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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignment of the reduced form of methionine sulfoxide reductase A from *Escherichia coli*

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Biological context

Reactive oxygen species (ROS) generated by cells during aerobic respiration or after exposure to various agents, can oxidize proteins. One of the targets in proteins is methionine (Met) which is oxidized into methionine sulfoxide (MetSO). This post-translational modification can provoke loss of protein function. Met oxidation is reversed *in vivo* by methionine sulfoxide reductases (Msr). Therefore, one of the major roles of Msrs is to protect cells against oxidative damage.

There exist two structurally-unrelated classes of Msrs, called MsrA and MsrB. The first one is specific for the S enantiomer while the second one is specific for the R enantiomer at the sulfur atom of the sulfoxyde. However, both classes of Msrs display a similar new catalytic mechanism that includes at least three steps (Olry et al., 2002). In the first step, a sulfenic acid intermediate is formed on Cys-51 with a concomitant release of one mol of Met per mol of Msr. Then, in the case of the *Escherichia coli* MsrA, two successive disulfide bonds are formed between first Cys-51 and Cys-198 and then between Cys-198 and Cys-206 (Boschi-Muller et al., 2000). In the last step, the Cys-198/Cys-206 disulfide bond is reduced selectively by thioredoxin (Trx).

Three X-ray structures of MsrAs from *E. coli*, *Bos taurus* and *Mycobacterium tuberculosis* (Tête-Favier et al., 2000; Lowther et al., 2000; Taylor et al., 2003) and one NMR assignment of MsrA from *Erwinia chrysanthemi* (Beraud et al., 2001) have been described so far. In all of the X-ray structures, the active site is occupied by molecules that can mimic either a Michaelis complex or an intermediate/transition state whereas in the NMR structure, no molecule is present in the active site. In all cases, the distances between Cys-51/Cys-198/Cys-206 are too long for disulfide bond formation and therefore the positions of Cys-206 relative to Cys-198 and of Cys-198 relative to Cys-51 which for the latter is situated at the beginning of an α helix, are not representative of those leading to formation of Cys-51/Cys-198 and Cys-198/Cys-206 disulfide bonds. This suggests that there exists a great flexibility of the C terminus of the MsrAs. In that context, the knowledge of the 3D NMR structure of another MsrA, such as the *E. coli* MsrA, not only in its reduced forms but also in its disulfide states will be very informative.

In a first approach, we report here the backbone and side-chain nearly complete assignment of the reduced form of MsrA from *E. coli* as well as one of the putative secondary structures.

Methods and experiments

Production and purification was done as described by Boschi-Muller et al. (2000). Sample purity and molecular mass were checked by SDS-PAGE and electrospray mass spectrometry, respectively.

The NMR sample contained 0.9 mM protein concentration (95% H_2O, 5% D_2O) in 10 mM phosphate buffer and 50 mM 1,5-dithiotreitol-d_{10} at pH 7.1. All spectra were acquired at 298 K on a Bruker DRX 600 MHz spectrometer equipped with a 3-axis gradient TXI probe. Spectra were processed using the program XWINNMR (Bruker) and analyzed with

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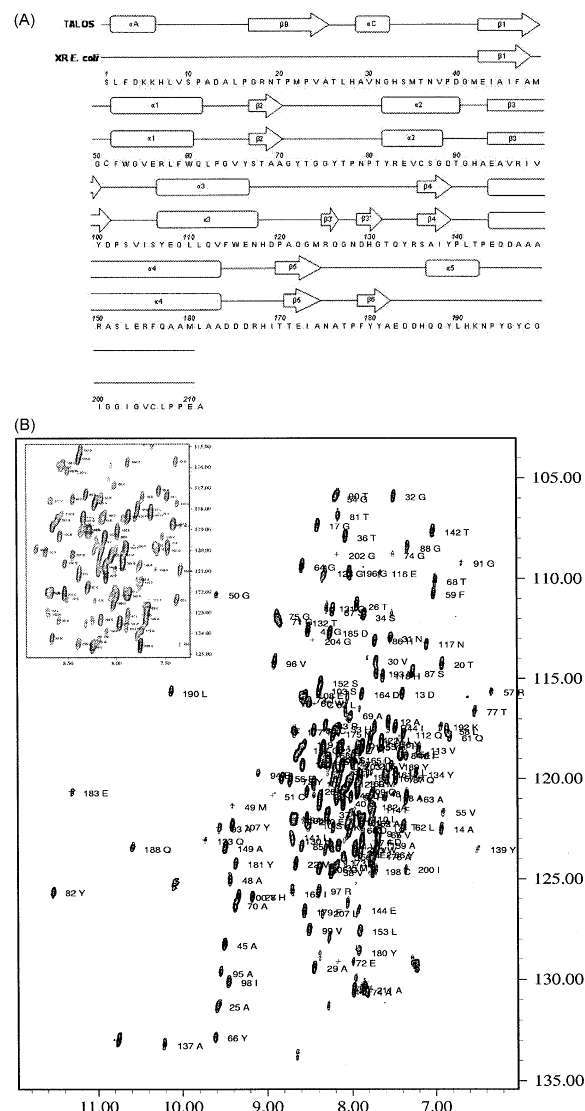

