Published in final edited form as:

Biomol NMR Assign. 2014 April ; 8(1): 63–66. doi:10.1007/s12104-012-9453-3.

¹H, ¹³C, and ¹⁵N Chemical Shift Assignments of Neuronal Calcium Sensor Protein, Hippocalcin

Congmin Li and James B. Ames*

Department of Chemistry, University of California, Davis, CA 95616

Abstract

Hippocalcin, a member of the neuronal calcium sensor (NCS) subclass of the calmodulin superfamily, serves as an important calcium sensor for the slow afterhyperpolarizing (sAHP) current in the hippocampus, which underlies some forms of learning and memory. Hippocalcin is also a calcium sensor for hippocampal long-term depression (LTD) and genetically linked to neurodegenerative diseases. We report NMR chemical shift assignments of Ca²⁺-free hippocalcin (BMRB no. 18627).

Keywords

Hippocalcin; VILIP-3; calcium; EF-hand; NCS protein; NMR

Biological Context

Hippocalcin (Kobayashi et al., 1993) belongs to the NCS branch of the calmodulin superfamily (Ames et al., 1996; Burgoyne, 2007) (Fig. 1) and confers Ca²⁺-induced activation of sAHP channels in hippocampal neurons that are important for learning and memory (Tzingounis et al., 2007; Villalobos and Andrade, 2010). Hippocalcin also functions as a calcium sensor for hippocampal LTD by binding to the beta2-adaptin subunit of the AP2 adaptor complex that couples NMDA receptor-dependent activation to regulated endocytosis of AMPA receptors during LTD (Jo et al., 2010).

Three-dimensional structures are known for many NCS proteins, including recoverin (Ames et al., 1997; Flaherty, 1993), frequenin (Bourne et al., 2001), Frq1 (Strahl et al., 2007), neurocalcin (Vijay-Kumar and Kumar, 1999), NCS-1 (Lim et al., 2011), and guanylate cyclase activating proteins (GCAPs) (Ames et al., 1999; Stephen et al., 2007). The Ca²⁺-bound NCS proteins all share a common fold with four EF-hands arranged in a tandem array and an exposed N-terminus. Binding of Ca²⁺ to recoverin leads to extrusion of its myristoyl group, termed the calcium-myristoyl switch, that enables recoverin to bind to retinal membrane targets only at high Ca²⁺ levels (Dizhoor et al., 1993; Zozulya and Stryer, 1992). A similar Ca²⁺-myristoyl switch has been reported for hippocalcin in the hippocampus (O'Callaghan et al., 2003).

The N-terminal myristoyl group attached to NCS proteins is sequestered inside unique protein environments in the Ca²⁺-free forms of recoverin (Tanaka et al., 1995), NCS-1 (Lim et al., 2011), and GCAP1 (Stephen et al., 2007). The structure of Ca²⁺-free recoverin contains a covalently attached myristoyl group buried inside a protein hydrophobic cavity formed by the first and second EF-hands in the N-terminal domain (residues highlighted blue in Fig. 1). The structure of Ca²⁺-free myristoylated NCS-1 contains a covalently

*To whom correspondence should be addressed: ames@chem.ucdavis.edu. .

attached myristoyl group buried in a cavity located near the C-terminus (see residues highlighted magenta in Fig. 1) and looks very different from that of recoverin. Finally, the structure of myristoylated GCAP1 (Stephen et al., 2007) contains a sequestered myristoyl group flanked by both N-terminal and C-terminal helices that looks different from the myristoyl environment in either recoverin and NCS-1. Thus, the myristoylated NCS proteins each fold differently around the covalently attached myristoyl group, which helps explain how these highly homologous proteins adopt unique structures to carry out distinct biological functions. 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 hippocalcin in the Ca^{2+} -free state as a first step toward elucidating its protein structure and environment around the N-terminal myristoyl group.

Methods and Experiments

Expression and Purification of hippocalcin

Recombinant myristoylated hippocalcin was uniformly ^{15}N - or $^{15}\text{N}/^{13}\text{C}$ -labeled by co-expressing hippocalcin and yeast N-myristoyl CoA-transferase in *E. coli* strain, BL21(DE3) grown on M9 medium supplemented with ^{15}N - NH_4Cl and/or $^{13}\text{C}_6$ -glucose (Ames et al., 1994). 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 10 mg of myristoylated protein. Detailed procedures for purifying myristoylated NCS proteins are described elsewhere (Zozulya et al., 1995). Triply labeled (perdeuterated) $^2\text{H}/^{15}\text{N}/^{13}\text{C}$ -labeled hippocalcin was expressed in the similar way described above with the exception that $^1\text{H}_2\text{O}$ and $^{13}\text{C}_6$ -glucose were substituted with $^2\text{H}_2\text{O}$ and $^{13}\text{C}_6$ - $^2\text{H}_7$ -glucose.

