Published in final edited form as:

Biomol NMR Assign. 2016 April ; 10(1): 219–222. doi:10.1007/s12104-016-9670-2.

Chemical Shift Assignments of the C-terminal EF-hand Domain of α -actinin-1

Matthew Turner¹, David E. Anderson¹, Sahana Rajan¹, Johannes W. Hell², and James B. Ames^{1,*}

¹Department of Chemistry, University of California, Davis, CA 95616

²Department of Pharmacology, University of California, Davis, CA 95616

Abstract

The regulation and localization of the neuronal voltage gated Ca^{2+} channel $\text{Ca}_v1.2$ is important for synaptic plasticity associated with learning and memory. The cytoskeletal protein, α -actinin-1 is known to interact with $\text{Ca}_v1.2$ and stabilize its localization at the postsynaptic membrane. Here we report both backbone and sidechain NMR assignments for the C-terminal EF-hands (EF3 and EF4) of α -actinin-1 (residues 824-892, called ACTN_EF34) bound to the IQ-motif (residues 1644-1665) from $\text{Ca}_v1.2$ (BMRB accession no. 25902).

Keywords

α -actinin-1; EF-hand; $\text{Ca}_v1.2$; IQ-motif; synaptic plasticity; Long-term depression

Biological Context

The neuronal L-type voltage-gated Ca^{2+} channel $\text{Ca}_v1.2$ is localized primarily at the postsynaptic membrane in hippocampal neurons, where it plays an important role in controlling neuronal excitability associated with learning and memory (Hell et al, 1993; Vogl et al, 2015). The IQ motif in the cytosolic C-terminal region of $\text{Ca}_v1.2$ (residues 1644-1665) is important for controlling Ca^{2+} -dependent surface expression and postsynaptic localization of $\text{Ca}_v1.2$ (Gao et al, 2000; Hall et al, 2013). The cytoskeletal protein, α -actinin-1 interacts with the IQ motif to control the surface expression of $\text{Ca}_v1.2$ (Hall et al, 2013). α -actinin-1 contains four EF-hands at its C-terminus and two of the EF-hands (EF3 and EF4, called ACTN_EF34) bind to the IQ motif in $\text{Ca}_v1.2$. In order to understand the structural basis of this neuronal recognition, an atomic-resolution structure of α -actinin-1 bound to $\text{Ca}_v1.2$ is needed. We report detailed NMR resonance assignments for ACTN_EF34 bound to the IQ-motif of $\text{Ca}_v1.2$ as a first step toward achieving this goal.

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

Methods and Experiments

Preparation of ACTN_EF34 bound to the Ca_v1.2 IQ-motif

A cDNA of the third and fourth EF-hands of Homo sapiens α -actinin-1 (residues 824-892, called ACTN-EF34) was subcloned into pET-15b vector (Novagen) that produced recombinant ACTN_EF34 protein with an N-terminal His(6)-tag (MGSSHHHHHSSGLVPRGSHM). Uniformly ^{15}N -labeled and ^{13}C , ^{15}N -labeled ACTN_EF34 were prepared as described previously using M9 minimal media (Turner et al, 2015).

NMR spectroscopy

Samples of ACTN_EF34 for NMR analysis (prepared as described above) were exchanged into a buffer containing 20 mM Tris- d_{11} (pH 7.5) with 1 mM DTT- d_{10} , 1 mM EDTA- d_{12} and 95% H_2O /5% D_2O . The Ca_v1.2 IQ-motif (residues 1642-1663) was purchased from China Peptides, suspended in $\text{DMSO}-\text{d}_6$ and quantified using UV-Vis absorption. A 1.5-fold excess of Ca_v1.2 IQ-motif was added to ACTN_EF34 and incubated at 15° C for 1hr. The complex was subsequently concentrated to 500 μM using a 3K Amicon concentrator. All NMR experiments were performed at 303K on a Bruker Avance 800 MHz spectrometer equipped with a four channel interface and triple resonance cryogenic (TCI) probe. The ^{15}N - ^1H HSQC spectrum (Fig. 1A) was recorded with 256 x 2048 complex points for ^{15}N (F1) and ^1H (F2), respectively. Assignment of backbone resonances was obtained by analyzing the following spectra: HNCA, HNCACB, CBCA(CO)NH, HNCO (Ikura et al, 1990). The assignment of side chain resonances was obtained by analyzing HCCH-TOCSY, CCCONH, C13 NOESY, and CTHSQC (Fig. 1B). The NMR data were processed using NMRPipe (Delaglio et al, 1995) and analyzed using Sparky NMRFAM (Lee et al, 2015).

Assignments and Data Deposition

Two-dimensional NMR spectra of ACTN_EF34 bound to unlabeled Ca_v1.2 IQ peptide, ^{15}N - ^1H HSQC (Fig. 1A) and constant-time ^{13}C - ^1H HSQC (Fig. 1B), are presented to illustrate representative NMR assignments for backbone and side-chain methyl resonances, respectively. NMR assignments were based on 3D heteronuclear NMR spectra recorded from $^{13}\text{C}/^{15}\text{N}$ -labeled ACTN_EF34 (residues 824-892) bound to unlabeled IQ peptide. The ACTN_EF34 protein sample in this study consisted of 68 native residues (residues 824-892) along with a 6His-tag and linker (MGSSHHHHHSSGLVPRGSHM). The NMR spectra exhibited well-dispersed peaks with uniform intensities indicative of a stably folded structure. A few amide resonances exhibit noteworthy downfield shifts, including T844, G869, and L877 that are flanked by nearby aromatic rings (Y842, Y867 and Y879 respectively), which may explain the large ring current shifts. All backbone resonances (^1HN , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO) and 95% of side-chain ^1H and ^{13}C resonances were assigned. The chemical shift assignments (^1H , ^{15}N , ^{13}C) of ACTN_EF34 bound to unlabeled Ca_v1.2 IQ peptide have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 25902.

