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Letter to the Editor: Sequence-specific ^{1}H, ^{15}N and ^{13}C resonance assignments for the third EH domain of Eps15

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Biological context

Eps15 homology (EH) domains are a family of domains that mediate protein interactions for processes including endocytosis, actin cytoskeleton organization, and signal transduction (reviewed in Di Fiore et al., 1997). EH domains are ∼95 residues in length with ∼50% sequence homology and share a double EF-hand fold (Di Fiore et al., 1997; de Beer et al., 1998). A canonical calcium-binding sequence is usually found in the second EF-hand of EH domains. However, EH_3 is unusual in that it is one of the few EH domains that has a calcium-binding sequence in the first EF-hand. Here we present the assignments for EH_3, the first report of ^{1}H, ^{15}N, and ^{13}C resonance assignments for an EH domain with a calcium-binding site in the first EF-hand.

Methods and results

Escherichia coli B834 pLys S cells were transformed with the pRSET expression vector (Invitrogen) containing amino acids 214–317 of human Eps15. Unlabeled EH_3 was produced by isopropyl β-D-thiogalactopyranoside (IPTG) induction in Luria–Bertani broth (LB) supplemented with vitamins and glucose. ^{15}N or ^{13}C, ^{15}N labeled EH_3 was produced with IPTG induction in minimal media supplemented with vitamins, $^{15}NH_4Cl$ and ^{15}N-methionine or $^{15}NH_4Cl$, ^{15}N,^{13}C-methionine and ^{13}C-glucose, respectively. The cell cultures were grown at 37 °C, induced with IPTG at an optical density (600 nm) of 0.8–1.0, and grown for an additional 5 h. The histidine tagged fusion protein was immobilized on Talon resin (Clontech) and eluted by cleavage with enterokinase (Invitrogen). The protease was removed with EK-Away beads (Invitrogen). The yield of unlabeled and ^{15}N-labeled EH_3 was 30 mg L^{-1} and that of ^{15}N,^{13}C-labeled EH_3 was 5 mg L^{-1}. Samples containing 0.5–1 mM protein were prepared in 20 mM Tris-d_{11}, 100 mM KCl, 2 mM NaN_3, 2 mM $CaCl_2$, 10 μM 4-amidinophenylmethane sulfonyl fluoride (APMSF), 100 μM to 10 mM perdeuterated dithiothreitol (d-DTT), and 10% or 99.99% D_2O at pH 7.8. EH_3 forms apparent disulfide-linked dimers through C^{274} that are reduced by the repeated addition of 10 mM d-DTT and incubation at 37 °C for 1 h as determined by diffusion coefficients measured with pulsed field gradient NMR experiments (Altieri et al., 1995).

NMR experiments were performed at 25 °C on Varian INOVA 500 MHz and 600 MHz spectrometers equipped with triple resonance shielded probes. Spectra were processed with the NMRPipe package (Delaglio et al., 1995) and analyzed with PIPP (Garret et al., 1991) and in-house software on Sun Microsystems and Silicon Graphics workstations. The CBCA(CO)NH, HNCACB, and HNCO (Muhandiram et al., 1994) experiments were used to assign main-chain ^{1}H, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ resonances and also to establish segments of sequential connectivity (Figure 1). $^{1}H_\alpha$ and side chain ^{13}C and ^{1}H resonances were assigned using HCC-TOCSY, CCC-TOCSY (Grzesiek et al., 1993), and HCCH-TOCSY (Kay et al., 1993) experiments. Aromatic side chain resonances were assigned using 2D spectra correlating C_β with H_δ or H_ϵ (Yamazaki et al., 1993).

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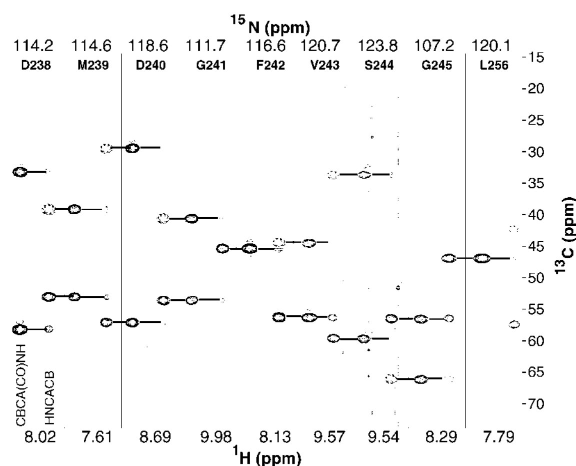

Figure 1. Representative strips from the CBCA(CO)NH and HNCACB spectra showing connectivities for residues D238 to L246 in the first EF-hand loop of EH_3. Backbone amide ^{1}H and ^{15}N chemical shifts are shown below and above each panel.

Gln side chain ^{15}N and ^{1}H were assigned using a 3D-^{15}N-NOESY experiment (Marion et al., 1989).

Extent of assignments and data deposition

The EH_3 construct includes five N-terminal residues from the vector; these residues were not included in the assignment statistics. The ^{15}N and ^1H_N resonances for 91 of 101 possible (non-Pro residues) backbone amides were assigned. All Gln side chain NH_2 resonances and side chain ^{13}CO resonances were assigned. 93.5% of C_α, 91.7% C_β, and 83.3% of backbone $^{13}C'$ resonances were assigned. The resonance assignments for the aromatic side chains of W218, Y228, F252, and F286 are complete. The H_ζ and C_ζ resonances for F232, F242, and F282 could not be assigned due to overlap. The three His $^1H_{\delta 2}$, $^1H_{\epsilon 1}$, and $^{13}C_{\epsilon 1}$ resonances were assigned. The γ methyl protons of Val residues 219, 243, and 248 and the δ methyl protons for Leu residues 233, 253, 261, 277, 288, and 293 have been stereospecifically assigned. No assignments were made for the N-terminal residues K214, D215, and K216 or for S259, which is preceded by a proline.

Two backbone ^{1}H and ^{15}N resonances for S311 and D312 are observed; the reported values reflect the major conformation as determined by peak intensity. The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4381.

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