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ARTICLE

^{1}H, ^{15}N, and ^{13}C chemical shift assignments of neuronal calcium sensor-1 homolog from fission yeast

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Abstract The neuronal calcium sensor (NCS) proteins regulate signal transduction processes and are highly conserved from yeast to humans. We report complete NMR chemical shift assignments of the NCS homolog from fission yeast (*Schizosaccharomyces pombe*), referred to in this study as Ncs1p. (BMRB no. 16446).

Keywords NCS · Ncs1p · Fission yeast · Calcium · EF-hand · NMR · *S. pombe*

Biological context

Neuronal calcium sensor (NCS) proteins belong to a sub-branch of the calmodulin superfamily that regulate a variety of physiological target proteins in the brain and retina (Ames et al. 1996; Braunewell and Gundelfinger 1999; Burgoyne et al. 2004). The best characterized NCS protein is recoverin that serves as a Ca^{2+} sensor in retinal rod and cone cells where it controls the desensitization of rhodopsin (Dizhoor et al. 1991; Erickson et al. 1998; Kawamura 1993). The NCS family also includes neuronal Ca^{2+} sensors such as neurocalcin (Hidaka and Okazaki 1993), hippocalcin (Kobayashi et al. 1993), and frequenin (Pongs et al. 1993), as well as yeast homologs, *S. cerevisiae* Frq1 (Hendricks et al. 1999) and *S. pombe* Ncs1p (Hamasaki-Katagiri et al. 2004). All members of the NCS family have around 200 amino acid residues, contain N-terminal myristoylation, and possess four EF-hands.

Three-dimensional structures are now known for many NCS proteins, including recoverin (Ames et al. 1997; Flaherty et al. 1993), frequenin (Bourne et al. 2001), Frq1 (Strahl et al. 2007), neurocalcin (Vijay-Kumar and Kumar 1999), and GCAPs (Ames et al. 1999; Stephen et al. 2007). The Ca^{2+}-bound NCS proteins share a common fold with four EF-hands arranged in a tandem array and an exposed N-terminus. The structure of Ca^{2+}-free recoverin contains a covalently attached myristoyl group buried inside the protein hydrophobic core (Tanaka et al. 1995). Binding of Ca^{2+} to recoverin leads to extrusion of its myristoyl group, termed the calcium-myristoyl switch, that enables recoverin to bind to membrane targets only at high Ca^{2+} levels (Dizhoor et al. 1993; Zozulya and Stryer 1992). By contrast, frequenin (NCS-1) and yeast Frq1 contain exposed myristoyl groups in their Ca^{2+}-free state and therefore lack a Ca^{2+} myristoyl switch (Ames et al. 2000; O'Callaghan and Burgoyne 2004). Also, the recent x-ray structure of Ca^{2+}-bound GCAP1 (Stephen et al. 2007) showed the myristoyl group to be sequestered in a unique environment flanked by N- and C-terminal helices, very different from the myristate binding pocket seen in Ca^{2+}-free recoverin. The atomic-resolution structures of other myristoylated NCS proteins are needed to better define the range and different types of NCS protein-myristate interactions. We report here NMR resonance assignments of myristoylated *S. pombe* Ncs1p in the Ca^{2+}-free state as a first step toward elucidating the protein structure and environment around the N-terminal myristoyl group.

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Methods and experiments

Expression and purification of Ncs1p

Recombinant myristoylated neuronal calcium sensor-1 (Ncs1p) was uniformly ^{15}N- or $^{15}N/^{13}C$-labeled by co-expressing Ncs1p and yeast N-myristoyl CoA-transferase in *E. coli* strain, BL21(DE3) grown on M9 medium supplemented with ^{15}N-NH_4Cl and/or $^{13}C_6$-glucose. Recombinant protein expression was induced by exogenously adding myristic acid (10 mg/l) and 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to cells grown overnight at 25°C. Typically, a 1-L culture yields about 30 mg of myristoylated protein. Detailed procedures for purifying myristoylated Ncs1p are described elsewhere (Hamasaki-Katagiri et al. 2004).

NMR spectroscopy

Samples of recombinant Ca^{2+}-free myristoylated Ncs1p (0.5–0.7 mM) were prepared in 90%/10% H_2O/D_2O or 100% D_2O with 5 mM Tris-d_{11} (pH 7.4), 4 mM DTT-d_{11} and 0.3 mM EDTA-d_{12}. NMR experiments were conducted using Bruker Advance 600 or 800 MHz spectrometer equipped with a triple resonance cryogenic probe. All experiments were performed at 310 K. Backbone and side-chain chemical shift assignments were obtained using ^{15}N-HSQC, HNCO, HNCACB, CBCACONH, HBHA-CONH and ^{15}N-HSQC-TOCSY (mixing time of 60 ms) spectra (Ikura et al. 1990). Methyl group side-chain resonances were assigned using ^{13}C-CT-HSQC and ^{13}C-HCCH-TOCSY (Kay et al. 1993). For aromatic side-chain chemical shift assignments, HBCBCGCDHD, HBCBCGCDCEHE, ^{13}C-CT-HSQC-TOCSY spectra (Yamazaki et al. 1993) along with ^{13}C-HSQC-NOESY, recorded with a mixing time of 120 ms, were used. NMR data were processed using NMRPipe (Delaglio et al. 1995) software package and analyzed using SPARKY.