The secondary structure of ACTN_EF34 was calculated based on the chemical shift index (Wishart et al, 1992) of each assigned amino acid residue and ANN-Secondary structure

prediction using TALOS (Shen et al, 2009) (Fig. 2A). As expected, ACTN_EF34 contains two EF-hands (EF3 and EF4) that each have two α -helices. For EF3, the helices are named $\alpha 1$ (residues 825–836) and $\alpha 2$ (residues 845–851). For EF4, the helices are named $\alpha 3$ (residues 854–862) and $\alpha 4$ (residues 879–886) depicted by purple cylinders in Fig. 2B. In addition, the two EF-hands are connected by an antiparallel β -sheet formed between strand $\beta 1$ (residues 841–843) and strand $\beta 2$ (residues 876–878) shown by blue arrows in Fig. 2B. The secondary structure of ACTN_EF34 is identical to that of the corresponding EF-hand region in the NMR structure of α -actinin-2 (Atkinson et al, 2001). The NMR assignments of ACTN_EF34 presented here are an important first step toward determining the full three-dimensional structure of ACTN_EF34 bound to Ca_v1.2.

Acknowledgments

We thank Jeff Walton and Bennett Addison for technical support and help with NMR experiments. Work supported by NIH grants (EY012347) to J.B.A and (AG017502) to J.W.H.

References

- Atkinson RA, Joseph C, Kelly G, Muskett FW, Frenkiel TA, Nietlispach D, Pastore A. Ca²⁺-independent binding of an EF-hand domain to a novel motif in the alpha-actinin-titin complex. *Nat Struct Biol.* 2001; 8:853–857. [PubMed: 11573089]
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeiffer J, Bax A. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR.* 1995; 6:277–293. [PubMed: 8520220]
- Gao T, Bunemann M, Gerhardstein BL, Ma H, Hosey MM. Role of the C terminus of the alpha 1C (Ca_v1.2) subunit in membrane targeting of cardiac L-type calcium channels. *J Biol Chem.* 2000; 275:25436–25444. [PubMed: 10816591]
- Hall DD, Dai S, Tseng PY, Malik Z, Nguyen M, Matt L, Schnizler K, Shephard A, Mohapatra DP, Tsuruta F, Dolmetsch RE, Christel CJ, Lee A, Burette A, Weinberg RJ, Hell JW. Competition between α -actinin and Ca²⁺-calmodulin controls surface retention of the L-type Ca²⁺ channel Ca_v1.2. *Neuron.* 2013; 78:483–497. [PubMed: 23664615]
- Hell JW, Westenbroek RE, Warner C, Ahljianian MK, Prystay W, Gilbert MM, Snutch TP, Catterall WA. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel $\alpha 1$ subunits. *J Cell Biol.* 1993; 123:949–962. [PubMed: 8227151]
- Ikura M, Kay LE, Bax A. A novel approach for sequential assignment of ¹H, ¹³C, and ¹⁵N spectra of proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. Application to calmodulin. *Biochemistry.* 1990; 29:4659–4667. [PubMed: 2372549]
- Lee W, Tonelli M, Markley JL. NMRFAM-SPARKY: enhanced software for biomolecular NMR spectroscopy. *Bioinformatics.* 2015; 31:1325–1327. [PubMed: 25505092]
- Shen Y, Delaglio F, Cornilescu G, Bax A. TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. *J Biomol NMR.* 2009; 44:213–223. [PubMed: 19548092]
- Turner M, Zhang Y, Carlson HL, Stadler HS, Ames JB. Chemical shift assignments of mouse HOXD13 DNA binding domain bound to duplex DNA. *Biomolecular NMR assignments.* 2015; 9:267–270. [PubMed: 25491407]
- Vogl AM, Brockmann MM, Giusti SA, Maccarrone G, Vercelli CA, Bauder CA, Richter JS, Roselli F, Hafner AS, Dedic N, Wotjak CT, Vogt-Weisenhorn DM, Choquet D, Turck CW, Stein V, Deussing JM, Refojo D. Neddylaton inhibition impairs spine development, destabilizes synapses and deteriorates cognition. *Nature neuroscience.* 2015; 18:239–251. [PubMed: 25581363]
- 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]

Fig. 1.

Two-dimensional NMR spectra ^1H , ^{15}N -HSQC (A) and ^1H , ^{13}C -HSQC (B) of ^{15}N , ^{13}C -labeled ACTN_EF34 bound to unlabeled $\text{Ca}_v1.2$ IQ at pH 7.5 recorded at 800-MHz ^1H frequency. Side chain amide resonances of Asn and Gln are connected with solid lines. An expanded view of the crowded spectral regions (inside the dashed box, panel B) are shown as inset below panel B. Representative assignments are indicated; complete assignments are available as BMRB accession no. 25902.

Fig. 2. Primary sequence and secondary structure of ACTN_EF34 bound to Ca_v1.2 IQ. (A) ANN-secondary structure probability plotted as a function of residue number. (B) Sequence alignment of the EF-hand region of α-actinin-1 and α-actinin-2. Secondary structure elements (cylinder for helix and arrow for b-strand) were calculated on the basis of CSI and sequential NOE patterns. Non-conserved residues are highlighted in red.