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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignments for the 18.7 kDa GTPase domain of *Escherichia coli* MnmE protein

Daniel Monleón[a], Lucía Yim[b], Marta Martínez-Vicente[b], M. Eugenia Armengod[b,*] & Bernardo Celda[a,*]

[a]*Department of Physical Chemistry, University of Valencia C/ Dr. Moliner, 50, Burjassot 46100 Valencia, Spain;* [b]*Laboratory of Molecular Genetics, Instituto de Investigaciones Citológicas, Fundación Valenciana de Investigaciones Biomédicas, C/ Amadeo de Saboya, 4, 46010 Valencia, Spain*

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Biological context

GTPases have wide-ranging functions in both prokaryotes and eukaryotes (Leipe et al., 2002). The common property shared by these proteins is the presence of a structural module, the G domain, which is usually involved in the switching of the protein between a GTP-bound and a GDP-bound conformation (Geyer and Wittinghofer, 1997). Classical GTPases, like Ras-related proteins, are often described as molecular switches because of their particular mode of action. These GTPases are in an active state when GTP-bound. The binding of GTP causes a conformational change in the proteins that allows interaction with a target (effector) molecule. Upon GTP hydrolysis, they become inactive. GDP has to be released from the inactive GTPase to allow new entry of GTP. This cyclic reaction usually involves several other factors that catalyze the hydrolysis step and the release of bound GDP since classical GTPases show a very low intrinsic GTPase activity and a very high affinity for guanine nucleotides (Geyer and Wittinghofer, 1997).

Escherichia coli mnmE gene product (MnmE) is a 50 kDa protein involved in tRNA modification (Cabedo et al., 1999). Unlike the G domain-only proteins of the Ras superfamily, MnmE is a large protein that consists of three domains, an ∼220 amino-acid N-terminal domain, a middle GTPase domain, of about 165 residues, and an ∼75 amino-acid C-terminal domain. Moreover, MnmE exhibits a very high intrinsic GTPase hydrolysis rate and very low affinity for GTP and GDP (Cabedo et al., 1999; van der Bliek, 1999). These biochemical parameters predict that the GTPase cycle of MnmE proceeds without auxiliary factors.

*To whom correspondence should be addressed. E-mail: bernardo.celda@uv.es; armengod@ochoa.fib.es

Strikingly, the isolated G domain of MnmE conserves the guanine nucleotide binding and GTPase activities of the intact MnmE molecule (Cabedo et al., 1999). This domain is essential for the MnmE tRNA modifying function, which requires effective GTP hydrolysis, and not simply GTP binding (Yim et al., 2003). Thus, the mechanism of MnmE function may not follow the general GTPase switch model. If so, MnmE would be a new member of the group of multidomain GTPases, such as EF-G and dynamins, that have been proposed to require GTP hydrolysis to be functionally active (Rodnina et al., 2000; Marks et al., 2001). The molecular basis of the peculiar biochemical properties of MnmE (or its G domain) as well as the mechanism of MnmE function are unknown. As a basis for ligand recognition investigations and for better understanding of GTP binding, backbone dynamics and interactions of the MnmE G-domain, we here report its ^{1}H, ^{13}C and ^{15}N backbone resonance assignment.

Methods and materials

pIC758, a pGEX2T derivative carrying the DNA sequence of the *E. coli* MnmE G-domain, was transformed into DEV16 strain, a null *mnmE* mutant (Cabedo et al., 1999). The GST-fusion protein was overproduced in M9 minimal medium containing 10 μg ml^{-1} thiamine, 100 μg ml^{-1} ampicillin, 1 g l^{-1} $^{15}NH_4Cl$ and 2 g l^{-1} UL-^{13}C6-D-glucose (Spectra Stable Isotopes). Cells were grown at 37 °C to an OD_{600} of 0.4 and synthesis of the protein fusion was induced with 0.5 mM IPTG for 4 hours. The protein was purified to homogeneity using glutation agarose resin. Subsequently, the N-terminal GST tag was removed using thrombin. The resulting protein contains residues G210 to G377 of MnmE with four addi-