Assignments and data deposition

Figure 1 presents HSQC spectra of myristoylated Ca^{2+}-free Ncs1p at pH 7.4 to illustrate representative backbone and side chain resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^{13}C/^{15}N$-labeled Ncs1p (residues 2–190). The protein sample in this study consists of 189 native residues with a myristoyl group covalently attached at the N-terminus (Gly 2). All non-proline residues exhibited strong backbone amide resonances with uniform intensities, indicative of a well-defined three-dimensional protein structure. More than 95% of the backbone resonances (^1HN, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$, and ^{13}CO) and

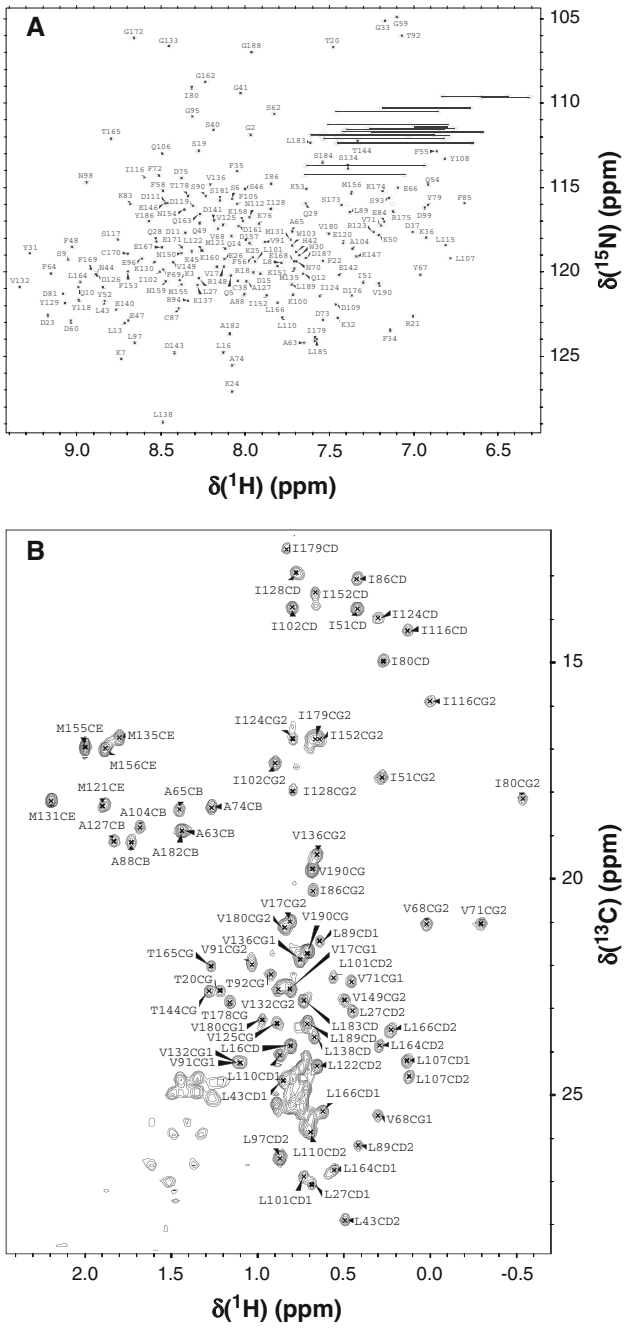

Fig. 1 Two-dimensional ^{15}N-HSQC **a** and ^{13}C-CT-HSQC **b** NMR spectra of Ca^{2+}-free Ncs1p recorded at 800 MHz proton frequency. Amide resonances from residues in the downfield region (N77, G78, F82, N113, G114, and indole amide protons of W30 and W103) are not shown in **a**. Side chain amide resonances of Asn and Gln are connected with *solid lines*. The assignment of backbone amide and side-chain methyl groups are indicated in **a** and **b**, respectively. Unresolved methyl groups of valine and leucine are designated as CG and CD, respectively

~82% of aliphatic side chain resonances were assigned. The chemical shift assignments (^1H, ^{15}N, ^{13}C) of Ca^{2+}-free Ncs1p have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 16446.

The chemical shift index of each amino acid residue reveals a protein secondary structure in Ncs1p that closely resembles the canonical secondary structure and topology seen in other NCS proteins. Ncs1p contains 10 α-helices and two antiparallel β-sheets (α1: 9–17; α2: 25–35; β1: 42–44; α3: 44–55; α4: 61–72; β2: 79–81; α5: 82–90; α6: 101–108; β3: 115–117; α7: 118–131; α8: 145–154; β4: 163–165; α9: 166–174; α10: 179–186). The NMR assignments reported here for Ca^{2+}-free Ncs1p are overall similar to those reported previously for Ca^{2+}-free recoverin (BMRB 4030). Similar chemical shifts are seen for conserved residues in the N-terminal region that might interact with the myristoyl group, suggesting that Ncs1p contains a myristoyl group in a sequestered environment similar to that seen previously in Ca^{2+}-free recoverin (Tanaka et al. 1995).

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