Journal of Biomolecular NMR, **27:** 89–90, 2003.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of rice telomere binding domain from *Oryza sativa*

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Received 31 January 2003; Accepted 9 April 2003

Key words: RTBP1 (rice telomere binding protein 1), sequence-specific assignments, telomere, telomere binding protein

Biological context

Telomere, the physical end structure of chromosomes, protects the chromosome end from recombination and regulates telomerase activity. It has been proposed that telomeric DNA has a specific repeat sequence for different species. Although telomeric repeat sequences are different among the species, the DNA binding domain of telomere binding proteins has a high sequence identity with a transcriptional activator, c-Myb.

The structural studies have shown that the Myb-like domains of telomere binding proteins all have in common a three-helix bundle motif (Horvath et al., 1998; Ogata et al., 1994). Recently in some plants – *Arabidopsis*, Rice, and Mung bean – telomere binding proteins have been cloned and characterized, showing that DNA binding domains of plant telomere binding proteins also possess a Myb-like domain. In addition, they reported that the binding mode and essential sequence of the DNA binding region are quite different from those of vertebrate. To determine DNA binding mode between vertebrate and plant telomere binding protein, we undertook a structural study of the DNA binding domain of rice telomere binding protein. Since the DNA binding domains of plant telomere binding proteins have high sequence identity, this study would provide the DNA-protein binding mode of plant telomere binding proteins and suggest how telomere binding proteins from different species are related evolutionally.

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Methods and experiments

Protein expression and isotope labeling

The cDNA segment encoding the DNA binding domain of RTBP1 (residues 506-615) was amplified using a primer pair by PCR from the full-length pBS-RTBP1 cDNA (Yu et al., 2000) and cloned into the *Bam*HI-*Sal I* sites of pGEX 4T-1 (Amersham Pharmacia Biotech), an *E. coli* expression vector. Uniformly ^{13}C/^{15}N- and ^{15}N-isotopically labeled protein samples were prepared by growing cells in M9 minimal media containing $^{15}NH_4Cl$, either with $^{13}C_6$-D-glucose or $^{12}C_6$-D-glucose. The protein was concentrated in buffer solution of 50 mM potassium phosphate, 100 mM NaCl at pH 7.0 using a Centricon concentrator (Milipore) and transferred to a 5 mm symmetrical micro cell (Shigemi) for NMR measurement.

NMR spectroscopy

All NMR experiments were performed at 303 K on Bruker DRX500, DRX800 and Varian INOVA 500 MHz equipped with a triple resonance probe head with gradients. All spectra were processed using NMRPipe/NMRDraw software (Delaglio et al., 1995) and analyzed using the program XEASY (Bartels et al., 1995). The backbone resonance assignments were accomplished using the ^{1}H-^{15}N HSQC and HNCO, HNCA, HNCACB and CBCA(CO)NH spectra. The sequential assignment data were finally confirmed by ^{15}N-edited 3D TOCSY-HSQC and NOESY-HSQC spectra as identifying $d_{NN}(i, i+1)$ and $d_{\alpha N}(i, i+1)$ NOEs (Cavanagh et al., 1996). The side chain assignments were completed by HCCH-TOCSY data (Kay et al., 1993). Proton chemical shifts were referenced

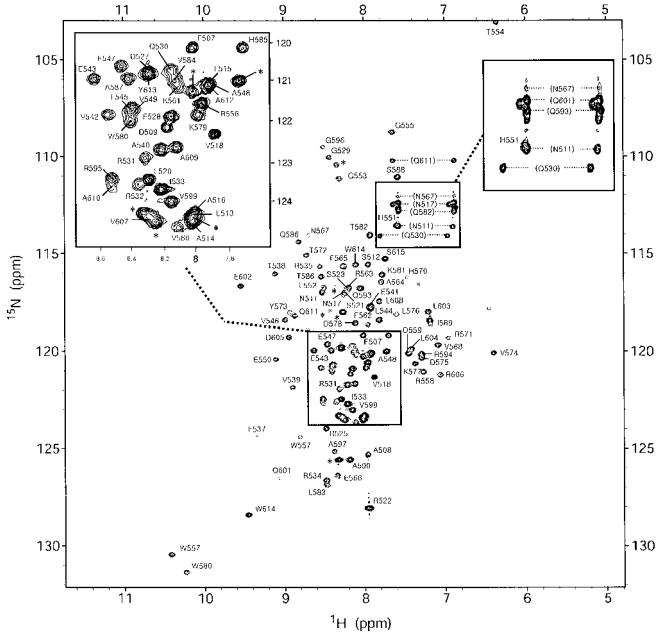

Figure 1. The 2D ^{1}H-^{15}N HSQC spectrum of uniformly ^{15}N-labelled telomere binding domain of RTBP1. * indicates the residues from the *E. coli* expression vector. The cross-peaks connected by dotted lines correspond to side chain NH_2 groups of Gln and Asn residues.

directly to internal DSS, while ^{15}N and ^{13}C shifts were indirectly referenced (Markley et al., 1998).

Extent of assignments and data deposition

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum for the DNA binding domain of RTBP1 with assignments. The secondary structural elements of the rice telomere binding domain of RTBP1 (506-615) based on CSI (Wishart and Sykes, 1994) predicted that the DNA binding domain consisted of four helices. It was further confirmed by $d_{NN}(i, i+1)$ and $d_{\alpha\beta}(i, i+3)$ NOEs from ^{15}N-edited NOESY-HSQC and ^{13}C-edited NOESY-HSQC. For backbone resonance assignment, 95% of ^{13}CO, NH, ^{15}N, C^{α} and $C^{\alpha}H$ were assigned. Excluding the aromatic rings, 92% of side chain ^{1}H and ^{13}C chemical shifts were assigned. Similar to mammalian telomere binding proteins, rice telomere binding protein has the Myb-like domain, too. However, the Myb-like domain alone does not bind to the telomeric DNA even though it has specific telomeric repeat sequence (Yu et al., 2000). The solution structure of the DNA binding domain of RTBP1 would provide key information to elucidate the structural differences between the mammalian and plant telomere binding protein in an evolutionary sense and it is currently in progress. The chemical shifts for ^{1}H, ^{13}C and ^{15}N have been deposited at BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5590.

Acknowledgements

This research was supported by the Korean Science and Engineering Foundation (KOSEF) through the center for Protein Network Research (PNRC) at Yonsei University (W.L. and I.K.C.) and the NRL program of MOST NRDP (M1-0203-00-0020) (W.L.). This work was also supported by grants from JSPS and TARA (T.T.).

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