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Letter to the Editor: Resonance assignments for the N-terminal domain from human RNA-binding protein with multiple splicing (RBP-MS)

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Biological context

Positional cloning studies in search of the Werner's syndrome gene have identified a unique gene for an RNA-binding protein with multiple splicing (RBP-MS) spanning over 230 kB (Shimamoto et al., 1996). The RBP-MS gene produces at least 12 distinct transcripts generated by alternate splicing of 16 identified exons, and thus codes for a family of proteins. The four 5′-proximal exons code for a sequence that is highly homologous (∼67% identical residues) to the RNA-binding domain of *Drosophila* couch potato protein that is involved in peripheral nervous system development (Bellen et al., 1992). This sequence also exhibits ∼22% sequence identity with the RNA-binding domain of the human spliceosomal protein snRNP U1A and contains highly conserved RNA-binding sequences RNP1 and RNP2 (Sahasrabudhe et al., 1998).

The 111-residue N-terminal RNA-binding domain of RBP-MS (RBP-MS) has been expressed in *E. coli*, and purified to homogeneity. Sedimentation equilibrium analysis and gel filtration chromatography reveal that this 12.5 kDa domain forms a trimer (MW ∼ 38 kDa) in solution (Sahasrabudhe et al., in preparation). It is unusual that an RNP-like protein forms a trimeric complex. The trimeric form of this RBP-MS domain exhibits broad NMR line widths and relatively short transverse relaxation times, making it a difficult sample to study using conventional triple resonance NMR methods. To alleviate the faster relaxation of ^{13}C nuclei and improve sensitivity, random partial deuteration and TROSY detection schemes have been used to provide an extensive set of backbone resonance assignments.

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Methods and experiments

E. coli strain BL21(DE3) cell cultures transformed with the pET22b expression vector encoding for RBP-MS, and acclimated for growth in 75% D_2O, were grown at 37 °C in 1× MJ minimal medium in 75% D_2O containing uniformly enriched $^{15}NH_4Cl$ and $^{13}C_6$-glucose as sole nitrogen and carbon sources. The production and purification of this sample will be described elsewhere (Sahasrabudhe et al., in preparation). The purity of the sample (>98%) was determined using SDS-PAGE and MALDI-TOF mass spectrometry. The molecular weight of RBP-MS determined by mass spectrometry, 13,503 Da, corresponds to ∼48% deuterium enrichment assuming 100% ^{13}C and 100% ^{15}N enrichment, as observed in parallel ^{13}C,^{15}N enrichment experiments in 100% H_2O. RBP-MS samples (270 μL) were prepared for NMR experiments at ∼1.8 mM monomer concentration in 95% H_2O/5% D_2O solution containing 50 mM sodium phosphate and 0.5 mM sodium azide at pH 6.0, in Shigemi susceptibility-matched NMR tubes.

All NMR data were collected at 15 °C on a Varian Inova 600 NMR spectrometer equipped with four channels. The programs VNMR (Varian), SPARKY (Goddard and Kneller, University of California, San Francisco) and AUTOASSIGN (Zimmerman et al., 1997) were used for data processing and analysis. Proton chemical shifts were referenced to internal DSS. ^{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 along with peaks from three intraresidue 3D [HNCA, CBCANH, and HN(CA)CO] and three interresidue 3D [HN(CO)CA, CBCA(CO)NH, and HNCO] experiments. These

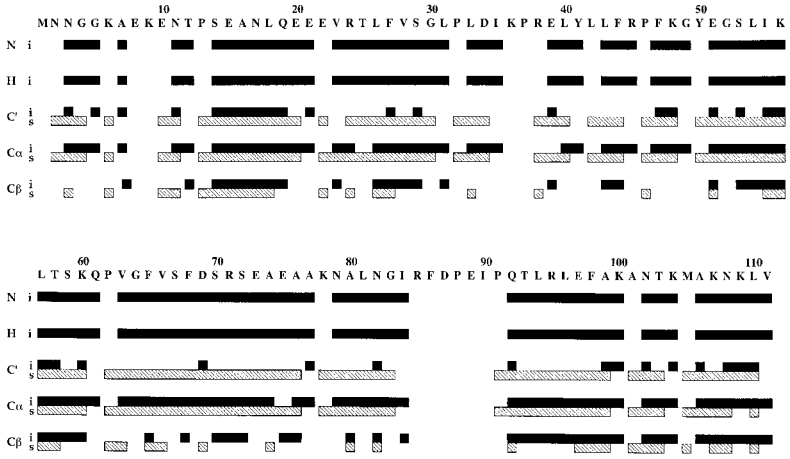

Figure 1. Summary of intraresidue and sequential connectivity data determined by AUTOASSIGN and subsequent manual analysis for RBP-MS. Solid and hatched lines indicate intraresidue (i) and sequential (s) residue connectivities, respectively.

pulse sequences were modified to implement TROSY sensitivity enhancement (Salzmann et al., 1998) and deuterium decoupling of transverse ^{13}C magnetization. 3D HCC(CO)NH-TOCSY, also modified to provide deuterium decoupling and TROSY detection, and 3D ^{15}N-edited NOESY-HSQC experiments were used to manually extend the results obtained from the automated analysis.

Extent of assignments and data deposition

The large size ($\sim$ 38 kDa) of the RBP-MS trimer particle makes it tumble slowly, resulting in significant line broadening and deterioration of 3D triple resonance spectral quality. Although TROSY implementation in the pulse sequences and partial ^{2}H enrichment of protein improved the quality of the spectra significantly, the number of peaks observed in the CBCANH and CBCA(CO)NH spectra was less than expected for a protein with 111 amino acids. The AUTOASSIGN program generally provides nearly complete backbone resonance assignments when good quality triple resonance data are available (Zimmerman et al., 1997). However, as a result of the peak overlaps and absence of many required peaks, only $\sim$40% of the backbone resonances of RBP-MS were assigned by AUTOASSIGN analysis. Subsequent manual analysis of these same triple resonance NMR data along with ^{2}H-decoupled 3D HCC(CO)NH-TOCSY and 3D ^{15}N-edited NOESY-HSQC data confirmed the assignments made by AUTOASSIGN and also increased the total of the assigned atoms to $\sim$85% of the assignable backbone atoms (85/103 ^{15}N-^{1}H^{N} sites, 92/111 C′, and 100/111 C$^{\alpha}$). Many side chain ^{13}C assignments were also obtained (70/104 C$^{\beta}$, 15/97 C$^{\gamma}$, 11/87 C$^{\delta}$, and 3/31 C$^{\varepsilon}$). Figure 1 shows the summary of intraresidue and sequential connectivity data determined by AUTOASSIGN and subsequent manual analysis. Backbone resonance assignments were obtained for most residues. Polypeptide segments Glu8–Glu10, Lys36–Arg38, and Arg85–Pro91 that could not be assigned are in surface loops or uncharacterized regions in the homology modeled structure (Sahasrabudhe et al., 1998) of this domain. These ^{1}H, ^{13}C, ^{15}N chemical shift data have been deposited in BioMagResBank (http://www.bmrb.wisc.edu; accession number 4772).

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