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Letter to the Editor: Resonance assignment and topology of the ^{2}H, ^{13}C, ^{15}N labelled 29 kDa N-terminal fragment of the polypyrimidine tract binding protein (PTB)

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Biological context

The polypyrimidine tract binding protein (PTB) is a key regulator of alternative splicing of pre-mRNA, a process that affects the majority of mammalian genes. Interest in PTB has intensified in recent years as investigators have revealed an ever-growing number of genes whose splicing is affected by the protein (reviewed in Smith and Valcarcel, 2000; Valcárcel and Gebauer, 1997). PTB has also been firmly established as an important stimulator of the process of translation initiation directed by the internal ribosome entry sites (IRES) of most picornaviruses (Valcárcel and Gebauer, 1997). PTB is thus a central player in several nuclear and cytoplasmic RNA manipulations. The protein is a 114 kDa homodimer containing four ribonucleoprotein consensus sequence (RNP-CS) domains (also known as RNA recognition motifs - RRMs) per monomer. Human PTB exists as three known isoforms (PTB1, PTB2, PTB4) and there are several closely-related homologues.

We have already solved the structure of the C-terminal fragment of PTB1 (PTB1-34) and characterised its binding to intron RNA sequence using chemical shift mapping studies (Yuan et al., 2002). We now aim to complete our analysis of the PTB monomer by determining the structure of the N-terminal fragment PTB1-12, which contains RRMs 1 and 2 (MW = 28.7 kDa). Although these RRMs do not appear to interact strongly with RNA in UV-crosslingking assays (Pérez et al., 1997; Oh et al., 1998), other experimental approaches suggest they make and important contribution to RNA binding (Kaminski et al., 1995). In addition, RRM2 is believed to be an important determinant of PTB dimerisation (Pérez et al., 1997).

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Methods

A 250-residue N-terminal fragment of human PTB (Gil et al., 1991), which contains the first and second RNP-CS domains (residues 51–301; designated PTB1-12) was sub-cloned by PCR into expression vector pQE9 (Qiagen) using engineered BamHI and HindIII sites which adds an N-terminal poly-Histidine tag (MRGSHHHHHHGS). The protein was over-expressed in *E.coli* host strain SG13009 grown on D_2O minimal media containing 0.7 g/l ^{15}N-ammonium chloride and 2 g/l ^{13}C-glucose and purified on TALON beads (Clontech) and gel-filtration using a Sephadex 75 10/30 column (Pharmacia). For NMR analysis the purified PTB protein was concentrated to ca. 0.5 mM in 300 µl of 50 mM, pH 6.0 sodium phosphate buffer containing 150 mM NaCl, 10 mM DTT and 0.1 mM PMSF.

The temperature was maintained at 298 K throughout the NMR experiments. The following spectra were recorded for assignment of the backbone and $^{13}C_\beta$ resonances: d-HNCA, d-HN(CO)CA (Yamazaki et al., 1994a, b), HNCO, d-HN(CA)CO (Matsuo et al., 1996a, b), d-HN(CA)CB (Yamazaki et al., 1994b) and a CBCA(CO)NH 'straight-through' experiment (Grzesiek and Bax, 1992) which used the $^{13}C_\alpha$, $^{13}C_\beta$ steady-state magnetisation. A 3D ^{15}N-separated NOESY spectrum using a mixing time of 400 ms was also recorded to help in the elucidation of secondary structure.

Extent of assignments and topology

Sequence-specific assignments were made primarily *via* the HNCA/HN(CO)CA pair, using the carbonyl

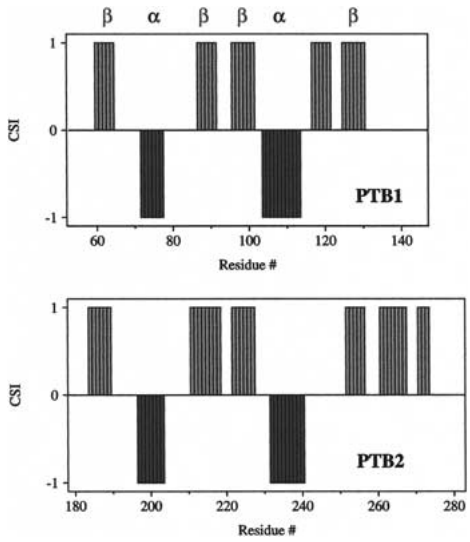

Figure 1. CSI consensus plot (Wishart and Sykes, 1994) of PTB1-12 generated using $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ chemical shifts. Chemical shifts were adjusted for deuterium isotope effects (Gardner and Kay, 1998).

experiments to resolve any ambiguities arising from degeneracy in the $^{13}C_\alpha$ frequency. Using this strategy, backbone assignments could be made for the majority of residues from the structured regions of PTB1-12 and these were confirmed using the HN(CA)CB. One exception was the first β strand from RRM2 (see below); a number of residues from this region had severely attenuated amide signals and as a consequence were barely distinguishable from the noise in the 3-dimensional experiments. The assignment of this region could only be completed by analysis of the 3D NOESY spectrum where a number of cross-strand H^N-H^N NOEs were identified to residues within the β strand. Attenuation of these signals could be caused by local conformational exchange processes on an intermediate timescale or by incomplete amide reprotonation.

Assignments could not be made for the majority of residues within the extensive inter-domain linker sequence (residues 143–184 approximately) and also at the N- and C-termini. ^{15}N-relaxation data confirm that these regions are highly flexible (data not shown) and are likely to be unstructured. The NMR assignment of ^{1}HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ nuclei of the RNP-CS domains in PTB1-12 is approximately 95% complete. The chemical shift data were used to identify secondary structure elements (Figure 1). These data clearly show the presence of two domains possessing the βαββαβ fold which is characteristic of RRM domains. In addition, further regions of β sheet-type secondary structure are also found in domain 1 as an inserted strand after the second helix of the βαββαβ motif (residues 117–121 inclusive) and within the C-terminal extension to domain 2 (residues 260–267 and 271–273; Figure 1). The strand comprising residues 271–273 is at an equivalent position to the additional strand which has been previously reported for RRM3 of PTB (Conte et al., 2000), whilst the extra segment of β structure around residues 260–267 appears to be an unprecedented feature of RRM motifs unique to this domain.

A table of the assignments (including ^{1}HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$) is available as supplementary material and has been deposited in the BioMagResBank in Madison, WI, U.S.A. (accession code 5409).

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