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Letter to the Editor: NMR backbone assignments of the cold-regulated RNA-binding protein, RbpA1, in the cyanobacterium, *Anabaena variabilis* M3

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Biological context

RNA-binding proteins are involved in various forms of RNA metabolism, such as splicing, modification, stability, and translation (Kenan et al., 1991; Birney et al., 1993; Nagai et al., 1995). One of the most well-characterized RNA-binding domains is the RNA-recognition motif (RRM). Nearly 100 proteins having one or more RRM have been found in various eukaryotic organisms (plants, animals, etc). The RRM-type RNA-binding protein gene (rbpA1) in a cyanobacterium, *Anabaena variabilis*, was discovered (Sato, 1994). The expression of this gene is enhanced at low temperature (Sato, 1994). This gene was shown to be a member of a multigene family (Sato, 1995), and the expressed protein (RbpA1) consists of 102 amino acids, and contains a single RRM domain and a glycine-rich C-terminal region. RbpA1 exhibits affinity to poly(U) and poly(G) rather than poly(A) and poly(C) (Sato, 1995), and this binding mode is similar to that of plant RNA-binding proteins from chloroplasts (Ye and Suigiura, 1992) and plant nuclei (Ludevid et al., 1992). In spite of this sequence specificity, the target sequence for RbpA1 in vivo remains unclear. We have proposed that RbpA1 recognizes the structure of double-stranded RNA, which is generated from complementary partial sequences in the RNA at low temperature.

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To examine this proposal and to clarify the functions of the two domains (RRM and glycine-rich C-terminal region), we have studied the solution structure of RbpA1 by NMR spectroscopy. We report here the NMR backbone assignments of RbpA1.

Methods and results

In minimal medium (Miller) containing $^{15}NH_4Cl$ (0.5 g/l) and [$^{13}C_6$]-D-glucose (3 g/l), RbpA1 was expressed in *E. coli* BL21(DE3)/pLysS cells harboring a plasmid containing the complete RbpA1 sequence. The majority of RbpA1 was expressed in the soluble fraction, with a yield of approximately 6 mg/l. The purified RbpA1 was obtained by a two-step protocol on HiTrap-Q strong anion exchange (5 ml; Pharmacia) and HiLoad Superdex75$_{pg}$ gel filtration (26 mm × 60 cm; Pharmacia) columns. N-terminal amino acid sequence analysis of the purified RbpA1 showed that the N-terminal methionine residue was deleted. The size of the purified RbpA1 was determined by TOF-MASS spectroscopy and sedimentation equilibrium analyses, and it was found that RbpA1 is a monomeric 11 kDa protein. A 0.5 mM NMR sample of RbpA1 was prepared in 250 μl of 90% H_2O/10% D_2O or 99% D_2O in the following NMR buffer: 50 mM K_2HPO_4/KH_2PO_4, 50 mM KCl, 1 mM DTT, 1 mM EDTA, pH 6.9. 2D ^{1}H-^{15}N HSQC and 3D HSQC-NOESY (^{1}H-^{15}N and ^{1}H-^{13}C), ^{1}H-^{15}N HSQC-TOCSY, CBCA(CO)NH, HNCACB, and

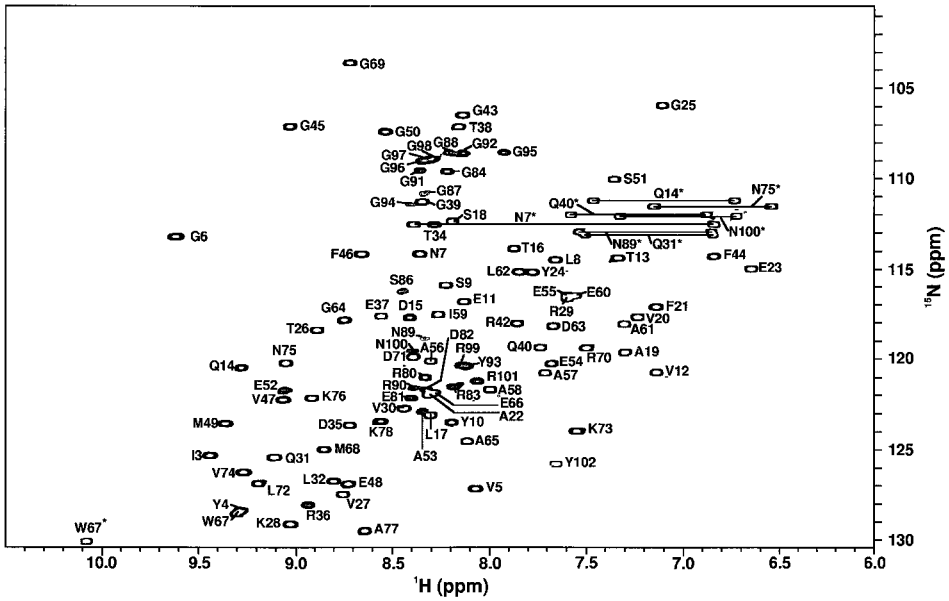

Figure 1. Two-dimensional HSQC spectrum of RbpA1 (0.5 mM, NMR buffer, pH 6.9, 30 °C) collected at a ^{1}H resonance frequency of 500 MHz. To optimize the resolution in the nitrogen dimension, a ^{15}N spectral width of 1500 Hz was used (128 (t_1) × 512 (t_2) complex points; and spectral widths of 1500 and 8000 Hz in F_1 and F_2, respectively). The cross-peak assignments denoted by asterisks are the side-chain resonances of Asn, Gln and Trp.

HCCH-TOCSY data were collected at 30 °C using Bruker DMX 500, DRX 600 and DRX 800 spectrometers. The data were processed using NMRPipe (Delaglio et al., 1995) on SGI Indigo and O2 workstations. The ^{1}H, ^{13}C, and ^{15}N chemical shifts were referenced according to the method of Wishart et al. (1995).

Extent of assignments and data deposition

Figure 1 shows a 2D HSQC spectrum of RbpA1 obtained at a ^{1}H resonance frequency of 500 MHz. The ^{1}HN resonances in Figure 1 were assigned primarily using the HNCACB and CBCA(CO)NH data in conjunction with the amide-to-amide region of the ^{1}H-^{15}N HSQC-NOESY data. Using these assignments, the ^{13}CO and Hα resonances were determined from the results of the HNCO, HCACO and HCCH-TOCSY experiments. All pairs of cross peaks for the side-chain resonances (Asn and Gln) in Figure 1 could be unambiguously assigned.

In total, 96 of 96 possible ^{1}HN resonances (102 residues minus four prolines, one methionine and the terminal amino group) were observed (100%). A list of the chemical shifts has been deposited in the BioMagResBank (accession number 4711). On the basis of these backbone assignments for RbpA1, 3D structure calculation is in progress.

Acknowledgements

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