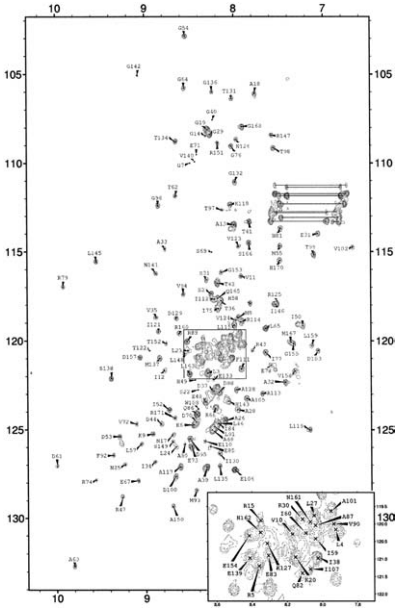

Figure 1. Assigned 2D ^{1}H,^{15}N HSQC spectrum with a expanded view of central highly overlapped region for GTPase domain of MnmE from *E. coli* at 25 °C and pH 7.0 recorded on a 500 MHz Bruker Avance DRX spectrometer. Side chain amide resonances are connected with horizontal lines.

tional C-terminal residues (IHRG). $^{13}C/^{15}N$-labeled protein was prepared in phosphate-buffered saline (PBS), pH 7.0. After column elution, the protein was concentrated using Microsep 10 K Omega (Pall Life Sciences), to a final concentration of about 0.3 mM.

All NMR spectra used for backbone resonance assignments were recorded at 25 °C on a Bruker AVANCE AMX 500 MHz spectrometer equipped with pulse-field gradient triple-resonance $^{15}N/^{13}C/^{1}H$ cryoprobes. Cryoprobe provided the necessary signal-to-noise ratio for the analysis of this low-solubility highly-unstable protein sample. All backbone assignment experiments were recorded in less than 2 days, just before sample degradation. Spectra were processed using the XwinNMR 3.1 software package. Sparky 3.91 (Goddard and Kneller, 1999). Backbone assignments of the MnmE G-domain were obtained mainly by combined analysis of CBCA(CO)NH and HNCACB and verified using HNCA and HN(CO)CA experiments (Bax et al., 1994). An additional HN(CO)(CA)HA experiment was collected for H_α chemical shifts identification. Final peak lists were initially analyzed with AutoAssign (Moseley et al., 2001). Initial AutoAssign results were manually confirmed and extended to obtain the final set of assignments. In the ^{1}H-^{15}N HSQC, ∼97% backbone amide resonances were assigned (Figure 1). The ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to DSS according to the IUPAC recommendation (Markley et al., 1998).

Extent of assignment and data deposition

The combined use of automated and manual analysis of triple resonance 3D data along with the distance connectivities extracted from a ^{15}N-HSQC-NOESY spectrum provided assignments for ∼95% of assignable backbone atoms of the MnmE G-domain. Definitive ^{15}N and H^N assignments for amides of residues His51, Val156, Lys162 and Ile169 could not be determined unambiguously as they exhibit severe degeneracy and overlapping. The chemical shifts for backbone resonances of G domain of the *E. coli* MnmE protein have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number BMRB-5861. Overall, based on these chemical shifts and H_N-H_α NOE connectivities, secondary structure for this G domain is very similar to that reported for other GTPases like human Ras or Rac1, or bacterial ERA protein. Interestingly, a detailed comparison of secondary structure shows more similarities of MnmE G-domain to Ras than to ERA protein, in spite of sequence homology between MnmE and ERA is higher.

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References

Bax, A. et al. (1994) *Meth. Enzymol.*, **239**, 79–105.
Cabedo, H. et al. (1999) *EMBO J.*, **18**, 7063–7076.
Geyer M. and Wittinghofer A. (1997) *Curr. Opin. Struct. Biol.*, **7**, 786–792
Goddard, T. D. and Kneller, D.G. (1999) *SPARKY 3*, University of California, San Francisco.
Leipe, D.D. et al. (2002) *J. Mol. Biol.*, **317**, 41–72.
Marks, B. et al. (2001) *Nature*, **410**, 231–235.
Markley, J.L. et al. (1998) *J. Mol. Biol.*, **280**, 933–952.
Moseley, H.N.B. et al. (2001) *Meth. Enzymol.*, **339**, 91–108.
Rodnina, M.V. et al. (2000) *Biol. Chem.*, **381**, 377–387.
Yim, L. et al. (2003) *J. Biol. Chem.*, **278**, 28378–28387.