hCCcoNH-TOCSY and HcccoNH-TOCSY, identifying N-H^N resonances belonging to or following Val, Leu, Ile residues, are also indicated.

2D ^{15}N-^{1}H HSQC, 3D HNCACB, 3D HNcoCACB, 3D HNCO and 3D HNcaCO spectra, together with *Spin System TypeAssignment Constraints* (STAC) derived from HcccoNH-TOCSY and hCCcoNH-TOCSY spectra (Zheng et al., 2003). Minimal editing of the peak lists generated by SPARKY was necessary to separate two cases of severe overlapping H^N-N resonances. The resulting intra-residual and sequential connections for SR10 established by automated analysis and extended by manual analysis are shown in Figure 1. An annotated [^{15}N-$^{1}H^N$] HSQC spectrum is available from the authors. Stereospecific assignments of methyl resonances were obtained from ^{13}C-^{1}H HSQC data recorded on a 10% ^{13}C enriched sample (Neri et al., 1989). The following residues were identified to have two different H^N-N resonances, but one set of C^α, C^β and C' resonances: S60, T68, K69 and F103. The peptide segment N110-R113 also exhibits two sets of backbone resonances. Furthermore, the H^N values of E38 and S118 are unusual, 5.23 ppm and 13.13 ppm respectively. These residues are close in space and near the proposed enzymatic active site of MsrB. The biological implications of this conformational heterogeneity, reported here for the first time, are currently under investigation.

Extent of assignments and data deposition

The combined use of automated and manual analysis of triple resonance 3D data provided assignments for ~96% of assignable backbone atoms (i.e., 130/135 ^{15}N-$^{1}H^N$ sites, 138/143 C', 140/143 C^α, 130/133 C^β) of SR10 (excluding residues in His-tag). Based on these chemical shift data, the SR10 structure is composed mainly of a short β-sheet with two small α-helices at the N- and C-terminal, respectively. This secondary structure is consistent with the 3D X-ray crystal structure (Figure 1) reported for MsrB of *Neisseria gonorrhoeae* pilB (Lowther et al., 2002). Further analysis of HcccoNH-TOCSY, hCCco-NH-TOCSY, ^{15}N-edited NOESY, and ^{13}C-^{1}H HSQC data resulted in near complete assignment of 5/5 Ile δ, 21/22 Leu and 13/14 Val methyl resonances, as well as 4/9 Asn $N^\delta H_2$ and 2/4 Gln $N^\varepsilon H_2$ amide pairs. These ^{1}H, ^{13}C and ^{15}N chemical shift data have been deposited in BMRB (accession number 5619).

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