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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignments of calmodulin complexed with the calmodulin-binding domain of calcineurin

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Biological context

Calmodulin (CaM) is a 148-residue protein which regulates a large number of key enzymes and controls a wide spectrum of important biological responses. Unraveling its diversity in activation mechanisms and target recognition has received extensive attention (for a review, Hoeflich and Ikura, 2002). Calcineurin (CaN), also known as protein phosphatase 2B (PP2B), is a Ca^{2+}/CaM-dependent Ser/Thr protein phosphatase comprising two subunits, A and B. CaN is critically involved in T cell activation (for a review, Klee, 1998). The immunosuppressive drugs, cyclosporin A (CsA) and FK506 form complexes with cyclophilin and FK506-binding protein (FKBP), respectively. The complexes bind to and inhibit CaN activity and prevent the transcription of genes response for T lymphocyte activation. The crystal structures of catalytic domain of CaN bound with FKBP12-FK506 (Griffith et al., 1995; Kissinger et al., 1995), and with CsA-cyclophilin (Jin and Harrison, 2002) have been solved, however, lacking structural information on the Ca^{2+}/CaM dependent regulatory domain of CaN. In order to elucidate the regulatory role of CaM on CaN, and to gain more insight into the interactions between CaM and CaN from structural point of view, we have applied multidimensional heteronuclear NMR techniques to study the structure of CaM bound with the CaM-binding domain of CaN. It is likely that the peptide model can be the excellent mimetic for the interaction of CaM with CaM-dependent enzymes (Kranz et al., 2002). Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments of CaM when bound with the CaM-binding domain of CaN.

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Methods and experiments

The cDNA of vertebrate CaM was subcloned into a modified pET29a expression vector, then transformed into *E. coli* BL21 (DE3) host and expressed. The recombinant CaM was purified by a hydrophobic, phenyl sepharose column. An unlabeled 24-residue peptide, NaNp, which corresponds to the sequence of the CaM-binding domain of CaN (residue 391 to 414 in bovine CaN A subunit), was obtained commercially from Annaspec Inc (San Jose, CA).

The NMR sample was prepared in the following procedures: 10 mg ^{13}C, ^{15}N-labeled CaM was dissolved into 1 ml solution containing 90% H_2O/10% D_2O, 100 mM KCl, 5 mM $CaCl_2$, 0.02% NaN_3, at pH 6.5. The appropriate amount of unlabelled CaNp stock solution was added drop wise into the CaM solution with gentle mixing to ensure the CaM/CaNp complex formation. The solution was then concentrated by Centricon-10 ultrafiltration apparatus (Millipore Inc) to a final concentration of CaM/CaNp complex about 1.1 mM. NMR experiments were performed at 310 K on Bruker AVANCE-500 or -600 spectrometers. All the spectra were processed by XWINNMR and analyzed by AURELIA. All the chemical shifts were

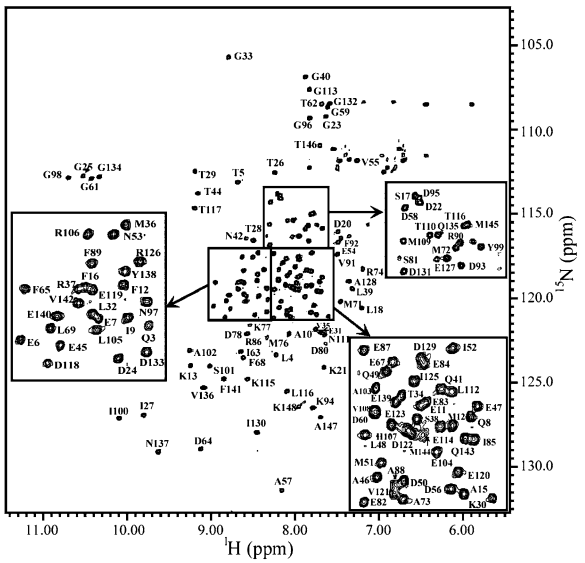

Figure 1. 2D ^{1}H-^{15}N-HSQC spectrum of 1.2 mM uniformly ^{15}N-enriched CaM complexed with the CaM-binding domain of CaN in 100 mM KCl, at pH 6.5, 310 K. Assignments of the backbone amide protons and ^{15}N cross peaks are indicated in the figure. The expanded region indicated by arrow is for the purpose of clarity.

referenced and calibrated to internal DSS as described (Wishart et al., 1995).

Backbone sequential assignments of CaM in the bound form were obtained using the following heteronuclear 3D spectra: HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, and CBCA(CO)NH. 2D ^{1}H-^{15}N-HSQC spectra of specifically ^{15}N-labeled (^{15}N-Lys and ^{15}N-Met) CaM were obtained to facilitate and confirm the assignments. Partial spin system and sequential assignments of the CaNp when bound with CaM were obtained 2D isotope-filtered experiments (Ikura and Bax, 1992).

Extent of assignments and data deposition

All of the ^{1}H and ^{15}N backbone resonances of CaM were assigned, except Lys^{75} and Thr^{79} and the N-terminal Ala^{1} and Asp^{2} residues. All the $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO resonances were assigned for all residues except Ala^{1} and Thr^{79} residues. All of the $^{1}H^{\alpha}$ and $^{1}H^{\beta}$ resonances were assigned based on 3D HBHA(CBCACO)NH and ^{1}H-^{15}N-TOCSY-HSQC spectra except Ala^{1}, Phe^{65}, Asp^{78}, Lys^{148} residues. Aliphatic side-chain proton and carbon chemical shifts were assigned primarily from C(CO)NH, H(CCO)NH, and ^{1}H-^{15}N-TOCSY-HSQC spectra. About 85% of the aliphatic side-chain proton resonances and 82% of side chain carbon were assigned. Assignments on the aromatic rings have not been performed. We have employed the consensus chemical shift index (CSI) (Wishart and Sykes, 1994) to identify the secondary structure of CaM when bound with CaNp. The CSI analysis suggest 8 α-helix regions (residues 6–18, 29–38, 45–54, 65–73, 79–92, 102–111, 118–127, and 138–144), 4 β-strand regions (residues 26–28, 62–64, 99–101, and 135–137). The predicted secondary structure distribution of bound CaM are the same as CSI analysis for the free Ca^{2+}-CaM except two differences in the central tether region, which predicted α-helices from residues 65–75, and 81–92 in free Ca^{2+}-CaM (BMRB accession number 547). The free CaNp has no regular secondary structure as judged by sharp NMR peaks and fast amide H/D exchange in D_2O solvent. The chemical shifts of α-protons of the bound CaNp are in the range of 3.49 to 4.58 ppm suggested the bound peptide is in a α-helical structure. Since the chemical shifts of α-, β- and amide protons are severely overlapping, only partial proton resonance assignments were achieved for the bound CaNp. Figure 1 shows the 2D ^{1}H-^{15}N-HSQC spectrum of uniformly ^{15}N-enriched CaM complexed with unlabelled CaNp. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6023.

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