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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N NMR sequence-specific resonance assignments for rat apo-S100A1(αα)

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Biological context

S100A1(αα) is a member of the highly homologous S100 family of Ca^{2+}-binding proteins (for reviews see Zimmer et al., 1995; Schafer and Heizmann, 1996). Similar to most other S100 proteins, S100A1(αα) is a noncovalent homodimer with each S100α subunit having a typical and a pseudo EF-hand (Amburgey et al., 1995). S100A1(αα) has no enzymatic activity of its own, but rather it regulates cellular processes by interacting with and modulating the function of other proteins termed 'target proteins'. As with calmodulin, S100A1(αα) interacts with some target proteins in a Ca^{2+}-dependent manner while other interactions are independent of Ca^{2+} (Landar et al., 1998). Here we report the sequential assignments for apo-S100A1(αα) which initiate the study of its solution structure by NMR spectroscopy.

Methods and results

Uniformly ^{15}N- and ^{13}C, ^{15}N-labeled protein was purified as previously described except that 0.5 mM DTT was used as a reducing agent throughout instead of β-mercaptoethanol. A 1.8 mM ^{15}N-S100A1(αα) and a 2.0 mM ^{13}C, ^{15}N-S100A1(αα) sample (monomer concentration) were prepared in 2–5 mM Tris-d_{11}, 0.1 mM EDTA, 0.3 mM NaN_3, pH 6.5 with enough added NaCl to bring the total ionic strength to 25 mM in 90% H_2O and 10% D_2O.

Spectra were recorded at 37 °C on a Bruker Avance-series DMX 600 MHz spectrometer. For H^N, N, H_α, C_α backbone and ^{1}H, ^{13}C side-chain sequential assignments, the following heteronuclear NMR experiments were performed: 2D ^{1}H-^{15}N HSQC, 3D ^{15}N- HOHAHA-HSQC (60 ms spin-lock duration), 3D CBCA(CO)NH, 3D HNCACB, 3D H(CCO)NH, and 3D C(CO)NH (Clore and Gronenborn, 1993). NOE-based data from the 3D ^{15}N-NOESY-HSQC (τ_m = 150 msec), 3D ^{15}N, ^{15}N-HMQC-NOESY-HSQC (τ_m = 100 ms) and 4D ^{15}N, ^{13}C-NOESY (τ_m = 100 ms) was also collected and used to confirm the resonance assignments as previously described (Wüthrich, 1986).

All observable resonances in the ^{1}H-^{15}N HSQC of S100A1(αα) have been sequentially assigned. A number of residues exhibit multiple peaks in the HSQC, including S2, E3, E5, T6, T10, E40, L41, S42, S43, F44, and L45. However, this multiplicity did not cause any problems in assignments because no doubling was observed in side-chain proton or carbon resonances. As was the case with another S100 protein, S100B(ββ) (Smith et al., 1997), the multiplicity observed here likely derives from proximity to a heterogeneous N-terminus (i.e. with or without an N-terminal Met). The detection of 91 of 93 backbone amides facilitated the sequential assignment process. The 3D CBCA(CO)NH and HNCACB experiments were used to sequentially assign the C_α and C_β resonances. These residue-specific assignments were extended to include the side-chain ^{1}H and ^{13}C resonances using 3D ^{15}N-separated HOHAHA-HSQC, 3D H(CCO)NH and the 3D C(CO)NH ex-

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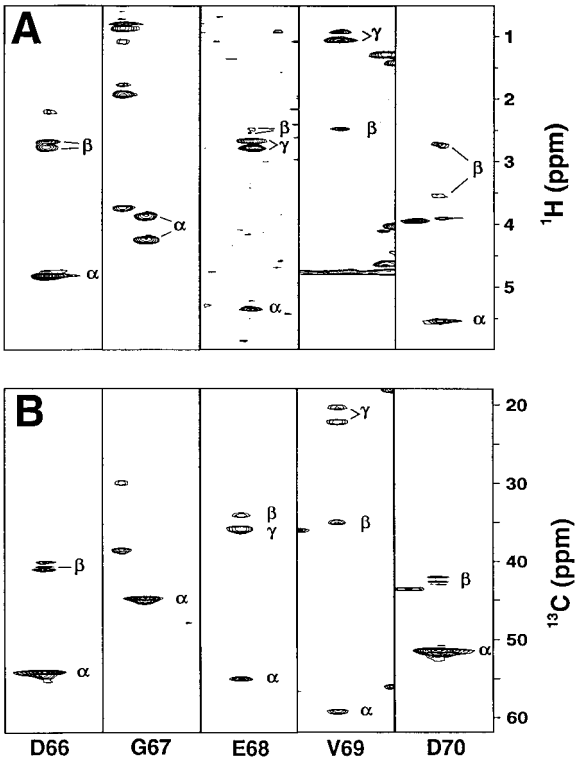

Figure 1. Strips from selected 2D planes of the (A) H(CCO)NH and (B) C(CO)NH data sets for residues D66–D70. Side-chain ^{1}H and ^{13}C resonance assignments are indicated.

periments. Figure 1 illustrates a representative stretch (residues D66–D70) of assignments made from the H(CCO)NH and C(CO)NH data sets. The completeness of the assignments is evidenced by the fact that only 1 of the 29 possible correlations is not detected in that five-residue stretch. A relatively few number of ambiguities in the 3D experiments were resolved using the 4D ^{15}N, ^{13}C-NOESY. 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)] was also used to confirm the resonance assignments, and to indicate the regions of secondary structure. In this regard, the secondary structure of S100A1(αα) is consistent with two helix-loop-helix Ca^{2+}-binding domains. These domains are brought together by a short antiparallel β-sheet similar to other S100 proteins for which 3D structures have been determined (Drohat et al., 1999).

Extent of assignments and data deposition

Only the backbone amide groups of S18 and F89 and the side-chain amide of N86 are not detected in the ^{1}H-^{15}N HSQC of S100A1(αα). The ^{1}H and ^{13}C side-chain assignments of S100A1(αα) are nearly complete except for the phenylalanine, tyrosine, and tryptophan aromatic ring resonances. For the eight aromatic rings, 21 of the total 63 resonances have been sequentially assigned, and the remainder will be obtained from the 3D HCCH-TOCSY and 4D ^{13}C, ^{13}C-NOESY experiments (Clore and Gronenborn, 1993). 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 accession number 4285 and are being used in the analysis of NOE-derived data as well as data from chemical shifts, coupling constants and dipolar couplings to obtain a high-resolution solution structure of S100A1(αα) as done previously for S100B(ββ) (Drohat et al., 1999).

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