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Letter to the Editor: Sequence-specific chemical shift assignment and chemical shift indexing of murine apo-Mts1

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Biological context

Members of the S100 family of calcium-binding proteins have been linked to a wide variety of cellular functions, including cell growth, differentiation, cytoskeletal dynamics, inflammation and calcium homeostasis. S100A4, also known as Mts1, has been linked to metastatic capabilities of cells (Ebralidze *et al.*, 1989; Davies *et al.*, 1993; Grigorian *et al.*, 1993; for reviews see Barraclough, 1998; Sherbet and Lakshmi, 1998) through several lines of evidence including: (1) Mts1 RNA is transcribed at detectable levels only in metastatic (and blood) cancers, and not in non-metastatic cancers; (2) introduction of Mts1 into non-metastatic cell lines derived from mammary tumors confers the metastatic phenotype; (3) cross breeding of mice prone to non-metastatic tumors with Mts1 transgenic mice results in offspring with substantially increased aggressiveness of tumors; (4) use of antisense ribozyme techniques to reduce available Mts1 RNA levels diminishes metastatic character of tumor cells. As with other S100 proteins, calcium binding is believed to trigger a conformational switch which is critical for Mts1 function. Here, we present the sequential heteronuclear chemical shift assignments of Mts1 in its calcium free form.

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Methods and experiments

Recombinant mouse Mts1 was expressed in *Escherichia coli* strain BL21(DE3) CodonPlus (Novagen) transformed with the plasmid H-MBP-3C containing the murine Mts1 gene preceded by a 6-histidine tag, maltose-binding protein (MBP), and a 3C protease cleavage site. This plasmid, modified from pMal-c2X (New England Biolabs) has been described previously (Alexandrov *et al.*, 2001). The correct DNA insert was verified by sequencing. The final protein product contains the full 101 amino acids of murine Mts1 plus 5 additional *N*-terminal residues (GPGSA), which we have numbered -5 to -1 (there is no residue zero). To produce ^{15}N-labeled protein, cells were grown in M9 minimal media using $^{15}NH_4Cl$ (Cambridge Isotope Labs, Andover, MA) as the sole nitrogen source , and double-labeled samples also used uniform ^{13}C-glucose (2 g l^{-1}) as the sole carbon source. Cells were lysed by passing three times through a French pressure cell, centrifuged at $20{,}000 \times g$ for 40 min, and the cleared cell supernatant was incubated for 3 h with 5 ml Talon cobalt affinity resin (Clontech, Palo Alto, CA) per liter of growth. After extensive washing of the Talon resin with PBS at pH 7.5, separation of the his-MBP tag from full length Mts1 was achieved by adding 50 μg of 3C protease (grown and purified as described in Alexandrov *et al.*, 2001) to the resin-bound fusion protein and incubating for 12 h at 4 °C. After elution of cleaved Mts1, further purification was achieved by anion exchange chromatography (Bio-Rad biologic workstation) on a Hi-Trap Q Sepharose column (Pharmacia Biotech) yielding $> 95\%$ pure protein. The sample was exchanged

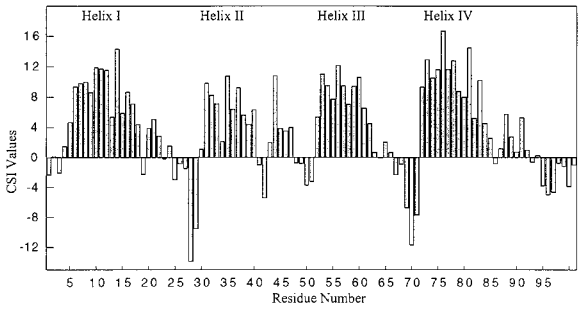

Figure 1. Secondary structure prediction of murine Mts1 based on chemical shift data.

into buffer containing 5 mM Tris-HCl, 1 mM EDTA, pH 6.0, concentrated to 1.5 mM, and supplemented with 10 mM d_{12}-dithiothreitol, 50 μM NaN_3 and 5% (v/v) D_2O. All NMR experiments were performed at 40 °C on a Varian INOVA 600 MHz spectrometer equipped with four rf channels and a triple-axis pulsed field gradient probe. Data were processed with VNMR (Varian) and nmrPipe (Delaglio *et al.*, 1995), and analyzed with and NMRview (Johnson and Blevins, 1994). Backbone chemical shifts were assigned principally through analysis of ^1H,^{15}N-HSQC, HNCO, HNCACB, CBCA(CO)NH and HBHA(CO)NH experiments. Residue types were confirmed and side chain resonances were assigned via analysis of C(CO)NH, H(CO)NH and HCCH-TOCSY spectra.

Chemical shift indexing was performed with Cα, Cβ and C-carbonyl chemical shifts to obtain an estimate of secondary structure (Wishart and Sykes, 1994). The four observed helices (I, L5-K18; II, K31-R40; III, E52-L62; IV, F72-M85) are consistent with the architecture of other S100 proteins, with the possible addition of approximately one helical turn to the *N*-terminus of helix I and to the C-terminus of helix IV (Figure 1). The data also suggest that separate helical structures may be present between helix I and II (S20-K22) and between helix II and III (S44-G47), with additional helical tendencies C-terminal to helix IV (N87-G92). Beta-like structure is indicated immediately N-terminal to helix II (D25-L29), helix III (K48-D51) and helix IV (D67-D71), with an additional beta-like stretch at the extreme C-terminus (D95-K101). Chemical shifts and the secondary structure of human Mts1 have recently been reported (Rustandi *et al.*, 2001). For human Mts1, the resonances and corresponding secondary structure assignment of ten residues, including four from the second putative calcium-binding loop, were unobservable due to exchange broadening.

Extent of assignments and data deposition

With the exception of the carbonyl carbons immediately preceding proline residues and the extreme C-terminal carbonyl carbon, 100% of the backbone resonances (HN, N, Cα, Cβ, C-carbonyl) were assigned. In total, 1011 chemical shifts are reported, representing approximately 88% of all resonances, and 95% of resonances excluding aromatic/guanidino/imidazole group side chains. Chemical shifts have been deposited in the BioMagResBank Database (accession code BMRB-5127).

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