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Letter to the Editor: NMR assignments of the cold-shock protein ribosome-binding factor A (RbfA) from *Thermotoga maritima*

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Biological context

Cold-shock proteins are produced in response to a sudden drop in temperature and help cells adapt to lower temperatures to resume their normal growth. Ribosome-binding factor A (RbfA) belongs to a family of cold-shock proteins with homologues in the genomes of archebacteria and eubacteria.

The rbfa gene of *E. coli* was isolated as a high copy repressor of a cold-sensitive mutation located in the 5′-terminal helix of 16S rRNA (Dammel and Noller, 1995). Immunolocalization studies showed that RbfA associates with free 30S ribosomal subunits, but not with polysomes or large ribosomal subunits (Dammel and Noller, 1995). The rbfA gene is required for normal cell growth at lower temperatures and its deletion results in slower growth (Jones and Inouye, 1996). This suggests that RbfA is an RNA-binding protein required for normal ribosomal function at low temperatures (Dammel and Noller, 1995). In ΔrbfA cells, the amount of pre-16S rRNA increases after cold shock with a concomitant reduction of the mature 16S rRNA. These findings indicated that the cold sensitivity of ΔrbfA cells is directly related to their lack of translation initiation-capable 30S subunits containing mature 16S rRNA (Xia et al., 2003). Furthermore, increased synthesis of RbfA can suppress the aberrant assembly of 30S subunits and the resulting translation deficiency and slow growth in *Escherichia coli* strains deficient for the ribosome assembly factor protein RimM (Bylund et al., 2001). The NMR solution structure of an N-terminal 108 aa fragment of *E. coli* RbfA revealed that RbfA contains a KH-domain (Huang et al., 2003).

Here we report the nearly complete ^{1}H, ^{13}C and ^{15}N NMR resonance assignments for an N-terminal 120 residue fragment (14.2 kDa) of the RbfA protein from the thermophilic eubacterium *Thermotoga maritima*.

Methods and experiments

The gene coding for residues 1–120 of RbfA from *T. maritima* was cloned by PCR using *T. maritima* genomic DNA (ATCC 43589D) as a template. It was inserted into the pET11a – overexpression vector and overexpressed in ^{15}N or ^{15}N/^{13}C-labeled form in *E. coli* BL21(DE3) growing in M9 minimal medium containing only ^{15}N-labeled NH_4Cl and ^{13}C-labeled glucose as the sole nitrogen and carbon source. The purification of the protein was achieved by a cation-exchange chromatography step on a SP-sepharose-column (Pharmacia), followed by a size-exclusion chromatography step on a gelfiltration column (Superdex75, HiLoad 16/60, Amersham). Protein purified by this procedure was $>95\%$ pure and showed a molecular weight of 14.2 kDa verified by SDS-PAGE and MALDI. Samples for NMR-spectroscopy contained

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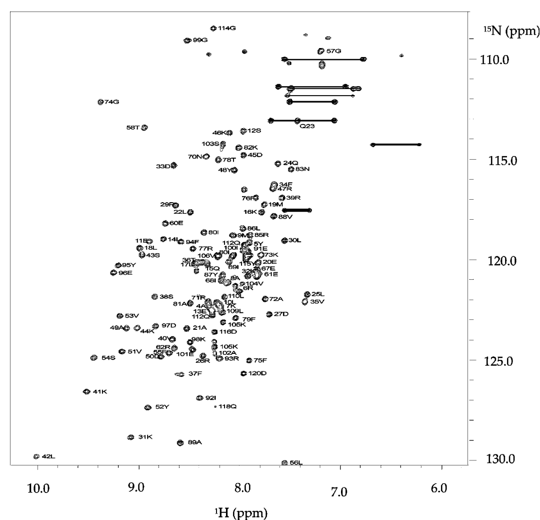

Figure 1. $^{1}H,^{15}N$-HSQC (700 MHz) spectrum of *Thermotoga maritima* RbfA recorded at 315 K with the assignments indicated.

~1.0 mM protein in 20 mM acetic acid, pH 4.5, 50 mM KCl and 10% $^{2}H_2O$.

Spectra were acquired at 315 K on Bruker DRX600, DRX700 and DRX800 spectrometers equipped with *z*-axis gradient 1H {13C, 15N} triple resonance cryogenic probes. Spectra were processed with XWINNMR 2.1 (Bruker) and analyzed with XEASY (Bartels et al., 1995). Sequential backbone resonance assignments were obtained with HNCA, HNCACO, HNCACB and HNCO experiments. Side-chain assignments were based on HBHA(CO)NH, HCCH-TOCSY, CC(CO)NH and HCCH-COSY experiments. ^{1}H chemical shifts were referenced to TMSP at 0.00 ppm and ^{13}C and ^{15}N chemical shifts were calculated from the ^{1}H frequency (Wishart et al., 1995).

Extent of assigment and data deposition

For the 120 residue fragment of *T. maritima* RbfA the backbone assigments are essentially complete with the exception of M1 and N2 preceding proline 3 and the N-atoms of all five prolines. In addition, there are no assignments for T65, V66 and R93.

From the triple resonance experiments, further backbone and non-aromatic side chain assignments were made to the following extents: 97% of C_α, H_α; 95% of C_β, 93% of H_β; 33% of C_γ and 66% of H_γ; 28% of C_δ and 67% of H_δ, and 97% of CO.

The assigned ^{1}H, ^{15}N, ^{13}C chemical shifts of the *T. maritima* RbfA have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number BMRB-6314.

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