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Letter to the Editor: Backbone H^N, N, C^α, C' and C^β assignment of the 25 kDa peptide methionine sulfoxide reductase from *Erwinia chrysanthemi*

Sabine Béraud[a], Jean-Pierre Chambost[b], Beate Bersch[a], Pierre Gans[a,*], Frédéric Barras[b] & Dominique Marion[a]

[a]*Institut de Biologie Structurale 'Jean-Pierre Ebel' (UMR 5075 CEA-CNRS-UJF), 41 rue Jules Horowitz, F-38027 Grenoble Cédex 1, France;* [b]*Institut de Biologie Structurale et Microbiologie (CNRS), 31 Chemin Joseph Aiguier, F-13402 Marseille Cédex 20, France*

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Biological context

In proteins, the sulfur atom of methionine residues is very susceptible to oxidation induced by oxidative stress that occurs during host/pathogen interactions, aging and some pathological processes (see Berlett and Stadtman, 1997 for a review). As a consequence, the function of the oxidised protein can be more or less affected but, especially if the oxidation occurs close to the active site, it may be completely abolished involving dramatic effects for the cell. Methionine oxidation to methionine sulfoxide can be reversed by methionine sulfoxide reductase (MsrA), an enzyme present in most living organisms. Three crucial biological roles have been allocated to MsrA: (1) restoration of protein function, (2) regulation of enzyme activities which involve methionine oxidation and (3) protection of proteins from oxidative damage due to its antioxidative activity (Moskovitz et al., 1999 and references cited therein). The importance of MsrA in response to oxidative stress has been illustrated recently in the plant pathogen *Erwinia chrysanthemi* (El Hassouni et al., 1999) where MsrA is required for full virulence in order to alleviate the active oxygen species produced by the plant defense.

The chemical mechanism of MsrA recently received much attention but now, little is known about its structural properties (Boschi-Müller et al., 2000; Lowther et al., 2000; Moskovitz et al., 2000). We have undertaken a structural study using NMR in order to get a better knowledge of this ubiquitous protein. Here we report the backbone H^N, N , C^α, C' and C^β assignment of MsrA from *Erwinia chrysanthemi*, a 221 residue protein.

*To whom correspondence should be addressed. E-mail: pierre@rmn.ibs.fr

Methods and experiments

The DNA fragment containing the msrA gene of *Erwinia chrysanthemi* (GenBank AJ012716) was cloned into the NdeI/XhoI sites of pET-22b+ expression vector (Novagen) as described previously (El Hassouni et al., 1999). The recombinant plasmid was introduced into *E. coli* strain BL21(DE3). The freshly transformed bacteria were grown at 37 °C in M9 minimal medium and protein expression was induced by addition of IPTG (1 mM) when cells reached an OD_{600} of 0.6. Incubation after induction was continued for 2 hours. After centrifugation of bacteria, the cell pellets were broken using a French press and centrifugated. MsrA was purified from the supernatant using a Nickel column (Qiagen). ^{15}N labeled and $^{2}H/^{13}C/^{15}N$ triply labeled MsrA samples were obtained with 1 g/l of $^{15}NH_4Cl$ and/or 2 g/l ^{13}C-glucose as sole nitrogen and carbon sources. For the triply labeled protein, cells were grown in 99.9% D_2O and the incubation delay was 9 hours. The deuteration level of the purified triply labeled protein was about 80% as determined by mass spectrometry. For both cultures, the yield was about 50 mg per liter of medium. Final NMR samples were prepared in 25 mM potassium phosphate buffer at pH 5.0 containing 0.02% sodium azide, 1 mM dithiothreitol, a protease inhibitor cocktail (Complete, Boehringer Mannheim) and 10% D_2O. The final protein concentration was about 1.5 mM.

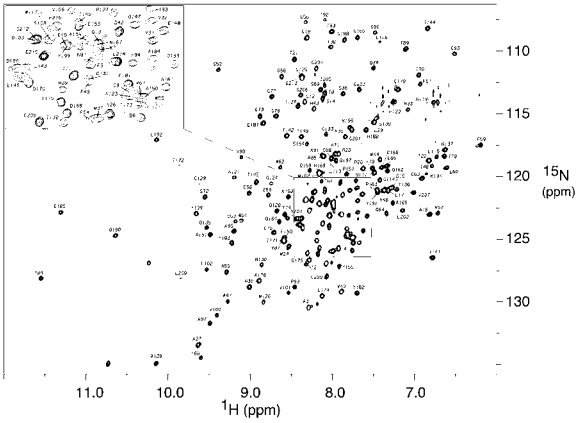

Figure 1. 2D ^{1}H-^{15}N TROSY spectrum of 1.5 mM ^{15}N labeled methionine sulfoxide reductase (MsrA) from *Erwinia chrysanthemi* recorded at 600 MHz and 25 °C.

For the H-D exchange experiment, ^{15}N labeled sample was lyophilised and dissolved in a 100% D_2O buffer.

NMR spectra were acquired at 25 °C on Varian Inova spectrometers (600 and 800 MHz) equipped with triple-resonance (^{1}H, ^{13}C, ^{15}N) probes including shielded z-gradients. The NMR experiments performed included 2D ^{15}N-TROSY, 3D d-HNCA, d-HN(CO)CA, d-HNCACB, d-HN(COCA)CB, d-HNCO, d-HN(CA)CO. The pulse sequences of 3D experiments (except for d-HN(COCA)CB) were taken as implemented from the Varian *Protein Pack* (available at ftp site: http://www.varianinc.com). Backbone assignment was obtained from the $^{2}H/^{13}C/^{15}N$ triply labeled sample. It was partially established and verified using the program package AUTOASSIGN (Zimmerman et al., 1997) as well as ALPS software (Morelle et al., 1995). All data were processed with Felix (MSI Technologies). Proton chemical shifts were reported with respect to the H_2O signal relative to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the $^{1}H/X$ frequency ratios of the zero-point according to Wishart et al. (1995).

Extent of assignments and data deposition

H^N and ^{15}N assignments of the 2D ^{15}N-TROSY backbone peaks were obtained for all but one (N184) of the expected 198 residues (221 residus minus N-terminal, 16 prolines and poly-histidine tag). Assignments for $^{13}C\alpha$, ^{13}CO and $^{13}C\beta$ were obtained for all residues except for P183 and N184. The chemical shift values of ^{1}H, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$ and ^{13}CO of the reduced form of MsrA have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number BMRB-4844.

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Note added in proof

The crystal structures of bovine MsrA [Lowther et al. (2000) *Biochemistry*, **39**, 13307-13312] and *E. coli* MsrA [Tete-Favier et al. (2000) *Structure*, **8**, 1167-1178] have been published since this paper was accepted.

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