Journal of Biomolecular NMR **29:** 99–100, 2004.

Letter to the Editor: NMR assignment of TM1442, a putative anti-σ factor antagonist from *Thermotoga maritima*

Touraj Etezady-Esfarjani* & Kurt Wüthrich
The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

Received 15 September 2003; Accepted 17 October 2003

Key words: NMR assignment, σ-factor, structural proteomics, *Thermotoga maritima*

Biological context

Based on 25% sequence identity to the SPOIIAA protein, which is the anti-σ^f factor antagonist of *B. subtilis*, the polypeptide with the TIGR identification TM1442 is believed to be involved in the regulation of gene expression as an anti-σ factor antagonist in *Thermotoga maritima.* As part of the structural proteomics project of The Joint Center for Structural Genomics (JCSG; Lesley et al., 2002), we initiated a NMR structure determination of this 110-residue protein, and report here the complete sequence-specific assignments for this molecule.

In bacteria, σ-factors are part of the regulatory system which controls gene expression at transcription initiation. During transcription, promotor recognition and promoter melting depend on the binding of a σ-factor to the core of RNA polymerase (RNAP) to form the RNAP holoenzyme (Lonetto et al., 1992). The σ-factor in turn is regulated by the anti-σ-factor, which functions by ATP-stimulated binding to the σ-factor (Duncan and Losick, 1993) to drive the σ-factor into a transcriptionally inactive state. The complex between σ-factor and anti-σ-factor remains stable until a third protein, the anti-σ-factor antagonist binds to the anti-σ-factor, and thus reduces the affinity of this molecule to the σ-factor and induces the release of the σ-factor from the anti-σ-factor (Diederich et al., 1994). From the structure determination of TM1442 we expect to obtain confirmation of its structural and functional homology to *B. subtilis* SPOIIAA, and hopefully to gain new insights into the mechanism of transcription initiation control in *T. maritima.*

*To whom correspondence should be addressed. E-mail: touraj@scripps.edu

Methods and experiments

Expression and purification of recombinant TM1442, which is a putative anti-σ-factor antagonist of *T. maritima*, was based on a previously reported three-step purification procedure for the isolation of thermophilic proteins without purification tag (Etezady-Esfarjani et al., 2003).

The presently used NMR samples contained, respectively, 4 mM unlabeled protein, 2 mM uniformly $^{13}C/^{15}N$-labeled protein, or 2 mM uniformly ^{15}N-labeled protein in 600 μl solution (solvent 95% H_2O/5% D_2O or 99.9% D_2O, 20 mM d_3-sodium acetate at pH 4.8, 150 mM sodium chloride, 2 mM sodium azide). The protein is unstable above pH 5.2, and it tends to form soluble aggregate. All NMR measurements therefore had to be performed with freshly prepared protein solutions. NMR measurements were performed at 313 K on Bruker Avance600 and Avance900 spectrometers using TXI-HCN-xyz gradient probes. Proton chemical shifts are referenced to internal 3-(trimethyl-silyl)-propane-1,1,2,2,3,3-d_6-sulfonic acid, sodium salt (DSS). Using absolute frequency ratios, the ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS.

2D [^1H,^{15}N]-HSQC, 3D HNCACB, 3D CBCA(CO) NH and 3D HNCO spectra (Bax and Grzesiek, 1993) were used to obtain sequence-specific assignments for the polypeptide backbone. Virtually complete ^1H and ^{13}C assignments of the non-aromatic side chain CH_n moieties were obtained using 2D [^{13}C,^1H]-HSQC, 3D ^{15}N-resolved [^1H,^1H]-TOCSY and 3D H(C)CH-TOCSY experiments. ^1H spin systems of the aromatic rings of His, Phe and Tyr were identified in a D_2O solution of the unlabeled protein, using 2D [^1H,^1H]-NOESY and 2D [^1H,^1H]-TOCSY experiments. Sequence-specific assignments

