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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of rabbit apo-S100A11

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Abstract

S100 proteins belong to the EF-hand family of calcium binding proteins. Upon calcium binding, these proteins undergo a conformational change to expose a hydrophobic region necessary for target protein interaction. One member of the S100 protein family is S100A11, first isolated from chicken gizzard and termed calgizzarin. It was later isolated from other organisms and tissues including human placenta, pig heart and rabbit lung. The physiological target of S100A11 is thought to be annexin I, a phospholipid-binding protein involved in EGF receptor sorting. This work reports the ^{1}H, ^{15}N and ^{13}C resonance assignments of rabbit apo-S100A11 determined using ^{15}N, ^{13}C-labelled protein and multidimensional NMR spectroscopy.

Biological context

Changes in calcium levels within a cell serve as chemical signals for many cellular processes such as contraction, metabolism and cell growth. Frequently, the responses to elevations in intracellular calcium levels are mediated by calcium binding proteins. S100 proteins belong to the EF-hand family of calcium binding proteins (Schäfer and Heizmann, 1996). When these proteins bind calcium, they undergo a conformational change to expose a hydrophobic region necessary for target protein interaction (Smith and Shaw, 1998). S100 proteins can exist as homo- or heterodimers with each S100 monomer having two EF-hand calcium-binding motifs connected by a flexible loop.

S100A11 (S100C or calgizzarin) is a member of the S100 protein family and was first identified in chicken gizzard smooth muscle (Todoroki et al., 1991). S100A11 binds to the N-terminus of annexin I, a phospholipid-binding protein, in the presence of calcium (Naka et al. 1994). This interaction may affect the phosphorylation of annexin I by epidermal growth factor (EGF) receptor kinase (Haigler et al., 1987) and therefore affect the sorting of EGF receptors to the lysosome (Futter et al., 1993). This work reports the NMR assignment of rabbit apo-S100A11 as a first step towards its three-dimensional structure determination.

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Methods and experiments

Rabbit lung S100A11 protein was over-expressed using the pAED4/S100A11 construct, cloned in a similar manner to the construct used for chicken gizzard S100A11 (Schönekess and Walsh, 1997). For the preparation of uniformly ^{15}N-labelled and ^{15}N,^{13}C-labelled S100A11, the *E. coli* strain BL21(DE3) was grown in M9 minimal medium containing 1 g l^{-1} 99% $^{15}NH_4Cl$ or 1 g l^{-1} 99% $^{15}NH_4Cl$ and 2 g l^{-1} ^{13}C-glucose, respectively. Cultures were grown at 37 °C with agitation to an OD_{600} of 0.4–0.6 and induced with 0.4 mM IPTG. Cells were harvested by centrifugation at 8000 × *g* for 10 min and lysed by French

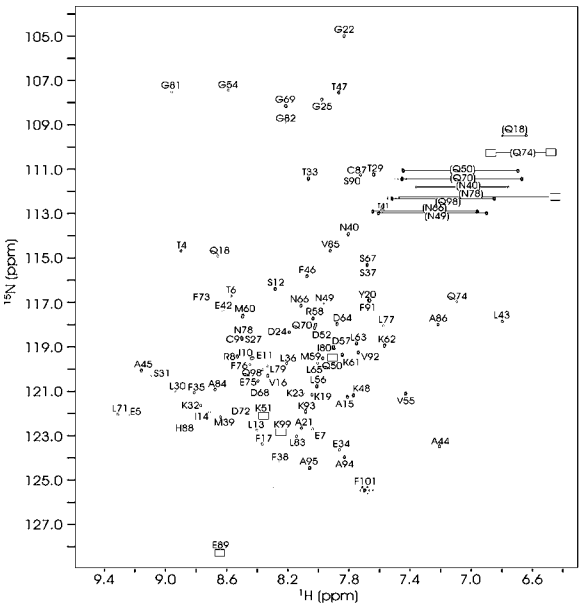

Figure 1. ^{1}H-^{15}N HSQC spectrum of 1 mM uniformly ^{15}N,^{13}C-labeled S100A11 in 50 mM KCl, 5 mM DTT and pH 7.25 acquired at 35 °C on a Varian INOVA 800 MHz spectrometer. Backbone amide cross peaks are indicated with their one letter amino acid code and number. Sidechain amide cross peaks have lines connecting pairs of resonances and boxes indicate residues that are visible at a lower contour level.

pressure cell. The supernatant was collected after centrifugation at 38 000 × *g* for 90 min. S100A11 was purified from the cell extract by phenyl-Sepharose chromatography via a Ca^{2+}-dependent hydrophobic interaction with the column. The cell extract was applied to the column with a $CaCl_2$ containing buffer and washed until the OD_{280} returned to baseline. Bound proteins were eluted using the same buffer containing EGTA and lacking calcium. Fractions containing S100A11 were pooled, dialyzed against 3 mM KCl and lyophilized. NMR samples were prepared at a dimer concentration of 1 mM in 90% H_2O/10% D_2O (v/v), 50 mM KCl, 5 mM DTT at a pH of 7.25.

NMR spectroscopy experiments were performed on Varian 500, 600 and 800 MHz spectrometers with pulse field gradient triple resonance probes. Sequential assignment of the polypeptide backbone resonances were made from HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH and ^{1}H-^{15}N HSQC experiments (Bax and Grzesiek, 1993). Sidechain resonance assignments were made from ^{15}N-edited TOCSY, C(CO)NH, HCCH-TOCSY and HC(CO)HN experiments. Data was processed and analyzed using NMRDraw, NMRPipe (Delaglio et al., 1995), Pipp and Stapp (Garrett et al., 1991) programs on a Sun Ultra 10 workstation.

Extent of assignments and data deposition

Figure 1 shows the assigned ^{1}H-^{15}N HSQC spectrum of apo-S100A11, acquired at 35 °C on a Varian INOVA 800 MHz spectrometer, indicating $^{1}H^{N}$ and ^{15}N resonances of the backbone and Gln and Asn sidechains of apo-S100A11. Excluding the four proline residues, the $^{1}H^{N}$ and ^{15}N resonance assignments of the backbone amides for 96 out of 97 have been assigned. Extents of assignments are: 99% of $^{13}C\alpha$, 97% of $^{1}H\alpha$, 92% of ^{13}CO, 88% of sidechain ^{13}C and 93% of sidechain ^{1}H. The ^{1}H, ^{15}N and ^{13}C resonance assignments have been deposited in the BioMagResBank under the accession number BMRB-5189.

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