Journal of Biomolecular NMR, **17:** 273–274, 2000.
KLUWER/ESCOM

Letter to the Editor: Assignment and secondary structure identification of the ribosomal protein L18 from *Thermus thermophilus*

Esmeralda A. Woestenenk[a], Peter Allard[a], George M. Gongadze[b], Svetlana E. Moskalenko[b], Dmitry V. Shcherbakov[b], Alexey V. Rak[b], Maria B. Garber[b], Torleif Härd[a] & Helena Berglund[a,*]

[a]*Department of Biotechnology, Royal Institute of Technology (KTH), Center for Structural Biochemistry, Novum, S-141 57 Huddinge, Sweden*

[b]*Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia*

Received 24 March 2000; Accepted 3 May 2000

Key words: resonance assignment, ribosomal protein, RNA-binding protein, *Thermus thermophilus*

Biological context

The ribosomal protein L18 is located in the central protuberance of the large subunit of the bacterial ribosome. The central protuberance is a relatively autonomous structure within the large subunit and in *E. coli* it is formed by the three proteins L5, L18, and L25, together with the 5S rRNA molecule and part of the 23S rRNA molecule. Binding of L18 to *E. coli* 5S rRNA increases the secondary structure content of the 5S RNA and stimulates binding of L5, as well as formation of the 5S RNA/23S RNA/protein complex (Bear et al., 1977; Newberry and Garrett, 1980).

With the crystal structure of the large subunit available at 5 Å resolution (Ban et al., 1999), high resolution structures of ribosomal proteins and RNA fragments are needed to shed light on ribosomal regions where the structure is still unclear at atomic level. Knowledge of the three-dimensional structure of L18 can help to understand the assembly of the 5S rRNA complex and to build a detailed molecular model of the ribosome.

Methods and results

A fragment of *Thermus thermophilus* DNA encoding L18 (111 amino acids) was cloned into the vector pET11c and expressed in *E. coli* strain BL21(DE3). Unlabeled NMR samples were purified from cells grown in LB medium at 37 °C; ^{15}N-labeled and ^{15}N,^{13}C-labeled samples were purified from cells grown in minimal medium, with $^{15}NH_4Cl$ (Martek) and D-glucose-U-$^{13}C_6$ (Cambridge Isotopes) as main nitrogen and carbon sources, supplemented with 0.8 g/l (^{13}C,)^{15}N-Isogro (Isotec Inc.). The supernatant of the cell lysate was heated to 55 °C for 10 min and precipitated *E. coli* proteins were spun down. L18 was purified from the supernatant using ion exchange chromatography followed by hydrophobic chromatography. The protein was precipitated with $(NH_4)_2SO_4$, dialysed against an NMR buffer (50 mM KH_2PO_4 pH 4.8, 150–200 mM LiCl) and concentrated. A typical NMR sample contained NMR buffer and 10% (v/v) D_2O, with a pH ranging from 5.4 to 6.5 and a protein concentration between 0.8 mM and 1.6 mM.

NMR spectra were recorded at 30 °C at 500 MHz on a Varian Inova, and at 500 MHz, 600 MHz, 700 MHz, and 800 MHz on Bruker *AVANCE* spectrometers. All spectrometers were equipped with three rf channels, pulsed field gradients and 5 mm triple resonance probes. All spectra were recorded using standard implementations of published pulse sequences as described in Cavanagh et al. (1996) and references therein. The peptide backbone was assigned using a series of 3D triple resonance experiments recorded at 500 MHz: HNCA, HN(CO)CA, HNCACB, and CBCA(CO)NH. Side-chain ^{13}C and ^{1}H chemical shifts were obtained from a 3D (H)C(CO)NH experiment and a 3D H(CCO)NH experiment, both recorded at 500 MHz. Further side-chain and ^{1}H chemical shifts were obtained from 3D HCCH-TOCSY and 3D

