Title

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Journal

Biomolecular NMR Assignments, 3(2)

ISSN

1874-270X

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Publication Date

2009-12-01

DOI

10.1007/s12104-009-9191-3

Peer reviewed

Biomol NMR Assign (2009) 3:269–271
DOI 10.1007/s12104-009-9191-3

ARTICLE

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

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Received: 12 August 2009 / Accepted: 8 October 2009 / Published online: 23 October 2009

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

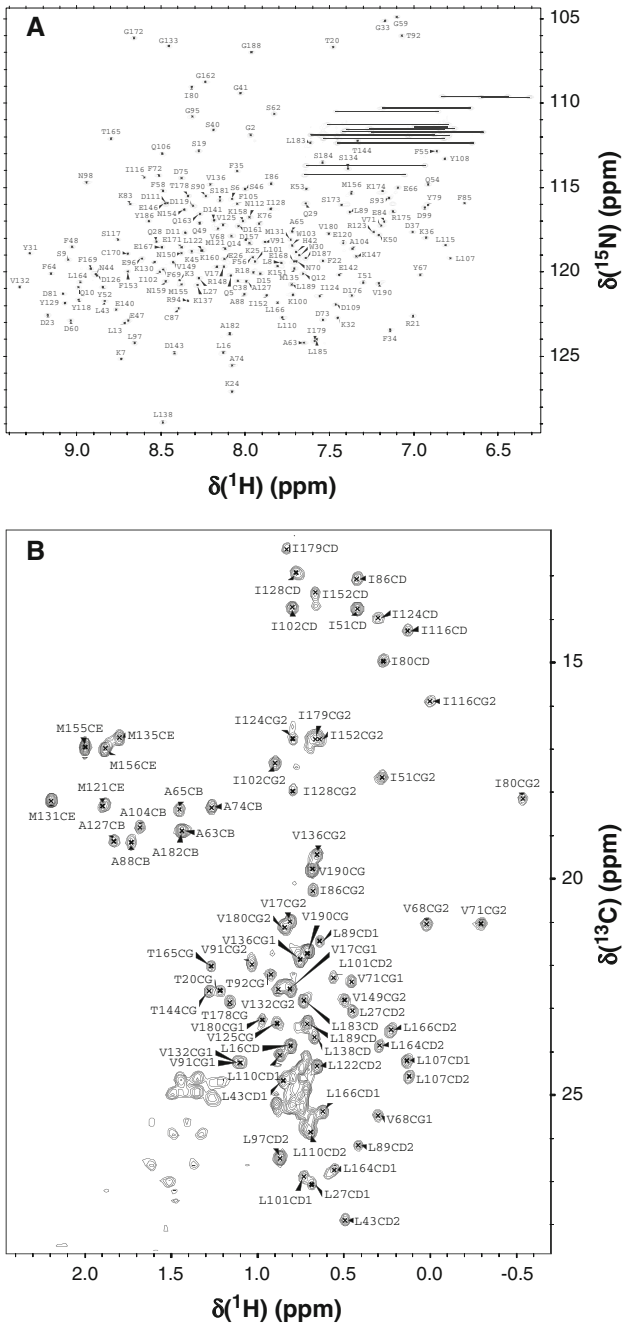

Fig. 1 Two-dimensional ^{15}N-HSQC **a** and ^{13}C-CT-HSQC **b** NMR spectra of Ca^{2+}-free Ncs1p recorded at 800 MHz proton frequency. Amide resonances from residues in the downfield region (N77, G78, F82, N113, G114, and indole amide protons of W30 and W103) are not shown in **a**. Side chain amide 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).

Acknowledgments We thank Jeff Walton for technical support and help with NMR experiments. Work supported by NIH grants (EY012347) to J.B.A and (RR11973) to the UC Davis NMR facility.

References

Ames JB, Tanaka T, Stryer L, Ikura M (1996) Portrait of a myristoyl switch protein. Curr Opin Struct Biol 6:432–438

Ames JB, Ishima R, Tanaka T, Gordon JI, Stryer L, Ikura M (1997) Molecular mechanics of calcium-myristoyl switches. Nature 389:198–202

Ames JB, Dizhoor AM, Ikura M, Palczewski K, Stryer L (1999) Three-dimensional structure of guanylyl cyclase activating protein-2, a calcium-sensitive modulator of photoreceptor guanylyl cyclases. J Biol Chem 274:19329–19337

Ames JB, Hendricks KB, Strahl T, Huttner IG, Hamasaki N, Thorner J (2000) Structure and calcium-binding properties of Frq1, a novel calcium sensor in the yeast Saccharomyces cerevisiae. Biochemistry 39:12149–12161

