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Backbone ^1H , ^{13}C , and ^{15}N resonance assignments of guanylyl cyclase activating protein-1, GCAP1

Sunghyuk Lim,

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

Igor V. Peshenko,

Basic Sciences, Pennsylvania College of Optometry, Salus University, Elkins Park, PA 19027, USA

Alexander M. Dizhoor, and

Basic Sciences, Pennsylvania College of Optometry, Salus University, Elkins Park, PA 19027, USA

James B. Ames

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

James B. Ames: ames@chem.ucdavis.edu

Abstract

Guanylyl cyclase activating protein 1 (GCAP1), a member of the neuronal calcium sensor subclass of the calmodulin superfamily, confers Ca^{2+} -dependent activation of retinal guanylyl cyclase that regulates the visual light response. GCAP1 is genetically linked to retinal degenerative diseases. We report backbone NMR chemical shift assignments of Ca^{2+} -saturated GCAP1 (BMRB no. 18026).

Keywords

GCAP1; Phototransduction; Calcium; EF-hand; Guanylate cyclase; NMR

Biological context

Guanylyl cyclase activating proteins (GCAPs) belong to the neuronal calcium sensor (NCS) branch of the calmodulin superfamily (Ames et al. 1996; Burgoyne 2007) and regulate Ca^{2+} -sensitive activity of retinal guanylyl cyclase (RetGC) in rod and cone cells (Palczewski et al. 1994). Phototransduction in retinal rods and cones is modulated by intracellular Ca^{2+} sensed by GCAPs (Palczewski et al. 2000) and defects in Ca^{2+} signaling by GCAPs are widely linked to retinal diseases (Kitiratschky et al. 2009; Payne et al. 1998).

The GCAPs (GCAP1 (Palczewski et al. 1994), GCAP2 (Dizhoor et al. 1995), GCAP3 (Imanishi et al. 2002) and GCAP4-8 (Imanishi et al. 2004) are all ~200-residue proteins

containing four EF-hand motifs (Fig. 1) and a covalently attached N-terminal myristoyl group. Mg^{2+} binds to three EF-hands (EF2, EF3 and EF4) in the light, when cytosolic Ca^{2+} levels are low, to activate RetGC. Ca^{2+} also binds functionally to these three EF-hands in the dark, when Ca^{2+} levels are high, to inhibit RetGC (Lim et al. 2009; Peshenko and Dizhoor 2007). The first EF-hand (EF1) is unable to bind Ca^{2+} or Mg^{2+} due to conserved substitutions in the binding loop (Cys29 and Pro30 in GCAP1). The x-ray crystal structure of Ca^{2+} -bound GCAP1 (Stephen et al. 2007) and NMR structure of GCAP2 (Ames et al. 1999) clearly showed that Ca^{2+} is bound at EF2, EF3 and EF4. In addition, the N-terminal myristoyl group in GCAP1 is buried inside the Ca^{2+} -bound protein flanked by hydrophobic residues at the N- and C-termini (see residues highlighted blue in Fig. 1). The structure of the RetGC-activator form of GCAPs (Mg^{2+} -bound/ Ca^{2+} -free state) is currently unknown.

Atomic resolution structures of GCAPs in both the Mg^{2+} -bound (activator) and Ca^{2+} -bound (inhibitor) states are needed to elucidate their Ca^{2+} -induced conformational changes that control activation of RetGC during visual recovery. We report here detailed NMR resonance assignments for Ca^{2+} -bound GCAP1 as a first step toward this end.