*To whom correspondence should be addressed. E-mail: hb@csb.ki.se

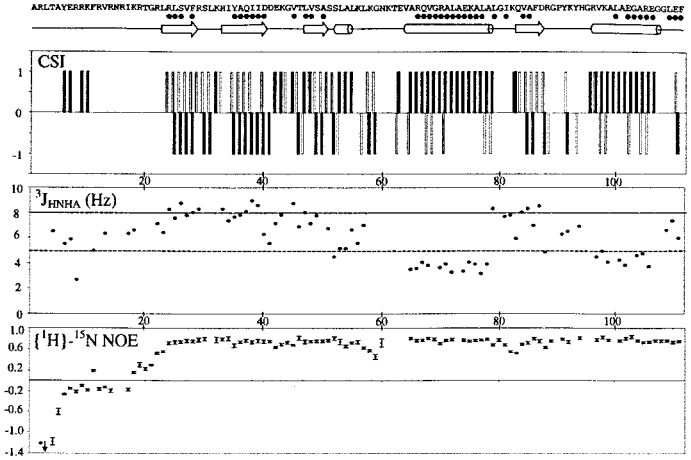

Figure 1. Identification of secondary structure features in L18. Top: amino acid sequence of L18. Black dots mark residues whose amide protons exchange slowly (resonances that are still present in the ^{15}N-HSQC 10 min after dissolving lyophilised L18 in D_2O; after 10 h residues 36–40, 48, 68–73, 76, 77, 100, 102–105, and 109 could still be observed clearly). Secondary structure elements (arrows for β-strands, cylinders for α-helices) as identified by the data are indicated below the sequence. First panel: ^{13}C chemical shift index of L18. Filled bars represent data derived from C_α secondary shifts, open bars represent data derived from C_β secondary shifts. ^{13}C chemical shift data was analysed according to Wishart and Sykes (1994). Residue numbers are indicated between the panels. Second panel: $^3J_{HNHA}$ coupling constants calculated from integrals of non-overlapping peaks in a 3D ^{15}N,^{1}H-HNHA spectrum. Approximate limits for $^3J_{HNHA}$ coupling constants typical for α-helices (< 5 Hz) and β-strands (> 8 Hz) are indicated with dashed lines in the diagram. Third panel: {^{1}H}-^{15}N heteronuclear NOEs defined as $I_{saturated}/I_{unsaturated}$. Errors were estimated using the average noise level in the spectra. *: the value for Leu3 is not displayed in the graph; it was determined to be $-3.89 +/- 0.38$.

HCCH-COSY experiments recorded at 600 MHz. A 3D ^{15}N-TOCSY-HSQC recorded at 600 MHz was also used for assignment of side-chain resonances, in combination with a 3D ^{15}N-NOESY-HSQC (700 MHz) and a 2D NOESY (800 MHz). $^3J_{HNHA}$ coupling constants were calculated from peak intensities in a 3D ^{15}N-HNHA experiment recorded at 700 MHz. {^{1}H}-^{15}N heteronuclear NOEs were measured as peak height ratios in a steady state NOE experiment recorded at 500 MHz. Data was processed using VNMR 5.3, XWIN-NMR or NMRPipe (Delaglio et al., 1995) on Sun or SGI workstations. All spectra were analysed using the software ANSIG (Kraulis, 1989).

Extent of assignments and data deposition

Essentially complete assignments were obtained for all residues that appeared in the ^{15}N-HSQC. From 109 expected resonances (111 minus Pro91 and Ala1), 101 were observed in the ^{15}N-HSQC. The eight missing amide resonances were Arg2, Arg16, Leu31, Thr62, Glu63, Val 64, Gly95 and Arg96. No assignments could be obtained for Ala1. Gly95 could only be observed when the temperature was lowered to 293 K. Side-chain assignments are essentially complete except for arginines and lysines which are likely to be surface-exposed, approximately 70% of their side-chain proton resonances have been assigned. {^{1}H}-^{15}N heteronuclear NOE values indicate that the N-terminal part of L18 (up to Arg22) is unstructured. The rest of the protein appears to be well ordered, apart from some loop regions where residues have lowered NOE values and/or weak or even missing amide resonances. Based on CSI, $^3J_{HNHA}$ coupling constants, amide proton exchange data, and typical NOE patterns, we have identified four β-strands, two α-helices, and a short helical turn. Chemical shifts at pH 5.9 and 30 °C have been deposited in the BioMagResBank under accession number BMRB-4688.

References

Ban, N., Nissen, P., Hansen, J., Capel, M., Moore, P.B. and Steitz, T.A. (1999) *Nature*, **400**, 841–847.

Bear, D.G., Schleich, T., Noller, H.F. and Garrett, R.A. (1977) *Nucleic Acids Res.*, **4**, 2511–2526.

Cavanagh, J., Fairbrother, W.J., Palmer III, A.G. and Skelton, N.J. (1996) *Protein NMR Spectroscopy: Principles and Practice*, Academic Press, San Diego, CA.

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

Kraulis, P.J. (1989) *J. Magn. Reson.*, **24**, 627–633.

Newberry, V. and Garrett, R.A. (1980) *Nucleic Acids Res.*, **8**, 4131–4142.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.