Journal of Biomolecular NMR, **27:** 93–94, 2003.
KLUWER/ESCOM

Letter to the Editor: Resonance assignment and secondary structure of an N-terminal fragment of the human La protein

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Received 18 March 2003; Accepted 14 April 2003

Key words: La antigen, NMR assignment, RRM

Biological context

The La protein is an abundant RNA-binding phosphoprotein originally described as an autoantigen in systemic lupus erythematosus and Sjogren's syndrome patients. Ubiquitous in eukaryotic cells, La localises predominantly in the nucleus where it associates with the UUU-OH 3′ terminus of nascent RNA polymerase III transcripts, stabilising them against exonucleolytic digestion and ensuring the correct maturation process (Maraia and Intine, 2001; Wolin and Cedervall, 2002). High affinity poly (U) recognition resides in the N-terminal domain (NTD) of human La (hLa), highly conserved from vertebrates to yeasts, whereas hLa C-terminal domain has been shown to play a key role in RNA nuclear retention and 5′-ppp-RNA recognition. Interestingly, La also associates with a large number of viral and cellular mRNAs which do not in fact end in or contain a 3′ poly (U), and appears to be recruited (at least in some cases) in regulation of mRNA translation, but its function and mode of action remain elusive (Maraia and Intine, 2001; Wolin and Cedervall, 2002).

The NTDs of La proteins are predicted to contain two RNA recognition motifs (RRM) that are both critical for poly (U) binding; however, some authors contest that the first domain, called the 'La-motif', folds into a predominantly helical structure (Wolin and Cedervall, 2002). Here we report the essentially complete assignment for the RRM2 of hLa and delineate its secondary structure. Insights into the structure of the NTD will help the understanding of RNA recognition and mechanisms of action for La proteins.

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Methods and experiments

An N-terminal fragment of human La encompassing the putative RRM2 domain was subcloned by PCR into expression vector JM28 using BamH1 and Xho1 restriction sites. JM28 is a modified pET-28a vector (Novagen) in which the Nco1-BamH1 region has been replaced with a sequence encoding the peptide MGHHHHHHIEGRWIL. The hexahistidine-tagged recombinant protein was overexpressed in BL21(DE3) pLysS *E. Coli* cells grown minimal media containing 0.8 g l^{-1} ^{15}N-ammonium chloride and 2 g l^{-1} ^{13}C glucose. Cell pellets were lysed in 20 mM Tris-HCl, 300 mM NaCl, 10 mM Imidazole, pH 8 and purified by affinity chromatography on a Ni-NTA resin (Qiagen), using the manifacturer's protocol. The protein eluted was dialysed in 50 mM Tris-HCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 10% (v/v) glycerol pH 7.25 (buffer A), loaded on a 5 ml Hi-Trap Heparin column (Amersham-Pharmacia Biotech) and eluted with a linear 0–2.0 M KCl gradient in buffer A. NMR samples were prepared dialysing the purified protein against a buffer containing 20 mM sodium phosphate, 100 mM KCl, pH 6 and then concentrated to 0.6 mM in 700 μl. NMR spectra were recorded at 293 K on Varian Inova spectrometers operating at 14.1 and 18.8 T. HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNCO experiments were collected to obtain sequence specific assignments for the backbone (Bax and

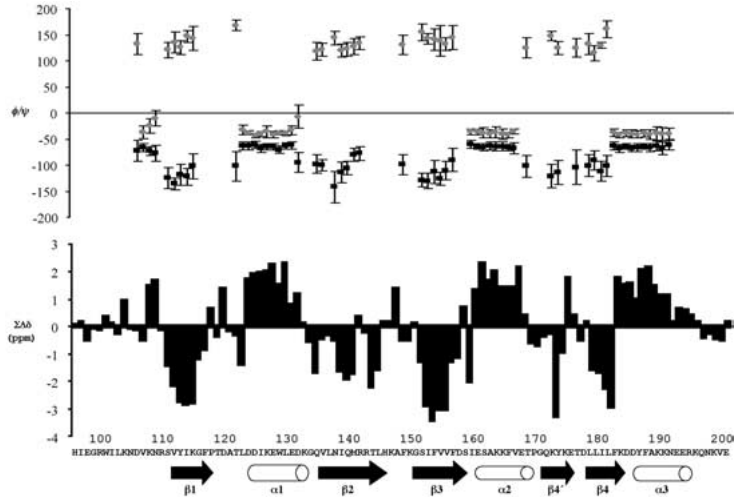

Figure 1. Plot of the chemical shift deviation from random coil and Talos derived ϕ/ψ predictions for La RRM2. The chemical shift deviation from random coil was obtained by subtracting the experimental values from the random coil chemical shifts and then deriving a weighted consensus value ($\Sigma\Delta\delta$) 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 9 or 10 database matches agree. The secondary structure features are also indicated.

Grzesiek, 1993). Side chains resonances were assigned by combining data from the following experiments: ^{15}N-edited TOCSY-HSQC and NOESY-HSQC (Fesik and Zuiderweg, 1988), HCCH-TOCSY and ^{13}C-edited NOESY-HSQC.

TALOS software was employed to predict backbone dihedral angles (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

Spectral analysis allowed the identification and sequential assignment for 106 residues out of the 113 of La RRM2 (i.e., not including the 7 N-terminal amino acid residues of the His-tag). For these, completeness levels of 98% for $^{1}H_N$, 97% for ^{15}N (excluding two Proline residues), 100% for $^{13}C_\alpha$, 96% for $^{13}C_\beta$, 93% for $^{13}C'$ and 98% for $^{1}H_\alpha$ were achieved. Side chain assignments are also almost complete. Gaps in the assignment for aliphatic side chains occur for K116, M142, R144, K151, S153 and K165, whereas of the 12 aromatic residues 74% of the ^{1}H and ^{13}C resonances have been unambiguously assigned.

Secondary structure elements were determined by the analysis of the chemical shifts of backbone atoms and $^{13}C_\beta$, by characteristic NOE patterns from the ^{15}N-edited NOESY-HSQC and by the results from TALOS software (Figure 1). These data clearly show the non-canonical nature of this RRM module, with an extra β-strand (β4′) and α-helix (α3) in addition to the archetypal βαββαβ RRM fold. Surprisingly, a remarkably similar topology has already been found in the C-terminal RRM (RRM3) of hLa protein (Jacks et al., 2002).

The chemical shifts for backbone and side chain assignments have been deposited in the BioMagResBank (accession number BRMB-7392).

Acknowledgements

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

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