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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C assignments of the regulatory domains of calcium-dependent protein kinase (CDPK)

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Biological context

Calcium-dependent protein kinases provide the basis for a unique Ca^{2+}-mediated signal transduction pathway in plants and protists (Harmon et al., 2000). They are closely related to calmodulin-dependent protein kinases but are unique since they have a calmodulin-like regulatory domain tethered to the C-terminal end of the kinase. The functional properties of this sequence have been studied through mutational analysis and synthetic peptides (Vitart et al., 2000). Because CDPKs are present only in plants and protozoa, they are potential targets for inhibitors designed for use as antimalarials and /or herbicides. On this basis we initiated structural studies on CDPK from *Arabidopsis thaliana*. We report here the ^{1}H, ^{15}N and ^{13}C assignments of the regulatory apparatus of calcium-dependent protein kinase, consisting of the Junction domain (J) and Calmodulin Like-domains (CaM-LD).

Methods and results

The junction and calmodulin-like regulatory domain (J+CaM-LD) of *Arabidopsis thaliana* CDPK isoform CPK-1 (consisting of *At*CPK1$_{428\text{-}594}$) was subcloned into the pET28b+ plasmid (Novagen) and overexpressed in *E. coli* (BL21-DE3). Full details of the expression and purification protocols used will be described elsewhere (JC, JFH, WJC, in preparation). For isotopic labeling, M9 minimal media was supplemented with uniform ^{13}C-glucose, ^{15}N-ammonium chloride and $^{2}H_2O$ (99.8%) purchased from CIL, Inc. (Andover, MA). The J+CaM-LD protein was isotopically labeled in various modes to facilitate assignment. Unlabeled, uniform single-labeled (^{15}N), uniform double-labeled (^{15}N,^{13}C) and uniform triple labeled (^{2}H, ^{13}C, ^{15}N) samples were prepared. Two additional samples containing specific ^{15}N-Lys labeling with and without uniform ^{13}C-labeling were also produced and analyzed.

NMR samples contained 0.5–1.0 mM protein in 50 mM Tris-d_{11} and 100 mM KCl. Above this concentration range, broadening of NMR lines consistent with aggregation was observed. All NMR samples contained 10 mM $CaCl_2$, 10 mM DTT and 5% (v/v) D_2O. Generally, samples were at pH 7.0 and 310 K, since these were determined to be optimal parameters for a combination of sample stability and spectral quality. However, in order to assign a limited subset of residues we found it necessary to record certain NMR spectra on a sample at pH 5. Further, to overcome loss of signal intensity due to exchange broadening of certain resonances, some spectra were recorded at an elevated temperature (322–330 K).

Experiments were performed at 600 and 800 MHz on Bruker DRX-600, Bruker AMX-800, Varian Inova 600 and Varian Inova 800 spectrometers. To assign the backbone resonances, deuterium decoupled versions of HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB were used (Yamazaki et al., 1994). Side-chain carbon and proton resonances were assigned using a range of experimental data sets: sensitivity-enhanced (Kay et al., 1992) versions of the 3D H(CCO)NH-TOCSY (Montelione et al., 1992; Grzesiek et al., 1993), the 3D (H)C(CO)NH-TOCSY

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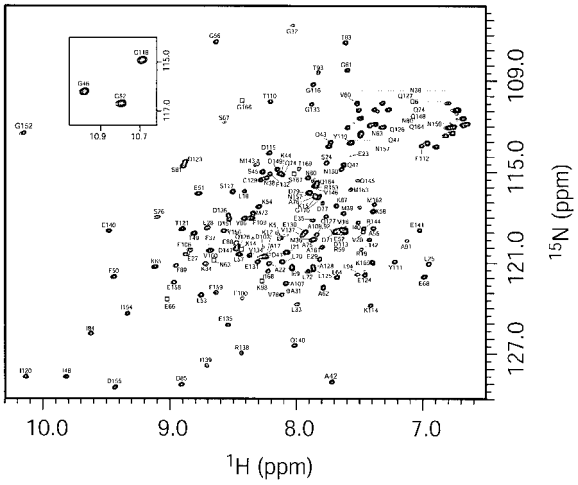

Figure 1. 600 MHz [^{15}N,^{1}H]- HSQC spectrum of 0.8 mM uniformly ^{13}C, ^{15}N, ^{2}H-labeled CDPK$_{428-594}$ in 50 mM Tris-HCl, 100 mM KCl, 10 mM $CaCl_2$ and 10 mM DTT, at 310 K. Resonances are labeled with residue type and sequence number.

(Grzesiek et al., 1993) and a double sensitivity-enhanced H(C)CH-TOCSY (Sattler et al., 1995). We also used a NOESY-[^{15}N]-HSQC with a mixing time of 150 ms and a 3D TOCSY-[^{15}N]-HSQC. For the latter experiment a sensitivity-enhanced version of the HSQC sequence was employed and the DIPSI2-rc TOCSY mixing sequence (Cavanagh et al., 1992, 1993) was used with a spin-lock period of 61 ms. Resonances for aromatic side-chains were assigned using (HB)CB(CGCD)HD, (HB)CB(CGCDCE)HE and HB(CBCGCD)HD (Yamazaki et al., 1993) experiments. The Assign module within FELIX2000 (Accelrys Inc.) was used for the assignment.

Extent of assignment and data deposition

The ^{15}N-^{1}H HSQC spectrum of Ca^{2+}-loaded J+CaM-LD at pH 7.0 and 310 K is shown in Figure 1. The inset shows 3 downfield shifted resonances, which are characteristic of the conserved glycine in position 6 from the four EF-hand Ca^{2+}-binding loops. The positions of amide resonances not observed in this plot are indicated using a small box.

Complete ^{1}H, ^{15}N and ^{13}C assignments for backbone and side-chain atoms have been determined for virtually all residues except for resonances of the disordered N-terminal residues of the Junction domain (residues 5–12) for which the assignments are partial. The assignments have been deposited in the BioMagResBank under accession code BMRB-5324. Analysis of chemical shifts by TALOS (Cornilescu et al., 1993) indicates the presence of eight alpha helices and four short beta strands in the CaM-LD, similar to that observed in calmodulin, and a helical segment (K14-E23) in the J region.

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