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NMR assignments of ^{1}H, ^{13}C and ^{15}N spectra of methionine sulfoxide reductase B1 from *Mus musculus*

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Abstract
Isotopically labeled, ^{15}N and $^{15}N/^{13}C$ forms of recombinant methionine-*R*-sulfoxide reductase 1 (MsrB1, SelR) from *Mus musculus* were produced, in which catalytic selenocysteine was replaced with cysteine. We report here the ^{1}H, ^{15}N and ^{13}C NMR assignment of the reduced form of this mammalian protein.

Keywords: methionine sulfoxide reductase, MsrB, selenium, selenocysteine

Biological context

Oxidation of methionine residues to an enantiomeric mixture of *R* - and *S* -isomers of methionine sulfoxide can influence protein function and has been implicated in neurological diseases and aging. Methionine oxidation can be reversed by methionine sulfoxide reductases, which reduce methionine sulfoxide back to methionine in the presence of thioredoxin (Brot et al. 1981, Kim and Gladyshev 2004). There are two classes of these enzymes: MsrA is specific for the *S*-isomer, and MsrB for the *R* -isomer of methionine sulfoxide (Hansel et al. 2005, Kryukov et al. 2002, Weissbach et al. 2005).

MsrBs are widely distributed in bacteria, archaea and eukaryotes, but only animals are known to have selenocysteine (Sec)-containing MsrBs. These selenoproteins co-occur with the Cys-containing forms of MsrBs. Mammalian MsrB1 (116 AA) contains Sec in the active site while two other mammalian MsrBs, MsrB2 and MsrB3, contain catalytic Cys (Kim and Gladyshev 2004, Kim and Gladyshev 2005a, b).

Methods and experiments

NMR assignment was carried out on a recombinant, bacterially expressed, mouse MsrB1 tagged at the C-terminus with a 6 His-tag and containing a Sec95Cys mutation (Kim and Gladyshev 2005a, b). To uniformly label MsrB1 with ^{15}N or $^{15}N/^{13}C$, cells were grown in M9 minimal media containing 99%-enriched $(^{15}NH_4)_2SO_4$ and 98%-enriched $^{13}C_6$-D-glucose (Spectra Stable Isotopes). MsrB1 was overexpressed in *Escherichia coli* ER2566 (New England Biolabs) by growing cells at 37°C until an OD_{600nm} of 0.6 was reached, followed by induction of protein synthesis with 1 mM IPTG and subsequent incubation for 3 h. Cells were collected by centrifugation and disrupted by sonication in 25 mM phosphate buffer, pH 7.0, containing 10 mM NaCl and 0.05% Triton X-100 (Sigma-Aldrich). Additionally, 1 mM lysozyme (Sigma-Aldrich) and half a tablet of Complete Protease Inhibitor cocktail (Roche) were added to the solution. Following centrifugation, the supernatant was applied onto a TALON Co-IMAC Sepharose column (BD Biosciences). The column was washed with 20 mM imidazole in 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 (Figure 1)