NMR spectroscopy

Samples of recombinant Ca^{2+} -free myristoylated hippocalcin (0.5 mM) were prepared in 90%/10% $\text{H}_2\text{O}/\text{D}_2\text{O}$ 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, HNCB, HNCACB, CBCACONH, HBHACONH 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. For aromatic side-chain chemical shift assignments, HBCBCGCDHD, HBCBCGCDCEHE, ^{13}C -CT-HSQC-TOCSY spectra along with ^{13}C -HSQC-NOESY, recorded with a mixing time of 120 ms, were used. NMR data were processed using NMRPipe software package and analyzed using SPARKY.

Assignments and Data Deposition

The ^{15}N - ^1H HSQC spectrum of Ca^{2+} -free myristoylated hippocalcin (Fig. 2A) illustrates representative backbone resonance assignments. A ^{13}C - ^1H constant-time HSQC spectrum (Fig. 2B) shows resonance assignments for side-chain methyl groups. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^2\text{H}/^{13}\text{C}/^{15}\text{N}$ -labeled hippocalcin (residues 2-193). The protein sample in this study consists of 193 native residues with an N-terminal myristoyl group covalently attached at Gly 2. Most of the 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}Ca , $^{13}\text{C}\beta$, and ^{13}CO) and 82% of side-chain resonances

were assigned. The chemical shift assignments (^1H , ^{15}N , ^{13}C) of Ca^{2+} -free hippocalcin have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 18627.

The chemical shift index (CSI) of each amino acid residue reveals a protein secondary structure in hippocalcin (Fig. 1) similar to that observed previously in recoverin (Tanaka et al., 1995) and NCS-1 (Ames et al., 1999). Hippocalcin contains 10 α -helices and two antiparallel β -sheets (α 1: 10-17; β 1: 40-43; α 2: 25-35; α 3: 44-56; β 2: 78-81; α 4: 62-72; α 5: 82-91; β 3: 114-117; α 6: 101-110; α 7: 118-131; β 4: 162-165; α 8: 146-157; α 9: 166-175; α 10: 178-187). The NMR assignments reported here for Ca^{2+} -free hippocalcin are overall different from those reported previously for recoverin (Tanaka et al., 1995), NCS-1 (Lim et al., 2011) and GCAP1 (Ames et al., 1999). The most noteworthy chemical shift differences are observed for residues known to interact with the N-terminal myristoyl group (highlighted blue for recoverin and magenta for NCS-1 in Fig. 1), consistent with the proposal that N-terminal myristoylation creates a unique binding site environment in each highly homologous NCS protein (Ames and Lim, 2012; Lim et al., 2011).

Acknowledgments

We thank Jerry Dallas for technical support and help with NMR experiments. Work supported by NIH grants (EY012347) to J.B.A., (EY11522) to A.M.D., and (RR11973) to the UC Davis NMR facility.

References

- Ames JB, Dizhoor AM, Ikura M, Palczewski K, Stryer L. Three-dimensional structure of guanylyl cyclase activating protein-2, a calcium-sensitive modulator of photoreceptor guanylyl cyclases. *J Biol Chem.* 1999; 274:19329–19337. [PubMed: 10383444]
- Ames JB, Ishima R, Tanaka T, Gordon JI, Stryer L, Ikura M. Molecular mechanics of calcium-myristoyl switches. *Nature.* 1997; 389:198–202. [PubMed: 9296500]
- Ames JB, Lim S. Molecular structure and target recognition of neuronal calcium sensor proteins. *Biochim. Biophys. Acta.* 2012; 1820:1205–1213. [PubMed: 22020049]
- Ames JB, Tanaka T, Stryer L, Ikura M. Secondary structure of myristoylated recoverin determined by three-dimensional heteronuclear NMR: implications for the calcium-myristoyl switch. *Biochemistry.* 1994; 33:10743–10753. [PubMed: 8075075]
- Ames JB, Tanaka T, Stryer L, Ikura M. Portrait of a myristoyl switch protein. *Curr. Opin. Struct. Biol.* 1996; 6:432–438. [PubMed: 8794166]
- Bourne Y, Dannenberg J, Pollmann VV, Marchot P, Pongs O. Immunocytochemical localization and crystal structure of human frequenin (neuronal calcium sensor1). *J. Biol. Chem.* 2001; 276:11949–11955. [PubMed: 11092894]
- Burgoyne RD. Neuronal calcium sensor proteins: generating diversity in neuronal Ca^{2+} signalling. *Nat. Rev. Neurosci.* 2007; 8:182–193. [PubMed: 17311005]
- Dizhoor AM, Chen CK, Olshevskaya E, Sinelnikova VV, Phillipov P, Hurley JB. Role of the acylated amino terminus of recoverin in Ca^{2+} -dependent membrane interaction. *Science.* 1993; 259:829–832. [PubMed: 8430337]
- Flaherty KM, Zozulya S, Stryer L, McKay DB. Three-dimensional Structure of Recoverin, a Calcium Sensor in Vision. *Cell.* 1993; 75:709–716. [PubMed: 8242744]
- Ikura M, Kay LE, Bax A. A novel approach for sequential assignment of ^1H , ^{13}C , and ^{15}N spectra of proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. Application to calmodulin. *Biochemistry.* 1990; 29:4659–4667. [PubMed: 2372549]
- Jo J, Son GH, Winters BL, Kim MJ, Whitcomb DJ, Dickinson BA, Lee YB, Futai K, Amici M, Sheng M, Collingridge GL, Cho K. Muscarinic receptors induce LTD of NMDAR EPSCs via a mechanism involving hippocalcin, AP2 and PSD-95. *Nat. Neurosci.* 2010; 13:1216–1224. [PubMed: 20852624]

