Journal of Biomolecular NMR **30:** 459–460, 2004.

Letter to the Editor: Complete ^{1}H, ^{13}C and ^{15}N NMR assignments of MTH0776 from *Methanobacterium thermoautotrophicum*

Godwin Amegbey[a], Zhan Chang[a], Paul Stothard[b], Adelinda Yee[c], Cheryl Arrowsmith[c] & David S. Wishart[b,*]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada T6G 2N8;* [b]*Department of Computing Science and Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E8;* [c]*Department of Medical Biophysics, University of Toronto and Ontario Center for Structural Proteomics, Ontario Cancer Institute, Toronto, Ontario, Canada M5G 2C4*

Received 26 July 2004; Accepted 28 September 2004

Key words: archaea, assignment, methanogenesis, NMR, protein

Biological context

MTH 0776 is a 101 amino acid residue protein originally isolated from *M. thermoautotrophicum*. It has been successfully cloned and expressed in *E. coli*. This small protein belongs to a distinct orthologous group (COG 4033) consisting of 11 proteins varying in size from 92–124 residues found only in methanogenic archaebacteria. MTH0776 is part of a two-protein operon with its upstream partner – MTH0777; which is also specific to methanogenic archaebacteria. The crystal structure of MTH0777 was recently determined (PDB 1KJN) and it exhibits a novel α/β fold that has not been seen in any other protein. Based on the MTH0776 sequence, its relationship to MTH0777 and some preliminary structural data, it is likely that MTH0776 also has a novel fold. In an effort to better understand its structure, its role in methanogenesis and the molecular interactions between MTH0776 and MTH0777, we have undertaken the NMR structure determination of MTH0776. Here we wish to report the complete sequence-specific assignments for MTH0776.

Methods and experiments

The MTH0776 gene was sub-cloned into a pET15b vector (Novagen) and transformed into BL21 (DE3) *E. coli* cells for expression. The construct expresses the native MTH0776 protein (101 residues) linked to a 20 residue, N-terminal affinity tag containing a His_6 sequence followed by a thrombin cleavage site (total length = 121 residues). Protein expression was accomplished by growing the transformed *E. coli* cells in a minimal (M9) growth medium. Uniform isotopic enrichment with ^{15}N or $^{15}N/^{13}C$ was accomplished by substituting the NH_4Cl and/or glucose in standard M9 media with $^{15}NH_4Cl$ and/or [U-^{13}C]-glucose respectively. Cells were grown at 37 °C in shaker flasks to an optical density of 1.0 and then induced with isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The cells were allowed to grow for an additional 8 h before harvesting and centrifugation. The cells were lysed and then centrifuged at 15,000 rpm for 30 min at room temperature to pellet any cellular debris. The protein was purified and re-natured using a nickel nitrilotriacetic acid-agarose (Ni-NTA) column (Qiagen) as described elsewhere (Holzinger et al., 1996). NMR samples were prepared by dissolving about 20 mg of the His-tagged protein in 500 µl of a buffer (pH 6.8) made up of 20 mM NaH_2PO_4, 300 mM NaCl, 50 µl of D_2O, 1 mM DSS, 15 mM DTT and 10 µl of a 3% solution of sodium azide (Figure 1). Spectra collected with and without the histidine tag were found to be essentially identical.

NMR experiments were recorded at 25 °C on Varian Inova 500 and 800 MHz spectrometers

