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Chemical Shift Assignments of Retinal Guanylyl Cyclase Activating Protein 5 (GCAP5)

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Abstract

Retinal membrane guanylyl cyclase (RetGC) in photoreceptor rod and cone cells is regulated by a family of guanylyl cyclase activating proteins (GCAP1-7). GCAP5 is expressed in zebrafish photoreceptors and promotes Ca^{2+} -dependent regulation of RetGC enzymatic activity that regulates visual phototransduction. We report NMR chemical shift assignments of the Ca^{2+} -free activator form of GCAP5 (BMRB no. 27705).

Keywords

Retinal guanylyl cyclase; RetGC; GCAP5; EF-hand; phototransduction

Biological Context

Guanylyl cyclase activating proteins (GCAP1-7) belong to a family of Ca^{2+} -binding proteins known as the neuronal calcium sensor (NCS) branch of the calmodulin superfamily (Burgoyne, 2007; Lim et al, 2014). Ca^{2+} -binding to the GCAP proteins regulates visual phototransduction in retinal photoreceptors (Palczewski et al, 1994). Light activation of retinal rod and cone cells causes cGMP-gated channels to close, which blocks the cytosolic entry of Ca^{2+} and generates a neural signal (Arshavsky & Burns, 2014). The light-induced decrease in cytosolic Ca^{2+} concentration is sensed by the binding of Ca^{2+} to the GCAPs that in turn modulates the Ca^{2+} -sensitive activation of RetGC needed to replenish cGMP levels during visual recovery (Koch & Helten, 2008; Koch & Stryer, 1988). Mutations in the GCAP proteins that disrupt their Ca^{2+} -sensitive activation of RetGC are genetically linked to retinal diseases (Jiang & Baehr, 2010; Payne et al, 1998).

GCAP5 in zebrafish photoreceptors not only serves as a Ca^{2+} sensor, but can also bind functionally to Fe^{2+} and Mg^{2+} , and the Fe^{2+} -bound GCAP5 inhibits RetGC (Lim et al, 2017). In essence, the Ca^{2+} -free/ Mg^{2+} -bound GCAP5 activates RetGC activity in light-adapted photoreceptors (Peshenko & Dizhoor, 2006), whereas the Ca^{2+} -bound and/or Fe^{2+} -bound GCAP5 strongly inhibits RetGC in dark-adapted photoreceptors (Peshenko & Dizhoor, 2007). Atomic resolution structures of GCAP5 in both the Ca^{2+} -free/ Mg^{2+} -bound (activator) and Ca^{2+} -bound/ Fe^{2+} -bound (inhibitor) states are needed to elucidate conformational changes that control light-dependent activation of RetGC during visual

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recovery. We report here NMR resonance assignments for the Ca^{2+} -free/ Mg^{2+} -bound activator form of GCAP5 as a first step toward determining its structure.

Methods and Experiments

Preparation of GCAP5.

Uniformly ^{15}N -labeled/ ^{13}C -labeled samples of recombinant myristoylated GCAP5 (residues 2-198) were prepared by co-expressing the GCAP5 D3N mutant and yeast N-myristoyl CoA transferase (NMT) in *E. coli* strain BL21(DE3) using M9 minimal media grown in the presence of ^{15}N -labeled ammonium chloride (0.5 g per liter) and ^{13}C -labeled glucose (3 g per liter) as described previously (Lim et al, 2016; Lim et al, 2017). The D3N mutation is required to enable myristoylation of GCAP5 by yeast NMT. The bacterial extract containing myristoylated GCAP5 was treated with 0.35 M ammonium sulfate and purified by Butyl-Sepharose chromatography (HiPrep Butyl FF 16/10, GE Healthcare). The GCAP5 protein was further purified by Q-Sepharose chromatography (HiTrap5 mL Q HP, GE Healthcare) and by size exclusion chromatography (HiLoad 26/600 Superdex, GE Healthcare). The final purified protein was more than 95% pure based on SDS-PAGE.

NMR spectroscopy.

Samples of myristoylated GCAP5 for NMR analysis consisted of native residues 2-198 (except for D3N) and did not contain any additional non-native residues or affinity tag. The NMR samples were prepared by exchanging the purified protein above into 3 mM MES (pH 6.5), 3 mM DTT- d_{10} , 3 mM MgCl_2 , 0.04% w/v NaN_3 , and 93% H_2O /7% D_2O . The final protein concentration was 0.5 mM. All NMR experiments were performed at 30°C on a Bruker Avance 600 MHz spectrometer equipped with a four channel interface and triple resonance cryogenic (TCI probe). The ^{15}N - ^1H HSQC (Fig. 1A) and constant-time ^{13}C - ^1H HSQC (Fig. 1C) spectra were recorded with 256×2048 complex points for $^{15}\text{N}(\text{F1})/^{13}\text{C}(\text{F1})$ and $^1\text{H}(\text{F2})$, respectively. Assignment of backbone resonances was obtained by analyzing the following spectra: HNCA, HNCACB, CBCA(CO)NH, HNCO (Ikura et al, 1990). Side chain resonances were assigned by analyzing HCCH-TOCSY (Ikura et al, 1991). 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 Ca^{2+} -free/ Mg^{2+} -bound GCAP5 (^{15}N - ^1H HSQC, Figs. 1A-B and ^{13}C - ^1H HSQC, Fig. 1C) illustrate representative NMR assignments determined from the analysis of 3D heteronuclear NMR spectra recorded from $^{13}\text{C}/^{15}\text{N}$ -labeled GCAP5. The NMR spectra of GCAP5 exhibited well-dispersed peaks with uniform intensities indicative of a stably folded structure. A few amide resonances exhibited noteworthy downfield shifts, including Q19, L33 and I70 that are flanked by nearby aromatic rings (W20, F35 and F72 respectively), which may explain the large ring current shifts (Fig. 1A). The downfield peak assigned to G68 is likely caused by a strong hydrogen bond between the backbone NH of G68 and side chain carboxyl group of D63 caused by Mg^{2+} binding to EF2 like that observed for GCAP1 (Lim et al, 2016). More than 86% of the main chain ^{13}C resonances

