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¹H, ¹⁵N, and ¹³C Chemical Shift Assignments of Calcium-binding Protein 1 (CaBP1)

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Abstract

Calcium-binding protein 1 (CaBP1) regulates inositol 1,4,5-trisphosphate receptors (InsP₃Rs) and a variety of voltage-gated Ca²⁺ channels in the brain. We report complete NMR chemical shift assignments of Ca²⁺-free CaBP1 (residues 1–167, BMRB no. 15197).

Keywords

calcium; CaBP1; magnesium; EF-hand; calcium channel

Biological Context

Neuronal calcium-binding proteins (CaBP1–5 (Haeseleer et al., 2000, Haeseleer et al., 2002)) are a sub-branch of the calmodulin EF-hand superfamily, expressed exclusively in the brain and retina where they regulate a variety of calcium channel targets in signal transduction. The calcium-binding protein 1 (CaBP1), also termed caldendrin (Laube et al., 2002), is comprised of at least two splice-variants (s-CaBP1 and l-CaBP1) with variable residues near the N-terminus (Haeseleer et al., 2000). Both forms of CaBP1 modulate the Ca²⁺-sensitive activity of inositol 1,4,5-trisphosphate receptors (InsP₃Rs) that serve as Ca²⁺ release channels on the endoplasmic reticulum membrane (Yang et al., 2002, Kasri et al., 2004). The Ca²⁺-free and Mg²⁺-bound form of CaBP1 facilitates channel opening, whereas Ca²⁺-bound CaBP1 induces channel closure. CaBPs also regulate a variety of voltage-gated Ca²⁺ channels (Lee et al., 2002, Haeseleer et al., 2004) and the transient receptor potential channel, TRPC5 (Kinoshita-Kawada et al., 2005). CaBP4 is genetically linked to retinal degenerative diseases (Zeitz et al., 2006). Thus, the CaBP proteins are emerging as a family of calcium sensors that modulate the activity of diverse neuronal Ca²⁺ channels in signal transduction and disease. We report here NMR assignments of Ca²⁺-free and Mg²⁺-bound CaBP1, as a first step toward elucidating the atomic-level structures and regulatory mechanisms of CaBPs generally.

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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 CaBP1, previously termed s-CaBP1 (Haeseleer et al., 2000) and referred to in this study as CaBP1. The recombinant CaBP1 protein (residues 1–167) was cloned into pET3b expression vector (Novagen) and over-produced in *Escherichia coli* strain BL21 (DE3) as described previously (Wingard et al., 2005). Briefly, $^{13}\text{C}/^{15}\text{N}$ -labeled protein expression was induced by the addition of 0.5 mM IPTG at 37 °C in M9 minimal medium containing $^{15}\text{NH}_4\text{Cl}$ and $[\text{U-}^{13}\text{C}]$ glucose. Cells obtained from M9 cultures were lysed by sonication. The 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, 25 mg of purified protein was obtained from a 1-liter culture. The identity and integrity of the final protein sample was confirmed by MALDI/TOF and SDS-PAGE.

NMR Spectroscopy.

Samples for NMR analysis were prepared by dissolving $^{13}\text{C}/^{15}\text{N}$ -labeled CaBP1 (0.5 mM) in 0.3 ml of a 95% $\text{H}_2\text{O}/5\%$ $[\text{2H}] \text{H}_2\text{O}$ solution containing 10 mM $[\text{2H}_{11}]$ Tris (pH 7.4), 5 mM $[\text{2H}_{10}]$ TCEP, 0.1 M KCl, and 5 mM MgCl_2 . 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 probe. The ^{15}N - ^1H HSQC spectra (see Fig. 1) were recorded on samples of ^{15}N -labeled CaBP1 (in 95% H_2O , 5% $^2\text{H}_2\text{O}$). The number of complex points and acquisition times were: 256, 180 ms (^{15}N (F_1)), and 512, 64 ms (^1H (F_2)). Assignment of backbone and side-chain resonances were obtained from the following spectra: HNCACB, HN(CO)CACB, HNCB, CBCACONH, HBHACONH, C(CO)NH-TOCSY, H(CCO)NH-TOCSY, HCCH-TOCSY, and H(CCH)-COSY. The NMR data were processed using NMRPipe and analyzed using NMRview.

Assignments and Data Deposition

Figure 1 presents $^1\text{H}/^{15}\text{N}$ HSQC spectrum of Ca^{2+} -free/ Mg^{2+} -bound CaBP1 to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^{13}\text{C}/^{15}\text{N}$ -labeled CaBP1 (residues 1–167). The protein sample in this study consists of 167 native residues and does not contain any affinity tags or extra residues. The first 10 residues from the amino-terminus exhibited weak NMR signals and could not be assigned in our analysis. The remaining residues (12–167) exhibited strong NMR signals with uniform intensities, indicative of a well-defined three-dimensional protein structure in this region. More than 95% the backbone resonances (^1HN , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO) and ~80% of aliphatic side chain resonances were assigned, including stereospecific assignment of valine and leucine methyl groups. More than 75% of aromatic side chain resonances were assigned except for residues F111 and F158 that exhibited overlapping ^1H and ^{13}C chemical shifts. The chemical shift assignments (^1H , ^{15}N , ^{13}C) of CaBP1 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 15197.

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FIG. 1. Two-dimensional ^{15}N - ^1H HSQC spectrum of Ca^{2+} -free/ Mg^{2+} -bound CaBP1 recorded at 600-MHz ^1H frequency. The protein sample was uniformly labeled with nitrogen-15. Side-chain amide proton resonances (NH_2) are connected by dotted lines.