*To whom correspondence should be addressed. E-mail: david.wishart@ualberta.ca

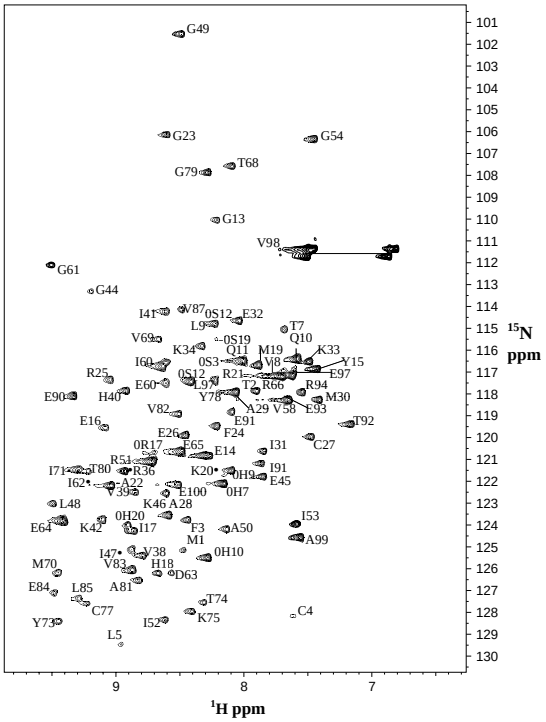

Figure 1. ^{1}H-^{15}N HSQC spectrum from 1 mM MTH0776 in 500 µl of a buffer solution pH 6.0, made up of 50 mM NaH_2PO_4, 300 mM NaCl, 50 µl D_2O, 1 mM DSS, 15 mM DTT and 10 µl of a 3% solution of sodium azide, collected on a Varian Inova 500 MHz spectrometer. Assignments for the histidine tag residues begin with '0'.

equipped with a 5 mm triple resonance and pulse gradient accessories. All spectra were processed with VNMR software or with NMRPIPE (Delaglio et al., 1995). Proton chemical shifts were referenced to internal DSS while ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS using the absolute frequency ratios (Wishart et al., 1995).

Sequential chemical shift assignments were obtained by identifying $^{13}C\alpha(i)$ to $^{13}C\alpha$ (i-1) and $^{13}C\beta(i)$ to $^{13}C\beta$(i-1) connectivities from the HNCACB (Kay et al., 1994) spectrum. These were confirmed and any existing ambiguities resolved using HNCA, CBCA(CO)NH spectra (Grzesiek and Bax, 1992) and NOEs measured from 2D homonuclear ^{1}H-^{1}H NOESY and ^{1}H-^{15}N NOESY-HSQC (Zhang et al., 1994) spectra. The backbone chemical shift assignment was then completed using additional data from HNCO and HNHA spectra. Side chain ^{1}H assignments for each amino acid were then added using data from a DE-HCCH_TOCSY experiment as well as C(CO)NH and H(CCO)NH (Grzesiek et al., 1993) spectra. Stereo-specific assignments of $^{1}H\beta$ protons were based on the intensities of ^{1}HN-$^{1}H\beta$ and $^{1}H\alpha$-$^{1}H\beta$ cross-peaks in the ^{1}H-^{15}N NOESY-HSQC and 2-D ^{1}H-NOESY spectra. The methyl groups of Val and Leu were assigned stereo-specifically based on the intensity of ^{1}HN-$^{1}H\gamma$, $^{1}H\alpha$–$^{1}H\gamma$ cross peaks.

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C backbone resonances for the native sequence of MTH0776 were assigned with the exception of Pro 56 and Pro 101 (98% complete). The amino acid side chains for all non-labile hydrogens of the native sequence were fully assigned except for the $H\epsilon$ atoms of all 5 methionines (99% complete). The ^{1}H, ^{15}N and ^{13}C backbone and side chain ^{1}H assignments were also completed for all visible resonances belonging to the 20 residue N-terminal affinity tag (90% complete). The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank under the BMRB accession number 6272.

Acknowledgements

GYA was supported by a Pfizer graduate student scholarship. Additional financial support from PENCE is gratefully acknowledged.

References

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Grzesiek, S. and Bax, A. (1992), *J. Am. Chem. Soc.*, **114**, 6291.

Grzesiek, S., Anglister, J. and Bax, A. (1993) *J Magn. Reson. B*, **101**, 114–119.

Holzinger, A., Philips, K.S. and Weaver, T.E. (1996) *BioTechniques*, **20**, 804–808.

Kay, L.E., Xu, Y.G. and Yamazaki, T. (1994) *J. Magn. Reson. A*, **109**, 129–133.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.

Zhang, O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) *J. Biol. NMR*, **4**, 845–858.