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Letter to the Editor: Resonance assignments for cold-shock protein ribosome-binding factor A (RbfA) from *Escherichia coli*

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Biological context

RbfA (ribosome-binding factor A) is a cold-shock adaptation protein from the bacterium *Escherichia coli* (Thieringer et al., 1998). Cold-shock proteins are produced in response to a sudden drop in temperature and help cells adapt at lower temperatures to resume their normal growth. The gene for RbfA was isolated as a high copy repressor of a cold-sensitive mutation located at the 5′-terminal helix of 16S rRNA (Dammel et al., 1995). Immunolocalization studies showed that RbfA associates with free 30S ribosomal subunits, but not with polysomes or other larger subunits (Dammel et al., 1995). The *rbfA* gene is required for the normal cell growth at low temperatures and its deletion results in slower growth (Jones et al., 1996). This suggests that RbfA is required for normal ribosomal function at low temperatures (Dammel et al., 1995; Jones et al. 1996; Thieringer et al., 1998). RbfA is probably involved in ribosome maturation and/or translation initiation (Dammel et al., 1995), although its precise cellular and biochemical functions are not yet known. RbfA is a member of a large protein family with homologues in archeabacteria, eubacteria, and eukaryotes, making it an important target for structural genomics efforts. No 3D structures are available for any of these homologues. To better understand the biochemical function and mechanistic role of RbfA at low temperatures, we have initiated a detailed study of its structure and dynamics by NMR spectroscopy.

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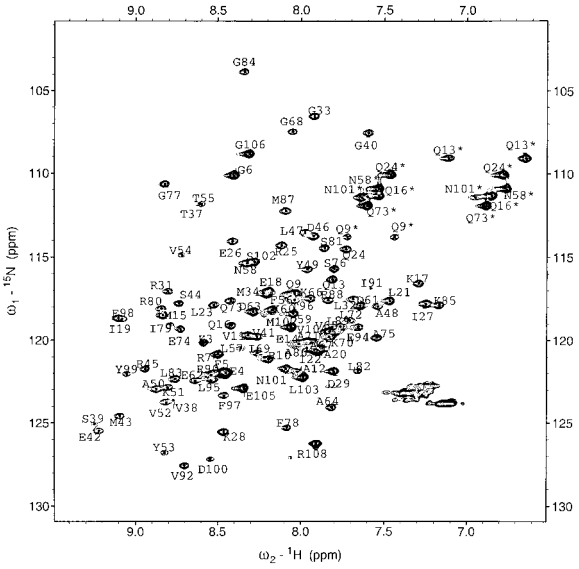

Figure 1. ^{1}H-^{15}N HSQC spectrum of *E. coli* RbfAΔ25 in 10 mM phosphate buffer, pH 5.05, 20 °C, recorded on a Varian INOVA 500 NMR spectrometer. Cross peaks are labeled with their assigned position in the amino acid sequence. Side chain NH_2 assignments of asparagines and glutamines are indicated by an *.

Here, we report the nearly complete ^{1}H, ^{13}C, and ^{15}N NMR resonance assignments.

For the NMR work reported here, we have used a truncated 108-residue, 12.5 kDa construct, RbfAΔ25, with 25 aa removed from the carboxy terminus of full-length RbfA. The full-length 133-aa protein slowly aggregates at room temperature, making it difficult to study by NMR. This smaller construct was identified by selective proteolysis experiments. Sedimentation

equilibrium analysis and gel filtration chromatography showed that RbfAΔ25 is monomeric in solution. Most of the ^{15}N-^{1}H^{N} cross peaks observed in the HSQC spectrum of the truncated construct (Figure 1) have identical chemical shift values in HSQC spectra of the full-length protein. Circular dichroism spectra of the full-length and truncated constructs are also very similar. These data demonstrate that the truncated construct RbfAΔ25 used in this work has a 3D structure similar to that of the corresponding portion of the full-length RbfA protein. The truncated protein supports normal cell growth at low temperatures in *rbfA*-deleted *E. coli*, indicating that RbfAΔ25 is biologically active in cold-shock adaptation.

