Journal of Biomolecular NMR, **22:** 387–388, 2002.
KLUWER/ESCOM

Letter to the Editor: Resonance assignment and secondary structure determination of a C-terminal fragment of the Lupus Autoantigen (La) protein containing a putative RNA recognition motif (RRM)

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Received 3 January 2002; Accepted 6 February 2002

Key words: Lupus Autoantigen protein, RNA binding, RRM, translation

Biological context

The Lupus Autoantigen (La) protein is a highly abundant and conserved nuclear phosphoprotein that binds to poly(U)-rich elements in RNA polymerase (Pol) III transcripts as well as several other cellular and viral RNAs (reviewed in Maraia and Intine, 2001). By binding to the common UUU-OH 3′ terminal sequence, La appears to stabilise these precursors against 3′-exonucleolytic digestion, ensuring that they are correctly folded, processed and assembled into functional RNA-protein complexes. Additional roles for La proteins, such as modulation of 5′ processing of tRNA precursors and involvement in Pol III transcription processes are still controversial (Maraia and Intine, 2001). La also appears to be recruited in regulation of viral and cellular mRNA translation, but its function in these processes is largely unknown (Svitkin et al., 1994; Maraia and Intine, 2001).

All the proposed functions of La require the recognition of, and binding to, cellular and viral RNA sequences. La belongs to a large family of RNA binding proteins, characterised by the possession of an 80–90 amino acid module, known as the RNA recognition motif (RRM), whose hallmark is the presence of two short but quite conserved sequence motifs. Human La has been predicted to possess three RRM motifs, two of which (RRM-1 and -3) are quite atypical (Kenan, 1991). The two putative RRMs in the N-terminal region of La appear to contain the determinants for polyU RNA binding, whereas the function of the third putative RRM, apparently acquired by the metazoan La proteins, is still unclear. It has been suggested that the C-terminal domain is involved in the interaction with the 5′-UTR of some cellular mRNAs and in the recognition of the 5′-ppp end of nascent RNA with a mechanism modulated by phosphorylation (Maraia and Intine, 2001).

The structure investigation of human La would provide new insights into its mechanisms of action in nuclear RNA processing and maturation, along with viral translation stimulation. We therefore have undertaken NMR-based structural studies of a C-terminal fragment of hLa, identified by limited proteolysis, containing the putative third RRM domain and a putative Nuclear Localisation Signal (Maraia and Intine, 2001). Herein we report an essentially complete backbone and side chain assignment, and the delineation of the secondary structure elements.

Methods and experiments

A C-terminal fragment of human La spanning residues 225–334, which contains the putative third RRM domain (residue 225–300) and a putative Nuclear localisation signal (NLS, residue 316 to 332) was obtained by proteolysis of La(225–408) sub-cloned by PCR into expression vector Qiagen pQE9, using BamH1 and HindIII restriction sites. The hexahistidine-tagged recombinant protein was over-expressed in *E. coli* host strain M15 (Qiagen) grown on minimal media containing 0.8 g l^{-1} ^{15}N-ammonium chloride and 2 g l^{-1}

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^{13}C-glucose and purified by affinity chromatography in a single step on a Ni-NTA resin (Qiagen). The sample was extensively dialysed against a buffer containing 20 mM Tris-HCl, 100 mM KCl, 1 mM DTT at pH 7, and then concentrated to 1 mM in 700 µl.

NMR experiments were acquired at 300 K on Varian Inova spectrometers operating at 14.1 and 18.8 T. CBCANH, CBCA(CO)NH, HNCO and HNCA experiments were employed to obtain sequence specific ^{1}HN, ^{15}N, $^{13}C_{\alpha}$, $^{13}C_{\beta}$ and $^{13}C'$ backbone assignments (Sattler et al., 1999; Bax and Grzesiek, 1993). H_{α} and H_{β} assignments, together with proton and carbon side chain assignments were achieved using a combination of ^{15}N-edited TOCSY-HSQC and NOESY-HSQC (Fesik and Zuiderweg, 1988), HCCH-TOCSY and HNHA spectra.

The HNHA experiment allowed the measurement of $^{3}J_{HN\alpha}$ coupling constants; values for ϕ and ψ dihedral backbone angles were obtained using the program TALOS (Cornilescu et al., 1999). All spectra were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analysed using XEASY (Bartels et al., 1995).

Extent of assignment, secondary structure and data deposition

The NMR assignment of ^{1}HN, ^{15}N, $^{13}C_{\alpha}$, $^{13}C_{\beta}$, $^{13}C'$ and H_{α} nuclei of La(225–334), i.e., excluding the hexahistidine tag, is 98% complete. The resonances of residues around Cys245 are quite weak and attenuation of these signals could be caused by internal mobility of these regions on a slow or intermediate timescale. The C-terminal residues appear to be highly flexible (data not shown). The side chain proton and carbon assignment is approximately 90% complete.

The backbone chemical shift data together with $^{3}J_{HN\alpha}$ coupling constants, the NOE pattern from the ^{15}N-edited NOESY-HSQC and the results from TALOS prediction were used to identify secondary structure elements (Figure 1). These data clearly show the presence of a $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$ fold, which is characteristic of RRM modules, in the predicted region spanning residues 225–300 (Kenan, 1991). However, additional secondary structure elements are also present, in particular a β-sheet type (β_5, 299–307) and a long α-helix (α_3, 309–329). This observation is particularly intriguing, as preliminary data show that all these secondary structure elements pack together to form a single well-structured domain, suggesting that the structure of La(225–334) differs somewhat from the archetypal

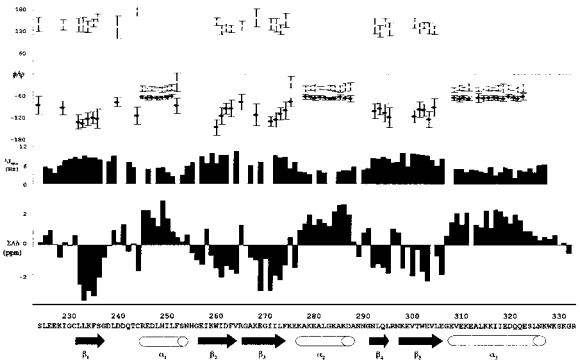

Figure 1. Plot of the chemical shift deviation from random coil, $^{3}J_{HN\alpha}$ coupling constants and Talos derived ϕ/ψ predictions for La(225–334). The chemical shift deviation from random coil was obtained by subtracting the experimental values from the random coil chemical shifts and then deriving a weighted consensus value ($\Sigma\Delta\delta$) using ($4\Delta\delta_{H\alpha} + \Delta\delta_{C\beta} - \Delta\delta_{C\alpha} - \Delta\delta_{C'}$) / number of assignments. ϕ and ψ values are denoted by black circles and grey squares respectively, for residues where all 10 database matches agree. The amino acid sequence is shown with the conserved regions of the RRM domain underlined (RNP-2 and RNP-1 from the N-terminus). The secondary structure features are also indicated.

RRM fold. Interestingly, the non-canonical RRM3 domain of the polypyrimidine tract binding protein (PTB) also revealed novel structural features (Conte et al., 2000).

The chemical shifts for backbone and side chain assignments have been deposited in the BioMagResBank (accession number 5235).

Acknowledgements

The authors are indebted for financial support to the Wellcome Trust. We are also grateful to Drs J. Keene and D. Kenan for the gift of human La cDNA.

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