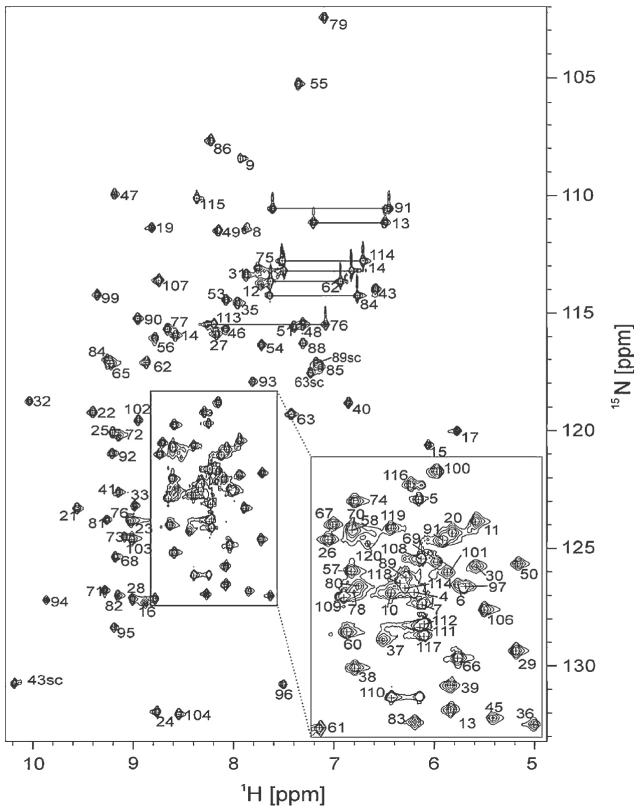

Figure 1 ^{1}H, ^{15}N HSQC spectrum of reduced ^{15}N-labelled 1.2 mM MsrB1 (Sec95Cys mutant) from *Mus musculus* in 90:10 H_2O:D_2O at pH 5.5, 298 K. Residue numbers are indicated. Side-chain amide resonances of Asn and Gln residues are connected by lines. Other side-chain amine resonances are indicated with amino acid number and sc

Samples for NMR studies contained 1–1.5 mM MsrB1 in 20 mM phosphate buffer, pH 5.5, 10 mM NaCl, 3 mM DTT, in the presence of either 95% H_2O/5% D_2O or 99.9% D_2O.

The NMR spectra were recorded at 298 K on a Bruker DRX600 spectrometer equipped with a 5 mm z-gradients TXI(H/C/N) cryogenic probe at the NT faculty NMR center at NTNU. The NOESY spectra were recorded at 298 K on a Bruker DRX800 spectrometer equipped with a 5 mm z-gradients TXI(H/C/N) cryogenic probe at the NMR center at the University of Florence. Proton chemical shifts were referenced to internal 3-(trimethylsilyl)-propane-sulfonic acid, sodium salt (DSS), 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 MsrB1 were accomplished using ^{15}N HSQC, ^{13}C HSQC, HNCA, HN(CO)CA, HNCO, HN(CA)CO, CBCANH, CBCA(CO)NH, HBHANH, HBHA(CO)NH, HCCH-TOCSY and HCCH-COSY spectra. Aromatic ring system assignments were obtained from 2D COSY, TOCSY and NOESY experiments as well as 2D (HB)CB(CGCD)HD and 3D [^{1}H–^{15}C] HSQC-NOESY of the aromatic region. The NMR data were processed with the BRUKER XWinNMR version 3.5 software and spectral analysis was performed using CARA version 1.4.1 (Keller 2004).

Assignments and data deposition

Herewith we report the assignment of the reduced form of mammalian MsrB1 from *Mus musculus*, which has 24.3% homology with respect to the bacterial MsrB from *Bacillus subtilis* for which the NMR assignment was reported (Zheng et al. 2003). 2D and 3D heteronuclear NMR experiments were used to obtain the resonance assignment on uniformly ^{13}C/^{15}N labeled reduced recombinant Sec95Cys MsrB1. The backbone and side-chain resonance assignment is essentially complete (H^N, N, C, C^α > 96%; H and C side chains >95%). The backbone H^N and N nuclei of Met1, Ser2, Phe3, His34 and Ser97 could not be found, although other nuclei of these residues were safely assigned. Except for NH^ε of Arg63 and Arg89, exchangeable side-chain protons of other Arg and Lys residues were not assigned, indicating fast exchange rate of these protons with water. Side-chain amide groups of all Asn and Gln were assigned. C^α and C^β chemical shifts of Cys 23, 26, 71, and, 74 suggest that these residues are involved in coordination of zinc ion, which is present in the protein (Kornhaber et al. 2006). The chemical shift index for MsrB1 indicates that the β-sheets are the main element for the protein secondary structure. The ^{1}H, ^{13}C, and ^{15}N chemical shifts have been deposited in BioMagResBank under the accession no. 15193.

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