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Letter to the Editor: Backbone NMR assignment of the 19 kDa translationally controlled tumor-associated protein p23^{fyp} from *Schizosaccharomyces pombe*

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Biological context

The translationally controlled tumor-associated proteins (TCTP; also known as p23, p21 or histamine releasing factor, HRF) are a family of proteins of ca. 19 kDa that are highly conserved across a wide range of eukaryotes, and found abundantly in many different cell types (Sanchez et al., 1997). The sequence conservation is high and no significant sequence homology to any other protein family has been reported. Two consensus sequences for the TCTP family have been identified in SWISSPROT (entry PDOC00768) but no structural information has been reported for the family. Despite the apparent ubiquity of the protein, there are conflicting reports of its function. It has variously been identified as an extracellular histamine releasing protein (Macdonald et al., 1995); a cytoplasmic microtubule associated protein (Gachet et al., 1997); a protein upregulated in earthworms in response to heavy metal pollution (Sturzenbaum et al., 1998); and a calcium binding protein (Sanchez et al., 1997). Therapeutically, it is a potential target of the antimalarial drug artemesinin (Bhisutthibhan et al., 1998). We report here the sequential backbone assignment of the TCTP protein from *Schizosaccharomyces pombe*, termed p23^{fyp}. This yields the intriguing result that one of the consensus sequences for the TCTP family is a highly disordered and mobile segment of ca. 20 amino acids. Determining the interactions that are capable of structuring this loop will be key to understanding the function and regulation of this family of proteins.

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Methods and results

p23^{fyp} was expressed in *E. coli* strain BL21(DE3) transformed with the plasmid pET22b containing the p23^{fyp} cDNA. Uniformly labelled samples were produced in M9 minimal medium with $^{13}C_6$-glucose and $^{15}(NH_4)_2SO_4$ as the sole carbon and nitrogen sources. The protein was purified by a combination of ion exchange, hydrophobic affinity and gel filtration chromatography. Protein samples for NMR spectroscopy (ca. 1 mM, 600 μl in 5-mm NMR tubes) contained 20 mM sodium acetate, 250 mM NaCl, 10% ^2H_2O, 5 mM NaN_3 and 1 mM PMSF, pH 5.1. Spectra were acquired at 500 or 600 MHz on Bruker DRX-series spectrometers at 308 K. Spectra used for the sequence specific assignment were ^{15}N-HSQC; HNCA, HNCO, HN(CA)CO (Ikura et al., 1990; Clubb et al., 1992); CBCA(CO)NH, HBHA(CBCACO)NH (Grzesiek and Bax, 1993) and HNCACB (Wittekind and Mueller, 1993). H^{α} assignments for residues preceding proline were obtained from a 30-ms mixing time ^{15}N-edited TOCSY-HSQC spectrum (Cavanagh et al., 1995). All spectra were processed, viewed, and analysed using FELIX97 (Molecular Simulations Inc.). Data reduction was achieved using manual peak picking within FELIX97, where all cross peaks were correlated with spin systems identified in high resolution HSQC spectra (Figure 1). These data were exported and further processed using in-house *awk* scripts to check data in-

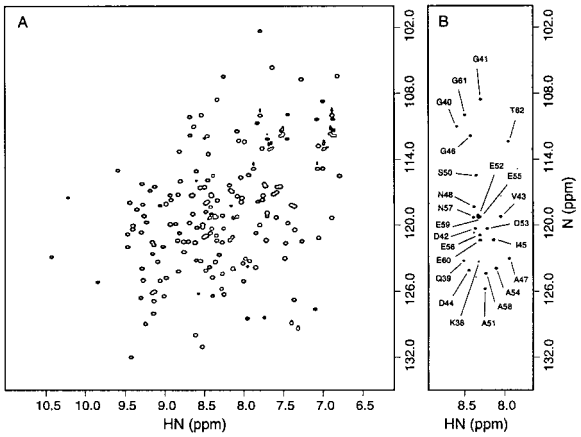

Figure 1. ^{1}H-^{15}N HSQC spectrum of p23^{fyp}. (A) HSQC spectrum of p23^{fyp} recorded at 308 K showing well-resolved ^{1}H-^{15}N cross peaks. (B) Central region of the HSQC spectrum plotted at a high contour level, highlighting the significant number of intense cross peaks at essentially random coil shifts, arising from the mobile loop of p23^{fyp}. All cross peaks (apart from P49) are present for the sequence K38 to T62. The mobile loop matches closely to the TCTP1 consensus sequence of the TCTP family, which corresponds to residues I45–E60 in p23^{fyp}.

tegrity and to reformat the data for the *asstools* suite of Monte Carlo-based assignment tools (in-house program of Leicester University Biological NMR centre), with which the sequential assignment was performed. Whilst C^{β}_{i-1} data was used for residue type information within the assignment procedure, sequential matching of C^{β} data was reserved as a cross-check of the final assignment.

Extent of assignments and data deposition

Complete assignment of the backbone nuclei (HN, non-proline N, C^{α}, H^{α}, C′) was achieved, along with assignment of all the C^{β} resonances. The assignment accounts for all but four of the main-chain amide cross peaks in the HSQC spectrum. These remaining four cross peaks correspond to duplication of the peaks for residues A47, N48, S50 and A51 arising from *cis-trans* isomerisation of the peptide bond between N48 and P49 (within the mobile loop). From peak intensities, the population of the minor conformer is estimated at ca. 7%. The fact that all the cross peaks in the HSQC spectrum could be accounted for, including the low population of peaks arising from *cis*-proline, confirms the high purity of the sample as visible by NMR and eliminates the possibility that the signals from mobile residues (Figure 1B) arise from a population of proteolytically truncated molecules. The chemical shift data have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4447.

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