Published in final edited form as:

Biomol NMR Assign. 2008 December ; 2(2): 199–201. doi:10.1007/s12104-008-9120-x.

¹H, ¹⁵N and ¹³C NMR Assignments of Mouse Methionine Sulfoxide Reductase B2

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Abstract

A recombinant mouse methionine-*r*-sulfoxide reductase 2 (MsrB2ΔS) isotopically labeled with ¹⁵N and ¹⁵N/¹³C was generated. We report here the ¹H, ¹⁵N and ¹³C NMR assignments of the reduced form of this protein.

Keywords

Methionine sulfoxide reductase; MsrB; MsrB2ΔS

Biological Context

Reactive oxygen species (ROS) can oxidize methionine (Met) residues in proteins to an enantiomeric mixture of *r*- and *s*-isomers of methionine sulfoxide (MetSO) (Weissbach, et al., 2005). Oxidation of Met to MetSO can influence protein function and has been associated with neurodegenerative diseases and aging (Kim and Gladyshev, 2007, Moskovitz, 2005). The methionine sulfoxide reductase (Msr) enzymes repair oxidatively damaged proteins by reducing MetSO back to Met in the presence of a reductant such as thioredoxin (Brot, et al., 1981, Kim and Gladyshev, 2004, Kim and Gladyshev, 2004). There are two known classes of Msr enzymes. MsrA is specific for the reduction of the *s*-isomer and MsrB for the *r*-isomer of MetSO residues (Hansel, et al., 2005, Kryukov, et al., 2002, Weissbach, et al., 2005).

Three MsrB isozymes have been identified in mammals. MsrB1 is present in cytosol and nucleus, MsrB2 in mitochondria, and MsrB3 in the endoplasmic reticulum or in mitochondria depending on the alternatively spliced N-terminal sequences (Kim and Gladyshev, 2004). These multiple cellular locations of MsrB isozymes provide cells with an ability to reduce MetSO in different compartments. MsrB1 is a selenocysteine (Sec)-containing enzyme. The Sec is located in the active site and replaces the Cys that normally serves as the catalytic residue (Kim and Gladyshev, 2005).

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The MsrBs catalyze in principal the same reaction, but might have different reaction mechanisms for the reduction of MetSO. Due to the presence of selenocysteine in the active site in MsrB1, it has been suggested that MsrB1 might have a different catalytic mechanism with respect to the cysteine-containing MsrB2 and MsrB3s (Kim and Gladyshev, 2005, Kim and Kim, 2008). The NMR structure of mouse MsrB1 has recently been solved (paper in preparation). An investigation of structural differences between Sec-containing MsrB1 and Cys-containing MsrB2 might lead to a better understanding of the structural basis for the suggested differences in the catalytic mechanisms. MsrB1 and MsrB2 have only a 21.9 % sequence identity, but they share conserved zinc-binding motifs and several residues critical to catalysis. Therefore, the structural elucidation of MsrB2 through NMR spectroscopy and its comparison with MsrB1 structure should provide further understanding of functioning for these enzymes.

Methods and Experiments

NMR assignment was carried out on the bacterially expressed recombinant mouse MsrB2ΔS (AA: 45–175) containing one extra amino acid in N-terminal (Met) and a C-terminal His-tag (AAALEHHHHHH), which result in a total length of 143 amino acids (Kim and Gladyshev, 2005). The N-terminal localization signal peptide of MsrB2ΔS was removed during cloning. To uniformly label MsrB2ΔS with ^{15}N or $^{15}\text{N}/^{13}\text{C}$, *Escherichia coli* cells were grown in M9 minimal media containing 99%-enriched $(^{15}\text{NH}_4)_2\text{SO}_4$ and 98%-enriched $^{13}\text{C}_6\text{-D-glucose}$ (Spectra Stable Isotopes). MsrB2ΔS was expressed in *E. coli* ER2566 (New England Biolabs) at 37° C until OD₆₀₀ reached 0.6, followed by addition of 1 mM IPTG and overnight protein expression at 16° C. The cells were collected by centrifugation and disrupted by sonication in 25 mM phosphate buffer, pH 7.0, containing 10 mM NaCl, 0.05% Triton X-100 (Sigma-Aldrich), and half a tablet of Complete Protease Inhibitor cocktail (Roche). Following centrifugation, the supernatant was applied onto a Ni-NTA His Bind Resin (Qiagen) column. The column was washed with 20 mM imidazole in a washing buffer (WEB - 50 mM phosphate buffer, pH 7.0, 300 mM NaCl), followed by elution of protein with 200 mM imidazole in WEB buffer at 4°C.

