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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N NMR sequence-specific resonance assignments for human apo-Mts1 (S100A4)

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Biological context

Mts1, also known as S100A4, is expressed in a wide range of normal tissues (Zimmer et al., 1995). Elevated levels of Mts1 expression correlate with the metastatic potential of tumor cells (Ebralidze et al., 1989), and protein levels are higher in malignant versus benign human breast tumors (Nikitenko et al., 2000), which correlates strongly with a decline in patient survival (Rudland et al., 2000). Mts1 is a member of the S100 protein family, which is a group of acidic, highly soluble dimeric Ca^{2+}-binding proteins with molecular masses ranging from 10 to 12 kDa per subunit. Consistent with its proposed role in regulating metastasis-associated motility, dimeric Mts1 displays Ca^{2+}-dependent interactions with non-muscle myosin II and tropomyosin (Watanabe et al., 1993; Takenaga et al., 1994).

Sequence analysis indicates that Mts1 has two EF-hand Ca^{2+}-binding domains in each monomer and is tightly regulated by intracellular calcium concentrations (Donato, 1999). To investigate the functional and cellular activities of Mts1, we have initiated a 3D-structure determination in solution by NMR spectroscopy. Here we report the ^{1}H, ^{13}C, and ^{15}N sequence-specific resonance assignments of apo-Mts1.

Methods and experiments

The cDNA for mts1 was subcloned into an expression vector (pET3a; Novagen) and transformed into *E. coli* BL21(de3) cells. Mts1 was isolated using phenyl sepharose and Fast Q anion exchange chromatography. The purified protein contained only Mts1 (>99%) based on SDS-PAGE and is fully active in myosin-II binding assays; however, further characterization using mass spectrometry showed that a majority of the protein (>95%) lacked the N-terminal Met residue, while in a minor component (<5%) the Met residue was intact. This slight amount of heterogeneity did not affect the NMR assignments because resonances from the first three residues of this protein (M1-C3) are missing.

For NMR spectroscopy, uniformly ^{15}N- and ^{13}C,^{15}N-labeled Mts1 was prepared and dialyzed against 0.1 mM Tris-d_{11} pH 7.5, 0.1 mM DTT, lyophilized, dissolved in a small aliquot of ddH_2O, and stored at −80 °C. The final NMR sample contained 2–3 mM Mts1 monomer concentration, 0.34 mM NaN_3, 8 mM NaCl, 16 mM Tris-d_{11}, 5 mM DTT, 0.2 mM EDTA, 2 mM EGTA, and 7% D_2O. The pH was adjusted with cold HCl to 6.5. All solutions were passed through a Chelex-100 column to remove any trace metals.

All NMR experiments were carried out at 37 °C with a Bruker DMX600 NMR spectrometer (600.13 MHz for protons) equipped with a pulse-field gradient unit, four frequency channels, and a triple resonance three-axis gradient probe. For backbone and side-chain sequential assignments, the following heteronuclear NMR experiments were performed: 2D ^{1}H,^{15}N-HSQC, 3D ^{15}N-edited HOHAHA-HSQC, 3D CBCA(CO)NH, 3D HNCACB and 3D C(CO)NH. NOE-based data from the 3D ^{15}N-edited NOESY-HSQC, 3D ^{15}N, ^{15}N-edited HMQC-NOESY-HSQC, 4D ^{13}C,^{15}N-edited NOESY-HSQC and 4D ^{13}C,^{13}C-edited NOESY-HSQC was also collected and used to confirm the resonance assignments.

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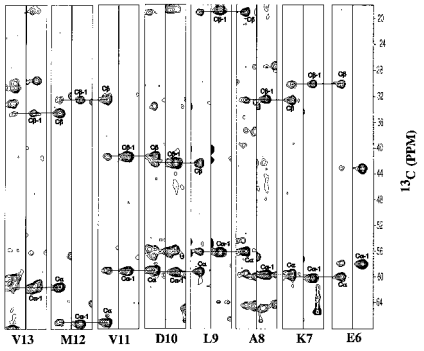

Figure 1. Assignment of main-chain resonances using strips derived from 3D CBCA(CO)NH and 3D HNCACB spectra of residues E6 through V13 of helix 1 of Mts1. Plots are provided as pairs of strips from CBCA(CO)NH spectra (on the right) and HNCACB spectra (on the left) for the given residues. CBCA(CO)NH strips indicate $C\alpha^{-1}$ and $C\beta^{-1}$ chemical shifts and HNCACB strips indicate Cα, Cβ, $C\alpha^{-1}$, and $C\beta^{-1}$ chemical shifts.

Mts1 is a 101-residue protein, and the 2D ^{1}H,^{15}N HSQC spectrum has 87 out of the 97 expected correlations (four proline residues) with 10 missing correlations being from M1–C3, D51, N65–N68, E88, and D95. Titrations of EDTA and/or EGTA to high concentrations (>50 mM) did not change the 2D HSQC spectrum, nor did any of the missing correlations appear. It is therefore very unlikely that any of these correlations are missing due to small amounts of contaminating metal ion; it is more likely that they are in regions of the protein that have significant exchange broadening effects due to local dynamic properties of the protein in the apo-state. The HSQC spectrum also shows all eight pairs of side-chain amide proton correlations belonging to glutamine and asparagine residues. Finally, the C-terminal residue, K101, and the side-chain amide correlations of N87 show multiple correlations, indicative of multiple electronic environments for these nuclei. The 3D CBCA(CO)NH and HNCACB experiments were used to sequentially assign the Cα and Cβ resonances. Typical data from these experiments are illustrated for the assignments of residues E6–V13 (Figure 1). The 3D ^{15}N-edited HOHAHA-HSQC and 3D C(CO)NH experiments were then used to make residue-specific assignments for the side-chain ^{1}H and ^{13}C resonances. 3D and 4D NOE data [NN(i,i+1), NN(i,i+2), αN(i,i+1), αN(i,i+2), αN(i,i+3), αN(i,i+4), βN(i,i+1), αβ(i,i+3)] were used to confirm resonance assignments and to indicate regions of secondary structure. These data indicate that each subunit of apo-Mts1 has four α-helices (helix 1, Glu6–Tyr19; helix 2, Lys31–Arg40; helix 3, Ala54–Asp63; helix 4, Phe72–Cys86), two β-strands (strand 1, Lys28–Asn30; strand 2, Glu69–Asp71), and five loops (loop 1, Met1–Leu5; loop 2, Ser20–Lys26; loop 3, Glu41–Ala53; loop 4, Ser64–Cys68; loop 5, Asn87–Lys101). This secondary structure is very similar to that observed for other S100 protein family members including S100B (Amburgey et al., 1995), S100A1 (Baldisseri et al., 1999), and S100A6 (Potts et al., 1996).

Extent of assignments and data deposition

All observable resonances of apo-Mts1 in the ^{1}H-^{15}N HSQC (S100A4) have been sequentially assigned. The ^{1}H and ^{13}C side-chain assignments are nearly complete (>98%), with the exception of some aromatic ring resonances that show extensive overlap in both ^{1}H and ^{13}C chemical shift. The ^{1}H, ^{13}C and ^{15}N sequence-specific assignments described here have been deposited in the BioMagResBank http://www.bmrb.wisc.edu) under BMRB accession number 4892.

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