Bourne Y, Dannenberg J, Pollmann VV, Marchot P, Pongs O (2001) Immunocytochemical localization and crystal structure of human frequenin (neuronal calcium sensor1). J Biol Chem 276:11949–11955

Braunewell KH, Gundelfinger ED (1999) Intracellular neuronal calcium sensor proteins: a family of EF-hand calcium-binding proteins in search of a function. Cell Tissue Res 295:1–12

Burgoyne RD, O'Callaghan DW, Hasdemir B, Haynes LP, Tepikin AV (2004) Neuronal Ca^{2+}-sensor proteins: multitalented regulators of neuronal function. Trends Neurosci 27:203–209

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeiffer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277–293

Dizhoor AM, Ray S, Kumar S, Niemi G, Spencer M, Rrolley D, Walsh KA, Philipov PP, Hurley JB, Stryer L (1991) Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. Science 251:915–918

Dizhoor AM, Chen CK, Olshevskaya E, Sinelnikova VV, Phillipov P, Hurley JB (1993) Role of the acylated amino terminus of recoverin in Ca(2+)-dependent membrane interaction. Science 259:829–832

Erickson MA, Lagnado L, Zozulya S, Neubert TA, Stryer L, Baylor DA (1998) The effect of recombinant recoverin on the photoresponse of truncated rod photoreceptors. Proc Natl Acad Sci USA 95:6474–6479

Flaherty KM, Zozulya S, Stryer L, McKay DB (1993) Three-dimensional structure of recoverin, a calcium sensor in vision. Cell 75:709–716

Hamasaki-Katagiri N, Molchanova T, Takeda K, Ames JB (2004) Fission yeast homolog of neuronal calcium sensor-1 (Ncs1p) regulates sporulation and confers calcium tolerance. J Biol Chem 279:12744–12754

Hendricks KB, Wang BQ, Schnieders EA, Thorner J (1999) Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase. Nat Cell Biol 1:234–241

Hidaka H, Okazaki K (1993) Neurocalcin family: a novel calcium-binding protein abundant in bovine central nervous system. Neurosci Res 16:73–77

Ikura M, Kay LE, Bax A (1990) A novel approach for sequential assignment of 1H, 13C, and 15 N spectra of proteins: heteronuclear triple-resonanc three-dimensional NMR spectroscopy. Application to calmodulin. Biochemistry 29:4659–4667

Kawamura S (1993) Rhodopsin phosphorylation as a mechanism of cyclic GMP phosphodiesterase regulation by S-modulin. Nature 362:855–857

Kay LE, Xu GY, Singer AU, Muhandiram DR, Forman-Kay JD (1993) A gradient-enhanced HCCH-TOCSY experiment for recording side-chain 1H and 13C correlations in H2O samples of proteins. J Magn Reson 101:33–337

Kobayashi M, Takamatsu K, Saitoh S, Noguchi T (1993) Myristoylation of hippocalcin is linked to its calcium-dependent membrane association properties. J Biol Chem 268:18898–18904

O'Callaghan DW, Burgoyne RD (2004) Identification of residues that determine the absence of a Ca(2+)/myristoyl switch in neuronal calcium sensor-1. J Biol Chem 279:14347–14354

Pongs O, Lindemeier J, Zhu XR, Theil T, Engelkamp D, Krah-Jentgens I, Lambrecht HG, Kock KW, Schwerner J, Rivosecchi R, Mallart A, Galceran J, Canal I, Barbas JA, Ferrus A (1993) Frequenin-a novel calcium-binding protein that modulates synaptic efficacy. Neuron 11:15–28

Stephen R, Bereta G, Golczak M, Palczewski K, Sousa MC (2007) Stabilizing function for myristoyl group revealed by the crystal structure of a neuronal calcium sensor, guanylate cyclase-activating protein 1. Structure 15:1392–1402

Strahl T, Huttner IG, Lusin JD, Osawa M, King D, Thorner J, Ames JB (2007) Structural insights into activation of phosphatidylinositol 4-kinase (Pik1) by yeast frequenin (Frq1). J Biol Chem 282:30949–30959

Tanaka T, Ames JB, Harvey TS, Stryer L, Ikura M (1995) Sequestration of the membrane-targeting myristoyl group of recoverin in the calcium-free state. Nature 376:444–447

Vijay-Kumar S, Kumar VD (1999) Crystal structure of recombinant bovine neurocalcin. Nat Struct Biol 6:80–88

Yamazaki T, Forman-Kay JD, Kay LE (1993) Two-dimensional NMR experiments for correlating 13C and 1H/chemical shifts of aromatic residues in 13C-labeled proteins via scalar couplings. J Am Chem Soc 115:11054–11055

Zozulya S, Stryer L (1992) Calcium-myristoyl protein switch. Proc Natl Acad Sci USA 89:11569–11573