Figure 1. (A) *E. coli* MsrA secondary structure from the TALOS data as compared to the X-ray data. Catalytic cysteines are C_{51}, C_{198} and C_{206}. (B) 2D ^{1}H-^{15}N TROSY spectrum of ^{15}N labelled *E. coli* MsrA at 298 K.

the program XEASY (Bartels et al., 1995). Backbone amide $^1H^N$, ^{15}N, $^{13}C^\alpha$, $^1H^\alpha$, $^{13}C'$, and side chain ^{1}H, ^{13}C resonances were assigned using ^{1}H-^{15}N HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNHA experiments. HCCH-TOCSY, H(CCCO)NH, CC(CO)NH and ^{1}H-^{15}N-, ^{1}H-^{13}C- 3D HSQC-NOESY spectra were also performed for side-chain assignments.

Torsion angles (ϕ, ψ) and secondary structure prediction are based on H^N, H^α, N, C^α, C^β and C' chemical shifts using the TALOS program (Cornilescu et al., 1999).

Extent of assignments and data deposition

The ^{15}N TROSY spectrum for *Escherichia coli* MsrA is shown in Figure 1. More than 91% of backbone H^N, N, C^α, C' and C^β nuclei have been assigned, in addition with most of the ^{1}H, ^{13}C side-chain nuclei. Comparing the predicted structure with that of the crystal structure of *E. coli* MsrA, more or less, the structural elements found in solution, *i.e.* 7 α-helices and 6 β-strands, coincide with X-ray data (Figure 1A). The main differences are the first two helices αA, αC, the final α-helix α5 and the β-strand βB which are only predicted in the NMR structure while the β6 is not. It is important to notice that the two Cys-198 and Cys-206, involved in the disulfide bond formation, are present in the C-terminal flexible fragment. The β-strands β3′ and β3″ from the X-ray structure are too small elements to be estimated by the NMR secondary structure prediction.

Chemical shifts were deposited in the BioMagResBank under the access number BMRB-6090 (http://www.bmrb.wisc.edu).

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References

Bartels, C., Xia, T., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

Beraud, S., Chambost, J-P., Bersh, B., Gans, P., Barras, F. and Marion, D. (2001) *J. Biomol. NMR*, **20**, 97–98.

Boschi-Muller, S., Azza, S., Sanglier-Cianferani, S., Talfournier, F., Van Dorsselear, A. and Branlant, G. (2000) *J. Biol. Chem.*, **275**, 35908–35913

Cornilescu, G., Delaglio, F. and Bax, A. (1999) *J. Biomol. NMR*, **13**, 289–302.

Lowther, W.T., Brot, N., Weissbach, H. and Matthews, B.W. (2000) *Biochemistry*, **39**, 13307–13312.

Olry, A., Boschi-Muller, S., Marraud, M., Sanglier-Cianferani, S., Van Dorsselear, A. and Branlant, G. (2002) *J. Biol. Chem.*, **277**, 12016–12022.

Taylor, A.B., Benglis, Jr., D.M., Dhandayuthapani, S. and Hart, P.J. (2003) *J. Bacteriol.*, **185**, 4119–4126.

Tête-Favier, F., Cobessi, D., Boschi-Muller, S., Azza, S., Branlant, G. and Aubry, A. (2000) *Structure*, **8**, 1167–1178.