Methods and experiments

Expression and purification of GCAP1

Uniformly ^{15}N - or $^{15}N/^{13}C$ -labeled unmyristoylated GCAP1 was expressed in *E. coli* strain, BL21(DE3) transformed with the plasmid encoding GCAP1 by growing it in M9 medium supplemented with ^{15}N - NH_4Cl and/or $^{13}C_6$ -glucose, and 100 $\mu g/ml$ ampicillin. Protein expression was induced by adding 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) to cells and growing for 5 h at 25°C. Typically, a 1-l culture yields about 10 mg of purified protein. Detailed procedures for purifying GCAP1 are described elsewhere (Lim et al. 2009). Triply labeled (perdeuterated) $^2H/^{15}N/^{13}C$ -labeled GCAP1 was expressed in the similar way described above with the exception that 1H_2O and $^{13}C_6$ -glucose were substituted with 2H_2O and $^{13}C_6$ - 2H_7 -glucose.

NMR spectroscopy

Samples of recombinant Ca^{2+} -bound unmyristoylated GCAP1 (0.7 mM) were prepared in 93%/7% H_2O/D_2O with 5 mM TRIS (pH 7.4), 5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM DTT, and 50 mM octylglucoside. NMR experiments were conducted using Bruker Advance 600 or 800 MHz spectrometers equipped with a triple resonance cryogenic probe. All experiments were performed at 317 K. Backbone chemical shift assignments were obtained using ^{15}N -HSQC, HNCB, HNCA, HNCACB, HNCOC, HNCOCACB spectra. NMR data were processed using NMRPipe (Delaglio et al. 1995) software package and analyzed using SPARKY.

Assignments and data deposition

Figure 2 presents HSQC spectra of Ca^{2+} -bound GCAP1 at pH 7.4 to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^2H/^{13}C/^{15}N$ -labeled GCAP1 (residues 2–204). The protein sample in this study consists of 204 native residues with an unmyristoylated N-terminus 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. The terminal 18 residues at both the N- and C-terminus are unstructured based on their chemical shift index (Wishart et al. 1992). More than 90% of the backbone resonances (^1HN , ^{15}N , ^{13}Ca , $^{13}\text{C}\beta$, and ^{13}CO) were assigned. The chemical shift assignments (^1H , ^{15}N , ^{13}C) of Ca^{2+} -bound GCAP1 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 18026.

The chemical shift index (CSI) of each amino acid residue reveals a protein secondary structure in GCAP1 in solution (Fig. 1) similar to that observed previously in the x-ray crystal structure (Stephen et al. 2007) and NMR structure of GCAP2 (Ames et al. 1999). GCAP1 contains 10 α -helices and two antiparallel β -sheets ($\alpha 1$: 18–29; $\beta 1$: 33–35; $\alpha 2$: 36–43; $\alpha 3$: 50–63; $\beta 2$: 70–72; $\alpha 4$: 73–83; $\alpha 5$: 89–99; $\beta 3$: 106–108; $\alpha 6$: 109–119; $\alpha 7$: 130–141; $\beta 4$: 149–151; $\alpha 8$: 152–160; $\alpha 9$: 163–171; $\alpha 10$: 175–183). The NMR assignments reported here for Ca^{2+} -bound GCAP1 are overall quite different from those reported previously for other NCS proteins, recoverin (Ames et al. 1997) and NCS-1 (Lim et al. 2011). The most noteworthy chemical shift differences are observed for non-conserved residues known to interact with the N-terminal myristoyl group (highlighted blue in Fig. 1), consistent with the recent proposal that N-terminal myristoylation creates a unique binding site environment in each highly homologous NCS protein (Lim et al. 2011).

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Fig. 1.

Alignment of the primary sequence of bovine GCAP1, GCAP2, GCAP3 and recoverin.

Secondary structural elements indicated schematically were derived from analysis of NMR data ($^3J_{\text{H}^{\text{N}}\text{H}^{\text{C}}}$, chemical shift index (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*

Fig. 2. Two-dimensional ^{15}N -HSQC NMR spectra of Ca^{2+} -bound GCAP1 (a) and expanded view of spectrally crowded region (b) recorded at 800 MHz proton frequency. Side chain amide resonances of Asn and Gln are connected with *solid lines*. Representative resonance assignments are indicated and the complete list of assignments can be found at the BMRB repository (accession no. 18026)