- Kobayashi M, Takamatsu K, Saitoh S, Miura M, Noguchi T. Molecular cloning of hippocalcin, a novel calcium-binding protein of the recoverin family exclusively expressed in hippocampus. *Biochemical and Biophysical Research Communications*. 1993; 196:1017. [PubMed: 8240319]
- Lim S, Strahl T, Thorner J, Ames JB. Structure of a Ca^{2+} -myristoyl switch protein that controls activation of a phosphatidylinositol 4-kinase in fission yeast. *J. Biol. Chem.* 2011 in press.
- O'Callaghan DW, Tepikin AV, Burgoyne RD. Dynamics and calcium sensitivity of the Ca^{2+} /myristoyl switch protein hippocalcin in living cells. *J. Cell. Biol.* 2003; 163:715–721. [PubMed: 14638856]
- Stephen R, Bereta G, Golczak M, Palczewski K, Sousa MC. Stabilizing function for myristoyl group revealed by the crystal structure of a neuronal calcium sensor, guanylate cyclase-activating protein 1. *Structure*. 2007; 15:1392–1402. [PubMed: 17997965]
- Strahl T, Huttner IG, Lusin JD, Osawa M, King D, Thorner J, Ames JB. Structural insights into activation of phosphatidylinositol 4-kinase (Pik1) by yeast frequenin (Frq1). *J. Biol. Chem.* 2007; 282:30949–30959. [PubMed: 17720810]
- Tanaka T, Ames JB, Harvey TS, Stryer L, Ikura M. Sequestration of the membrane-targeting myristoyl group of recoverin in the calcium-free state. *Nature*. 1995; 376:444–447. [PubMed: 7630423]
- Tzingounis AV, Kobayashi M, Takamatsu K, Nicoll RA. Hippocalcin gates the calcium activation of the slow afterhyperpolarization in hippocampal pyramidal cells. *Neuron*. 2007; 53:487–493. [PubMed: 17296551]
- Vijay-Kumar S, Kumar VD. Crystal structure of recombinant bovine neurocalcin. *Nature Struct. Biol.* 1999; 6:80–88. [PubMed: 9886296]
- Villalobos C, Andrade R. Visinin-like neuronal calcium sensor proteins regulate the slow calcium-activated afterhyperpolarizing current in the rat cerebral cortex. *J. Neurosci.* 2010; 30:14361–14365. [PubMed: 20980592]
- Wishart DS, Sykes BD, Richards FM. The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. *Biochemistry*. 1992; 31:1647–1651. [PubMed: 1737021]
- Zozulya S, Ladant D, Stryer L. Expression and characterization of calcium-myristoyl switch proteins. *Methods Enzymol.* 1995; 250:383–393. [PubMed: 7651166]
- Zozulya S, Stryer L. Calcium-myristoyl protein switch. *Proc. Natl. Acad. Sci. USA*. 1992; 89:11569–11573. [PubMed: 1454850]

Figure 1.

Alignment of the primary sequence of human hippocalcin, bovine recoverin, and *S. pombe* NCS-1. Secondary structural elements indicated schematically were derived from analysis of NMR data ($^3J_{\text{HNH}\alpha}$, chemical shift index (CSI) (Wishart et al., 1992) and sequential NOE patterns). The four EF-hands (EF1, EF2, EF3 and EF4) are highlighted green, salmon, cyan, and yellow, respectively. Residues that contact the myristoyl group are colored blue (recoverin) and magenta (NCS-1).

B

Figure 2.

Two-dimensional NMR spectra (^{15}N -HSQC, A and ^{13}C - ^1H constant-time HSQC, B) of Ca^{2+} -free myristoylated hippocalcin at 800 MHz proton frequency. Chemical shift assignments are shown for backbone amide groups (A) and side-chain methyl groups (B). The inset shows an expanded view of the spectrally crowded region. A complete list of assignments can be found at the BMRB repository (accession no. 18627).