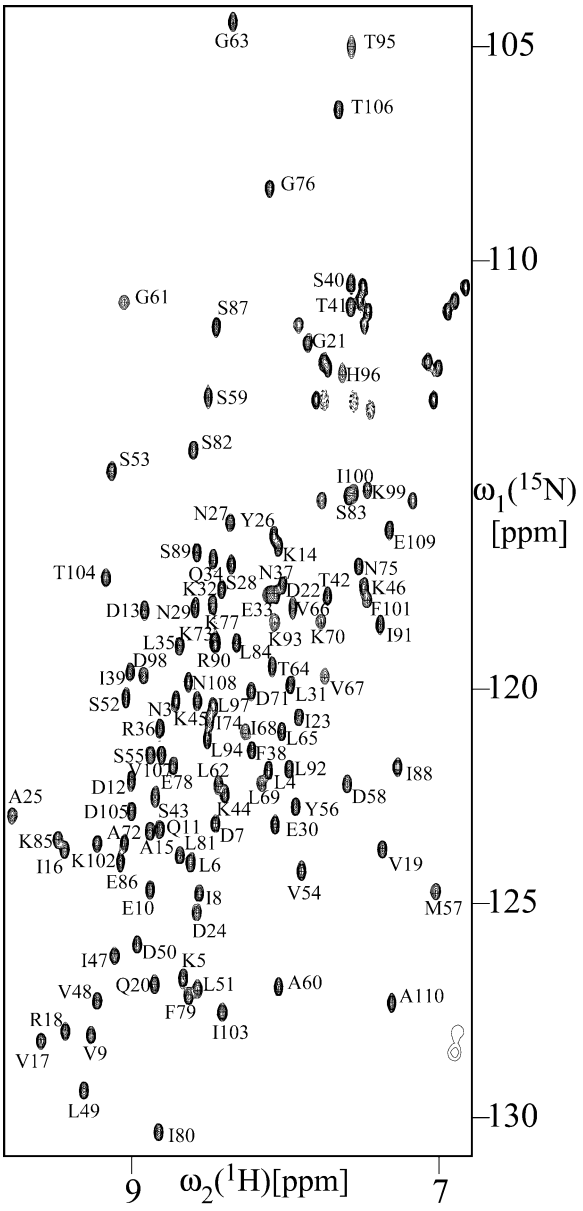

Figure 1. 2D [^{15}N,^{1}H]-HSQC spectrum of the uniformely ^{15}N-enriched protein TM1442, a putative anti-σ-factor from *T. maritima* recorded at 600 MHz and 313 K (protein concentration 2 mM in 600 µl of 95% H_2O/5% D_2O containing 20 mM d_3-sodium acetate at pH 4.8, 150 mM NaCl and 2 mM NaN_3). Resonance assignments of the backbone ^{15}N–^{1}H moieties are indicated by the one-letter amino acid code and the sequence number.

of the aromatic side chains were established from NOEs between the βCH_2 group and the aromatic protons (Wüthrich, 1986) using 2D [^{1}H,^{1}H]-NOESY. The ^{13}C chemical shifts of the aromatic rings were obtained from a 3D ^{13}C-resolved [^{1}H,^{1}H]-NOESY spectrum. The NMR spectra were processed with the program PROSA (Güntert et al., 1992), and analyzed with the XEASY software package (Bartels et al., 1995).

Extent of assignments and data deposition

All ^{1}H, ^{13}C and ^{15}N resonances of the polypeptide backbone were assigned, with the sole exceptions of Met1 and Asn2. The assignments of the non-labile protons of amino acide side chains are complete except for $C^{\alpha}H$, $C^{\beta}H_2$ and $C^{\gamma}H_2$ of Met 1, $C^{\alpha}H$, $C^{\beta}H_2$ of Asn 2, $C^{\delta}H_2$ and $C^{\varepsilon}H_2$ of Lys 46, and $C^{\zeta}H$ of Phe 38. The labile side-chain protons of Arg, Asn and Gln were completely assigned, except for H^{η} of Arg 18. The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the BMRB accession number 5921.

Acknowledgements

We thank Dr S.A. Lesley and H.E. Klock from GNF in the JCSG consortium funded by NIGMS GM062411 for providing us with the genomic materials, and Dr M. Hennig for helpful discussions. Financial support for T.E.-E. was obtained from the Schweizerischer Nationalfonds (project 31-66427-01); K.W. is the Cecil H. and Ida M. Green Visiting Professor of Structural Biology at TSRI.

References

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

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.

Diederich, B., Wilkinson, J.F., Magnin, T., Najafi, S.M.A., Errington, J. et al. (1994) *Genes Dev.,* **8**, 2653–2663.

Duncan, L. and Losick, R. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 2325–2329.

Etezady-Esfarjani, T., Peti, W. and Wüthrich, K. (2003) *J. Biomol. NMR*, **25**, 167–168.

Güntert, P., Dötsch, V., Wider, G. and Wüthrich, K. (1992) *J. Biomol. NMR*, **2**, 619–629.

Lesley, S.A., Kuhn, P., Godzik, A., Deacon, A.M., Mathews, I. et al. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 11664–11669.

Lonetto, M., Gribskov, M. and Gross, C.A. (1992) *J. Bacteriol.,* **174**, 3843–3849.

Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York, NY.