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ARTICLE

^{1}H, ^{15}N, and ^{13}C chemical shift assignments of calcium-binding protein 1 with Ca^{2+} bound at EF1, EF3 and EF4

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Abstract Calcium-binding protein 1 (CaBP1) regulates inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) and a variety of voltage-gated Ca^{2+} channels in the brain. We report complete NMR chemical shift assignments of the Ca^{2+}-saturated form of CaBP1 with Ca^{2+} bound at EF1, EF3 and EF4 (residues 1–167, BMRB no. 16862).

Keywords Calcium · CaBP1 · Magnesium · EF-hand · Calcium channel

Biological context

Neuronal calcium-binding proteins (CaBPs) belong to a subclass of the calmodulin (CaM) superfamily that regulates various Ca^{2+} channel targets in the brain and retina (Haeseleer et al. 2000). Multiple isoforms of CaBPs are localized in different neuronal cell types and perform specialized roles in signal transduction (Haeseleer et al. 2004). The CaBP1 isoform regulates the Ca^{2+} dependent activity of inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) that serve as Ca^{2+} release channels on the endoplasmic reticulum membrane (Kasri et al. 2004). CaBP1 also regulates P/Q-type voltage-gated Ca^{2+} channels (Haeseleer et al. 2004), L-type channels, and the transient receptor potential channel, TRPC5 (Kinoshita-Kawada et al. 2005). CaBP1 contains four EF-hand motifs, but the second EF-hand (EF2) lacks critical residues required for high affinity Ca^{2+} binding (Wingard et al. 2005). Calcium-induced conformational changes in CaBP1 are important for promoting Ca^{2+}-dependent regulation of $InsP_3Rs$ (Li et al. 2009) and other channel targets. Three-dimensional structures and NMR assignments are now known for CaBP1 in the Mg^{2+}-bound, Ca^{2+}-free state (Li et al. 2009) and for the protein with Mg^{2+} bound at EF1 and Ca^{2+} bound at EF3 and EF4 (Li et al. 2009). However, the structure is not yet known for Ca^{2+}-saturated CaBP1 with Ca^{2+} bound at EF1, EF3 and EF4, which is a key signaling state for ion channel regulation. We report here the NMR assignments of CaBP1 with Ca^{2+}-bound at EF1, EF3 and EF4, as a first step toward elucidating its atomic-level structure and regulatory mechanism.

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Methods and experiments

Expression and purification of human CaBP1

All NMR experiments were performed on a small splice-variant of human s-CaBP1 referred to in this study as CaBP1. The recombinant CaBP1 protein was cloned into pET3b expression vector (Novagen) and over-produced in *Escherichia coli* strain BL21(DE3) as described previously (Wingard et al. 2005). $^{13}C/^{15}N$-labeled protein expression was induced by the addition of 0.5 mM IPTG at 37°C in M9 minimal medium containing $^{15}NH_4Cl$ and [U-^{13}C] glucose. Cells obtained from M9 cultures were disrupted by sonication. The cell lysate was centrifuged and the supernatant was loaded onto a Phenyl-Sepharose 4B column (Amersham Biosciences) and CaBP1 protein was purified as described (Wingard et al. 2005). Typically, 50 mg of purified protein was obtained from 1L culture. The protein identity and purity were confirmed by SDS–PAGE.

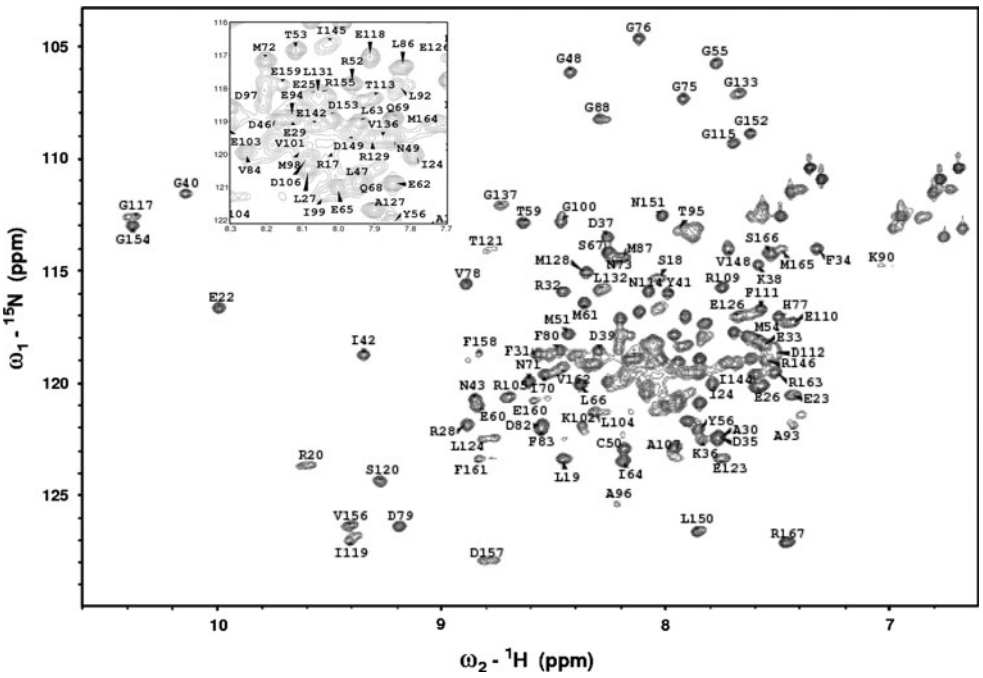

Fig. 1 Two-dimensional ^{15}N-^{1}H HSQC spectrum of Ca^{2+}-saturated CaBP1 recorded at 800-MHz ^{1}H frequency. The protein sample (1 mM) was uniformly labeled with nitrogen-15 and was dissolved in 0.3 ml of a 90% H_2O/10% [^{2}H] H_2O solution containing 10 mM [$^{2}H_{11}$] Tris (pH 7.4), 1 mM [$^{2}H_{10}$] dithiothreitol, and 5 mM $CaCl_2$. Under these conditions, CaBP1 contains Ca^{2+} bound at EF1, EF3 and EF4 (Wingard et al. 2005)

