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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of yeast *Saccharomyces cerevisiae* calmodulin in the Ca^{2+}-free state

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Abstract

Calmodulin (CaM) is a small Ca^{2+}-binding protein, which has been found in all of eucaryotic cells examined. CaMs isolated from various species have highly conserved amino acid sequence (more than 90% identical), and show the same biological functions. CaM isolated from the baker's yeast (*Saccharomyces cerevisiae*) (yCaM), however, shares only 60% identity in the amino acid sequence with CaM from vertebrate, and shows quite distinct conformational and biochemical properties compared with those of CaM from other species. The conformational details of yCaM, however, have not been revealed yet. We achieved the chemical shift assignments of yCaM (146 amino acids) in the apo-state using uniformly ^{15}N- and ^{13}C-labeled protein. Consequently, the resonances of 95% atoms in the backbone amides were successfully assigned.

Biological context

Calmodulin (CaM) is a small and ubiquitous eucaryotic protein (148 amino acids) which belongs to a family of Ca^{2+}-binding protein containing EF-hand Ca^{2+}-binding motifs. CaM binds four mols of Ca^{2+} in response to the rise in the intracellular Ca^{2+} concentration caused by extracellular stimuli and modulates the function of various target enzymes. The structures of this protein in both of the Ca^{2+}-binding and apo states were solved by X-ray crystallographic analysis and NMR structure determination, respectively (reviewed in Tjandra et al., 1999). CaM consists of the N-terminal and the C-terminal globular domain (N- and C-domain, respectively) connected by a flexible central linker. Each domain contains a pair of EF-hand joined with a short β-sheet. Any significant interaction has not been detected between the two domains, and Ca^{2+} binds to each domain independently. CaMs from a variety of species have highly conserved primary structure (more than 90% identical) and show the same functional properties (Klee and Vanamann, 1982). CaM isolated from the baker's yeast (*Saccharomyces cerevisiae*) (yCaM), however, shares only 60% identity in its sequence with vertebrate and other CaMs (Davis et al., 1986; Luan et al., 1987). yCaM binds only three mols of Ca^{2+} (Luan et al., 1987) and can poorly activate most of target enzymes of CaM from vertebrates or plants (Luan et al., 1987), while it can well activate target enzymes isolated from the yeast (Okano et al., 1998). The observed cooperative Ca^{2+}-binding occurs in all of the three Ca^{2+}-binding sites (Luan et al., 1987). Recently the N- and C- domain fragment of yCaM were reported to interact with each other in the high-concentration mixture (Lee & Klevit, 2000). Obviously, yCaM is expected to have quite distinct conformational and biological features compared with those of previously defined CaMs. Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments of the recombinant yCaM in the apo-state.

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Methods and experiments

Uniformly ^{15}N- and/or ^{13}C-labeled yCaMs (146 amino acids) were over-expressed in *E. coli* strain BL21 (DE3) transformed with plasmid pETYCM0 by growing cells in M10 minimal medium containing $^{15}NH_4Cl$ (0.5 g L^{-1}) and/or ^{13}C-glucose (4.0 g L^{-1}). yCaM was purified and decalcified using a previously published method (Ishida et al., 2000). The resulting NMR samples contained 1.2 mM protein, 50 mM KCl and 0.02% NaN_3 in 90% H_2O/10% D_2O or 99.99% D_2O. The pH/pD values of samples were adjusted to 6.9 ± 0.1 without consideration of the isotope effects. Temperature was kept at 30 ± 0.1 °C throughout the experiments. NMR spectra were acquired on Varian UNITY 500 MHz and JEOL JNM 600 MHz spectrometers. Each spectrometer is equipped with a triple resonance 5 mm probe with z-axis pulse field gradient coil. The sequential backbone resonance assignments were achieved using HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNHA, HNCO and HBHA(CBCACO)NH experiments. Side chain assignments were obtained from H(CCO)NH, C(CO)NH and HCCH-TOCSY experiments. 2,2-Dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal chemical shift standard at 0.00 ppm. The ^{15}N and ^{13}C chemical shifts were referenced as described in Wishart et al. (1995). All of the data were processed using NMRPipe (Delaglio et al., 1995) and analyzed with XEASY (Bartels et al., 1995) on SGI O_2 workstation (Molecular Simulations Inc., San Diego, CA).

Extent of assignments and data deposition

Figure 1 shows a 2D {^{1}H, ^{15}N} HSQC spectrum of ^{15}N-labeled yCaM in the apo state. A total of 141 out of 144 amide peaks except for one Pro were identified. The amide signal of Gly25 with large low-field shift in ^{1}H resonance (^{1}HN: 10.72 ppm, ^{15}N: 112.52 ppm) and low intensity is not included in Figure 1. The signals of two N-terminal residues, Ser1 and Ser2 could not be found. The amide resonance of Phe92 and Gly98 also could not be identified in the HSQC spectrum due to extremely weak intensity. The ^{1}HN resonance of Gly98 was, however, found in a 1D spectrum at 9.87 ppm. The chemical shift values of amide proton and nitrogen resonances were deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) in Madison, WI, U.S.A. (accession number: 5353).

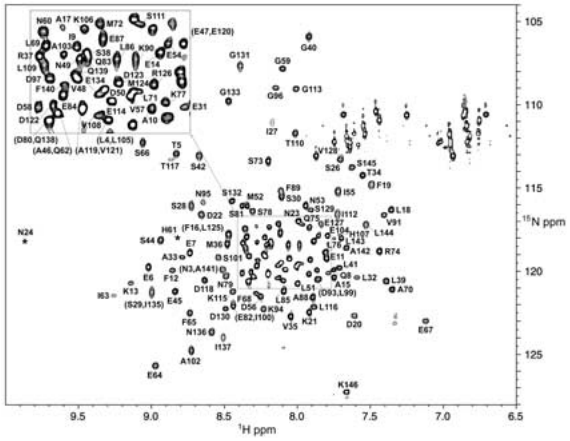

Figure 1. Two-dimensional {^{1}H, ^{15}N} HSQC spectrum of yCaM in the apo state (1.2 mM, pH 6.9 ± 0.1, 30 °C) acquired at 600 MHz. The resonance assignments of backbone amide are indicated by the one-letter amino acid code with residue number. The position of peak, which is invisible in this spectrum, is indicated with an asterisk. The labels of perfectly overlapped signals are grouped together.

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