($^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO), 84% of backbone amide resonances (^1HN , ^{15}N), and 75% of methyl side chain resonances (Fig. 1C) were assigned. The unassigned residues (marked by an asterisk in Fig. 2B) had severely overlapped backbone amide resonances and/or weak NMR intensities that obscured their assignment. In particular, a stretch of 10 residues in the fourth EF-hand (residues 136-146) could not be assigned due to very weak NMR intensities, perhaps caused by exchange broadening due to conformational heterogeneity in this region. Indeed, previous studies on GCAP1 suggest significant Ca^{2+} -induced conformational changes involving residues in the fourth EF-hand (Lim et al, 2013). The chemical shift assignments (^1H , ^{15}N , ^{13}C) of GCAP5 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 27705.

The secondary structure of Ca^{2+} -free/ Mg^{2+} -bound GCAP5 was determined based on the chemical shift index (Wishart et al, 1992) and secondary structure prediction software using TALOS+ (Shen et al, 2009) (Fig. 2A). GCAP5 contains ten α -helices named H1 (residues 8-14), H2 (residues 18-26), H3 (residues 35-41), H4 (residues 49-62), H5 (residues 74-82), H6 (residues 87-95), H7 (residues 110-117), H8 (residues 129-135), H9 (residues 150-160) and H10 (residues 161-172) depicted by cylinders in Fig. 2B. The overall secondary structure of Ca^{2+} -free/ Mg^{2+} -bound GCAP5 is comprised of four EF-hand motifs that are similar in structure to the EF-hands observed previously in GCAP1 (Lim et al, 2016; Stephen et al, 2007) (highlighted in color in Fig. 2B). A short β -strand is observed in all four EF-hands of Ca^{2+} -bound GCAP1 (depicted as arrow in Fig. 2B) that is partially missing in the third and fourth EF-hands of Ca^{2+} -free GCAP5. A short β -strand can be seen in the second EF-hand of Ca^{2+} -free/ Mg^{2+} -bound GCAP5 (residues 69-71) because it is stabilized by Mg^{2+} binding at this site (Lim et al, 2016). By contrast, the third and fourth EF-hands of GCAP5 do not exhibit the expected β -strand perhaps due to a lack of Mg^{2+} -binding at these sites. Another noticeable difference is that the exiting helix of EF3 (H7) in GCAP5 is one turn longer than that of GCAP1. The final 25 residues from the C-terminus in GCAP5 are dynamically disordered and unstructured, in contrast to GCAP1 that contains an extra helix near the C-terminus. The NMR assignments of Ca^{2+} -free/ Mg^{2+} -bound GCAP5 presented here are an important first step toward determining its full three-dimensional structure.

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Fig. 1:
(A) Two-dimensional ^{15}N - ^1H HSQC spectrum of ^{15}N -labeled Ca^{2+} -free/ Mg^{2+} -bound GCAP5 recorded at 600-MHz ^1H frequency and at 30 °C. (B) Expanded view of assignments from the spectrally crowded central region. (C) Constant-time ^{13}C - ^1H HSQC spectrum of ^{13}C -labeled Ca^{2+} -free/ Mg^{2+} -bound GCAP5. Representative chemical shift assignments are indicated by residue labels; complete assignments are available as BMRB accession no. 27705.

Fig. 2:

Primary and secondary structure of Ca^{2+} -free/ Mg^{2+} -bound GCAP5 and Ca^{2+} -bound GCAP1. (A) TALOS+ ANN-secondary structure probability for GCAP5 plotted as a function of residue number. (B) Secondary structure elements (cylinder for helix, line for random coil, and arrow for β -strand) for GCAP5 (shown above the amino acid sequence) were calculated on the basis of chemical shift index and sequential NOE patterns. The secondary structure of GCAP1 (below the sequence) was obtained from its crystal structure (Stephen et al, 2007). Residues marked with an asterisk are not assigned in GCAP5. The EF-hand motifs are highlighted in color (EF1: green, EF2: red, EF3: cyan, and EF4: yellow).