Samples for NMR studies contained 1–1.5 mM of MsrB2ΔS in 20 mM phosphate buffer (pH 6.0), 25 mM NaCl, 5 mM DTT, in the presence of either 95% H₂O/5% D₂O or 99.9% D₂O.

All NMR experiments were recorded at 298 K on a Bruker Avance 600MHz spectrometer equipped with a 5-mm z-gradient TXI(H/C/N) cryoprobe at the NT faculty, NMR center at NTNU and on a Bruker Avance 800MHz spectrometer at the Magnetic Resonance Center (CERM) in Florence, Italy. Proton chemical shifts were referenced to water, and ^{15}N and ^{13}C chemical shifts were referenced indirectly to DSS, based on the absolute frequency ratios (Zhang, et al., 2003). Sequence-specific backbone and side-chain assignments of MsrB2ΔS were accomplished using ^{15}N HSQC, ^{13}C HSQC, HNCA, HN(CO)CA, HNCO, HN(CA)CO, CBCANH, CBCA(CO)NH, HBHA(CO)NH, HCCHTOCSY, HCCH-COSY, and ^{15}N HSQC-NOESY. The NMR data were processed with the BRUKER XWinNMR version 3.5 software and spectral analysis was performed using CARA version 1.4 (Keller, 2004).

Assignments and data deposition

We report the assignment of the reduced form of recombinant mouse MsrB2ΔS. 2D and 3D heteronuclear NMR experiments were used to obtain the resonance assignment on uniformly $^{15}\text{N}/^{13}\text{C}$ labeled protein. The resonance assignment of backbone and side-chain is essentially complete (H^{N} , N, C, C^α > 95 %; H and C side chains > 92 %). The H^{N} and N of

Cys98 and Ser99 were not identified, although other nuclei of these residues were assigned. Like MsrB1, MsrB2 is a metalloprotein that binds zinc. From the NMR assignment it is evident that the Zn^{2+} ion is coordinated by four cysteine residues (i.e., at positions 83, 86, 139, 142 based on their ^1H chemical shifts (Kornhaber, et al., 2006). The assignment of ^1H , ^{13}C , and, ^{15}N nuclei has been deposited in BioMagResBank under the accession no. 15715. Preliminary results from TALOS (Cornilescu, et al., 1999) calculation, based on the obtained chemical shifts, indicate that MsrB2 has somewhat different secondary structure content compared to MsrB1.

Acknowledgments

This research was supported by the Faculty of Natural Sciences and Technology (NT faculty) at the Norwegian University of Science and Technology (NTNU), NIH AG021518 and KOSEF R13-2005-005-01004-0. The NMR data reported in this work was collected at the NT faculty NMR center at NTNU and the Magnetic Resonance Center (CERM) in Florence, Italy. We thank Dr. Reinhard Wimmer (AAU) for technical assistance.

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Figure 1.

$^1\text{H},^{15}\text{N}$ HSQC spectrum of a ^{15}N -labelled 1.2 mM sample of reduced recombinant mouse MsrB2 in 90:10 $\text{H}_2\text{O}:\text{D}_2\text{O}$ at pH 6.0, 298 K. Residue numbers are indicated. Side-chain amide resonances of Trp, Asn and Gln residues are indicated with amino acid number and sc. Lines connect the two resonances of the Asn and Gln side chains.