NMR spectroscopy

Samples for NMR analysis were prepared by dissolving ^{13}C/^{15}N-labeled CaBP1 (1 mM) in 0.3 ml of a 90/10% H_2O/D_2O or 100% D_2O with 10 mM [$^{2}H_{11}$] Tris (pH 7.4), and 5 mM $CaCl_2$. Under these conditions, CaBP1 contains Ca^{2+} bound at EF1, EF3 and EF4 (Wingard et al. 2005). All NMR spectra were recorded at 37°C on a Bruker Avance 600 or 800 MHz spectrometers with triple-resonance cryogenic probe. Backbone and side-chain chemical shift assignments were accomplished with ^{15}N-HSQC, HNCO, CACBCONH, HNCACB, HBHACONH, ^{15}N-HSQC-TOCSY, HCCH-TOCSY spectra. ^{1}H chemical shift assignments of aromatic side chains were based on HBCBCGCDHD, ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H NOESY spectra. Stereospecific assignments of valine and leucine methyl group were obtained by performing ^{13}C-CT-HSQC experiments on protein samples with directed ^{13}C labeling (Neri et al. 1989). NMR data were processed using NMR Pipe software package (Delaglio et al. 1995) and analyzed using SPARKY.

Assignments and data deposition

Figure 1 presents ^{15}N HSQC spectrum of Ca^{2+}-saturated CaBP1 (with Ca^{2+} bound at EF1, EF3 and EF4) to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on ^{13}C/^{15}N-labeled CaBP1 (residues 1–167). The first 15 residues from the amino-terminus exhibited weak NMR signals that could not be assigned in our analysis. The remaining residues (16–167) exhibited strong NMR signals with uniform intensities, indicative of a well-defined three-dimensional protein structure in this region. More than 95% of the backbone resonances (^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}CO), ~82% of aliphatic side chain resonances, and ~50% of aromatic side-chain resonances were assigned for residues in structured regions, including stereospecific assignment of valine and leucine methyl resonances. Three downfield shifted amide proton resonances at ~10.5 ppm are assigned to Gly40, Gly117 and Gly154 and verify that Ca^{2+} is bound at EF1, EF3 and EF4, consistent with our earlier Ca^{2+} binding analysis (Wingard et al. 2005). Chemical shift index analysis (Wishart and Sykes 1994) indicates the secondary structure of Ca^{2+}-saturated CaBP1 is nearly identical to that observed for Mg^{2+}-bound CaBP1 (Li et al. 2009). The chemical shift assignments (^{1}H, ^{15}N, ^{13}C) of Ca^{2+}-saturated CaBP1 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 16862.

Figure 2 presents amide chemical shift difference between residues of Ca^{2+}-free and Ca^{2+}-saturated CaBP1. The Ca^{2+}-saturated CaBP1 chemical shifts of residues in EF2 show almost no effect of Ca^{2+} within experimental error, consistent with a lack of Ca^{2+} binding at EF2. The largest Ca^{2+}-induced chemical shift changes are observed for residues in the Ca^{2+}-binding loops of EF3 and EF4, consistent with high affinity Ca^{2+} binding at these sites. Much smaller Ca^{2+}-induced chemical shift changes are observed for residues in the binding loop region of EF1. The relatively small Ca^{2+}-induced chemical shift changes

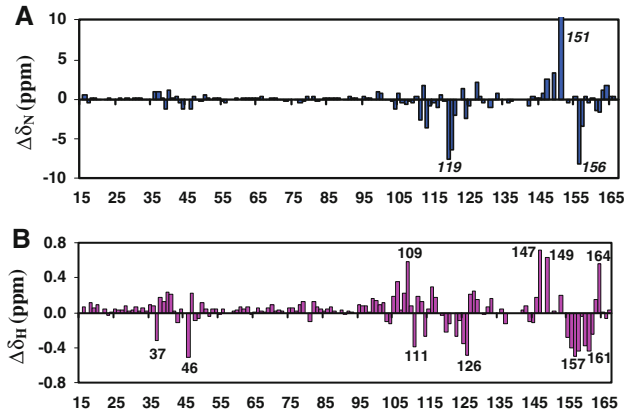

Fig. 2 Amide chemical shift differences between Ca^{2+}-free (Li et al. 2009) and Ca^{2+}-saturated CaBP1 (this study). **a** shows $\Delta\delta_N$(ppm) = δ_N(Ca^{2+}-free)—δ_N(Ca^{2+}-bound) and **b** shows $\Delta\delta_H$(ppm) = δ_H(Ca^{2+}-free)—δ_H (Ca^{2+}-bound). Residues in the EF-hand binding loops display the largest chemical shift differences and are highlighted

for residues in EF1 suggest that EF1 might remain in a closed conformation even in the Ca^{2+} bound state, in contrast to the Ca^{2+}-induced open conformation observed previously for EF3 and EF4 (Li et al. 2009). The Ca^{2+}-bound closed conformation for EF1 in CaBP1 is reminiscent of a Ca^{2+}-bound closed conformation seen previously in cardiac troponin C (Wang et al. 2002). We propose that the closed conformation of EF1 in CaBP1 would prevent adventitious binding to protein targets like that shown for cardiac troponin C (Wang et al. 2002), and therefore might be functionally important for promoting highly specific target binding to CaBP1.

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