*Journal of Biomolecular NMR* **30:** 223–224, 2004.



# Letter to the Editor: $^{1}$H, $^{13}$C and $^{15}$N resonance assignment of the methionine sulfoxide reductase B from *Neisseria meningitidis*

Aurélien Thureau[a], Alexandre Olry[b], Nicolas Coudevylle[a], Saïd Azza[b], Sandrine Boschi-Muller[b], Guy Branlant[b] & Manh-Thong Cung[a,*]

[a]*Laboratoire de Chimie-Physique Macromoléculaire, UMR 7568 CNRS-INPL, Groupe ENSIC, 1 rue Grandville, B.P. 451, 54001 Nancy Cedex, France;* [b]*Maturation des ARN et Enzymologie Moléculaire, UMR CNRS-UHP 7567, Faculté des Sciences, Bld des Aiguillettes, B.P. 239, 54506 Vandoeuvre-lès-Nancy Cedex, France*

Received 23 March 2004; Accepted 2 June 2004

*Key words:* methionine sulfoxide reductase B, *Neisseria meningitidis*, NMR assignment

## Biological context

The aerobic metabolism generates reactive oxygen species (ROS) that can easily oxidize accessible methionine (Met) residues of proteins into methionine sulfoxides (MetSO). This post-translational modification can provoke loss of protein function. Therefore, reduction of MetSO, back to Met, is crucial to protect the cells from the effect of ROS. This is the role of methionine sulfoxide reductases (Msr).

L-Met oxidation into sulfoxide leads to two [*R*/*S*] epimers at the sulfur atom. In that context, there exists two unrelated-structural classes of Msrs. MsrA reduces the L-Met-*S*-SO whereas MsrB reduces the L-Met-*R*-SO. Both Msrs display a similar new catalytic mechanism that includes formation of a sulfenic acid intermediate and then of an intradisulfide intermediate followed by a thioredoxin (Trx) regeneration step (Boschi-Muller et al., 2000; Olry et al., 2002).

Whereas several X-ray structures of MsrA have been already determined, only one MsrB structure, from *Neisseria gonorrhoeae*, (Lowther et al., 2002) has been described so far. More recently, a preliminary NMR study of the MsrB from *Bacillus subtilis* (Zheng et al., 2003), which presents 53% and 52% amino acid identity with the *N. gonorrhoeae* and *N. meningitidis* MsrBs, respectively has been done.

Here we report the backbone and side chain NMR assignment of the MsrB from *N. meningitidis* as well as its secondary structure as a first step in a full 3D structural study aimed at characterizing the amino acids involved in the MsrB chemical mechanism and MetSO and Trx substrate specificities.

*To whom correspondence should be addressed. E-mail: Manh-Thong.Cung@ensic.inpl-nancy.fr

## Methods and experiments

The *E. coli* strain used for MsrB production was BL21 transformed with a plasmidic construction containing the coding sequence under the T7 promoter. Samples of $^{15}$N and $^{15}$N/$^{13}$C labelled were prepared by growing cells in a minimal media with ($^{15}NH_4$)Cl as the sole nitrogen source and with glucose, $^{13}$C-labelled or not, as the only carbon source. Purification was done as described by Olry et al. (2002).

The NMR sample contained 2 mM protein concentration (95% $H_2O$, 5% $D_2O$) in 10 mM phosphate buffer and 50 mM 1,4-dithiothreitol-$d_{10}$ at pH 7.1. All spectra were acquired at 298 K on Bruker DRX 600 MHz spectrometer equipped with a 3-axis TXI probe and on Varian Inova spectrometer (800 MHz) equipped with triple-resonance probes including shielded z-gradients. Spectra were processed using the program XWINNMR (Bruker) and analyzed with the program XEASY (Bartels et al., 1995). Backbone amide $^{1}H^{N}$, $^{15}$N, $C^{\alpha}$, $C'$, and side-chain $C^{\beta}$ resonances were assigned using $^{1}$H-$^{15}$N HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH and CBCA(CO)NH experiments. Backbone $H^{\alpha}$, aliphatic sidechain protons and carbons resonances were assigned by analyzing the HNHA, HCCH-TOCSY and $^{15}$N- and $^{13}$C-NOESY spectra.