Methods and experiments

The full-length coding sequence of the *rbfA* gene was cloned into expression plasmid pET11a, generating plasmid pETrbfA. The expression plasmid used to produce the C-terminal truncated RbfA construct used for this NMR study, pETrbfAΔ25, was obtained by changing the 109th codon (ATG) of the wild-type *rbfA* gene to a TAA stop codon by site-directed mutagenesis. The sequence of this plasmid was verified by DNA sequencing. *E. coli* strain BL21(DE3) cell cultures transformed with the pETrbfAΔ25 expression plasmid were grown at 37 °C in M9 minimal media containing $(^{15}NH_4)_2SO_4$ and ^{13}C-glucose as sole nitrogen and carbon sources. The production and purification of RbfA will be described in detail elsewhere. Sample purity ($>$ 97%) and molecular weight (12.5 kDa) were verified by SDS-PAGE and MALDI-TOF mass spectrometry.

Uniformly ^{13}C, ^{15}N-enriched RbfA samples were prepared for NMR experiments at $\sim$ 1.6 mM concentration in 95% H_2O and 5% D_2O solution containing 10 mM sodium phosphate and 0.5 mM sodium azide at pH 5.00 $\pm$ 0.05, in 5-mm Shigemi susceptibilty-matched NMR tubes. All NMR data were collected at 20 °C on a four-channel Varian INOVA 500 NMR spectrometer. The programs VNMR (Varian), SPARKY (Goddard and Kneller, University of California, San Fransisco) and AutoAssign (Zimmerman et al., 1997; Moseley et al., 2001) were used for data processing, peak picking, and analysis. Proton chemical shifts were referenced to internal DSS, while ^{13}C and ^{15}N chemical shifts were referenced indirectly using the gyromagnetic ratios of ^{13}C:^{1}H (0.251449530) and ^{15}N: ^{1}H (0.101329118), respectively. The input for AutoAssign included peak lists from 2D ^{1}H-^{15}N HSQC and 3D CBCANH, haCANH, HAcaNH, CBCAcoNH, HAcacoNH, haCAcoNH, and HNCO experiments, recorded as described elsewhere (Montelione et al., 1999). Results obtained from the automated analysis were extended and in some cases corrected by manual analysis of these data together with 3D hCCcoNH-TOCSY, HcccoNH-TOCSY, and HCCH-COSY experiments. Sidechain aromatic, guanido, and amide ^{1}H, ^{13}C and ^{15}N assignments were made using homonuclear 2D TOCSY, 2D ^{1}H-^{13}C HSQC and ^{13}C-edited NOESY data.

Extent of assignments and data deposition

The combined automated and manual analysis of these triple resonance NMR data along with 3D ^{15}N-edited NOESY and ^{13}C-edited NOESY data provided assignments for $\sim$95% of the assignable backbone atoms (101/105 ^{15}N-^{1}H^{N} sites, 112/115 H$^{\alpha}$, 94/108 C$'$, and 106/108 C$^{\alpha}$). Most of the side chain ^{13}C, ^{15}N and ^{1}H assignments were also obtained (i.e., 100/101 C$^{\beta}$, 70/100 C$^{\gamma}$, 52/78 C$^{\delta}$ 18/31 C$^{\varepsilon}$, 1/5 C$^{\zeta}$, 170/174 H$^{\beta}$, 110/131 H$^{\gamma}$, 72/88 H$^{\delta}$, 33/58 H$^{\varepsilon}$, 1/5 H$^{\zeta}$, 2/2 N$^{\delta}$, 5/5 N$^{\varepsilon}$). Backbone NH assignments of Met^{35}-Thr^{36} could not be determined unambiguously. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum with labels indicating ^{15}N and ^{1}H^{N} assignments. These ^{1}H, ^{13}C and ^{15}N chemical shift data have been deposited in BioMagResBank (accession number 5093). Analysis of these chemical shift data indicate that RbfA is an α/β protein with $\alpha\beta\beta\alpha\beta$ fold topology.

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References

Dammel, C. S. and Noller, C. S. (1995) *Genes Dev.*, **9**, 626–637.
Jones, P. G. and Inouye, M. (1996) *Mol. Microbiol.*, **21**, 1207–1218.
Montelione, G. T., Rios, C. B., Swapna, G. V. T. and Zimmerman, D. E. (1999), In *Modern Techniques in Protein NMR*, L. Berliner and N.R. Krishna (Eds.), pp. 81–130.
Moseley, H. N. B., Monleon, D. and Montelione, G. T. (2001) *Meth.. Enzymol.*, **339**, 91–108.
Thieringer, H. A., Jones, P. G. and Inouye. M. (1998) *BioEssays*, **20**, 49–57.
Zimmerman, D. E., Kulikowski, C. A., Huang, Y., Feng, W., Tashiro, M., Shimotakahara, S., Chien, C. Y., Powers, R. and Montelione, G. T. (1997) *J. Mol. Biol.* **269**, 592–610.