Torsion angles ($\phi$, $\psi$) and secondary structure prediction are based on $H^{N}$, $H^{\alpha}$, N, $C^{\alpha}$, $C^{\beta}$ and $C'$

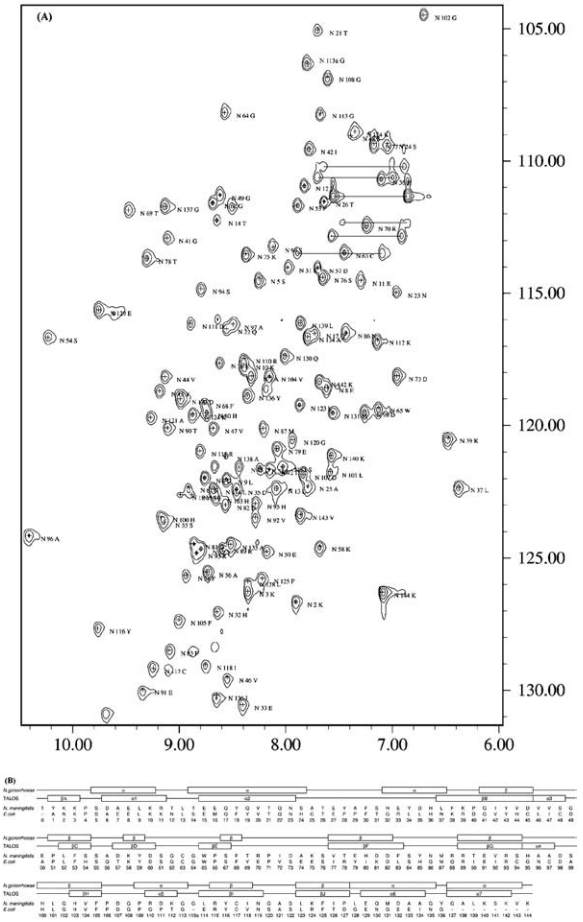


*Figure 1.* (A) $^{1}$H-$^{15}$N HSQC spectrum of MsrB at 298K. The backbone resonances of S67 and G135 are outside the region shown. Side chain amide protons of N and Q residues are indicated by solid horizontal lines. (B) The secondary structures from the X-ray structure of the *N. gonorrhoeae* MsrB and from the TALOS data of the *N. meningitidis* MsrB. The numbering of amino acid residues is based on that of the *Escherichia. coli* MsrB without the N-terminal M.

chemical shifts using the TALOS program (Cornilescu et al., 1999).

## Extent of assignments and data deposition

More than 91% of backbone $H^N$, N, $C^\alpha$, $C'$ and $C^\beta$ nuclei are assigned (i.e., 123/138 $^{15}$N-$H^N$ sites, 140/146 $C^\alpha$, 136/146 $C'$, 120/135 $C^\beta$). Proton and carbon chemical shift data for the majority of sidechain groups were determined using a combination of 3D spectra. Comparing the predicted structure with that of the crystal structure of *N. gonorrhoeae* (97% of identity), in overall, the structural elements in solution coincide with X-ray data. 7 α-helices and 10 β-strands were identified: βA (Y1-K3), α1 (D6-R11), α2 (E15-N23), βB (L37-D45), α3 (V46-S48), βC (L52-S54), βD (D57-D60), βE (W65-S67), βF (V77-F83), βG (R89-S94), α4 (H95-A97), βH (H103-F105), α5(R110-K112), βI (L114-N119), βJ (L123-P127), α6 (E129-A134), α7 (G137-K142).

The main differences are the first β-strand (βA) and the two helices (α3) and (α4) which are only seen in the NMR structure and the α-helix including residues H32 to L37 which is not present.

Some peaks present in the HSQC $^{1}$H $^{15}$N spectrum were not found in the other heteronuclear 3D spectra. Moreover the $H^N$ chemical shift value of the S67 (12.33 ppm) is unusual. This residue is closed to the active site which includes amino acids Y59, W65, F68 and P66. Chemicals shifts were deposited in the BioMagResBank under access number BMRB-6051 (http://www.bmrb.wisc.edu) in which the MsrB primary sequence is numbered from 1 to 146.

## Acknowledgements

This research was supported by the CNRS, the Universities of Nancy I and INPL, the IFR 111 Bioingénierie and the Association pour la Recherche sur le Cancer (ARC-No 5436). Access to the Bruker DRX 600 NMR facilities of the SCBIM Nancy I was deeply appreciated. We thank Dr P. Gans for doing HNCO, HNCACO spectra on the Varian Inova 800 of the NMR laboratory of the IBS Jean-Pierre Ebel (Grenoble, France). We are grateful to Dr C. Hemmerlin for her excellent technical assistance.

## References

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

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., Weissbach, H., Etienne, F., Brot, N. and Matthews, B.W. (2002) *Nat. Struct. Biol.*, **9**, 348–352.

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

Zheng, D., Cort, J.R., Chiang, Y., Acton, T., Kennedy, M.A. and Montelione, G.T. (2003) *J. Biomol. NMR*, **27**, 183–184.