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Structure of the Regulatory Apparatus of a Calcium-dependent Protein Kinase (CDPK): A Novel Mode of Calmodulin-target Recognition

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Calcium-dependent protein kinases (CDPKs) are a class of calcium-binding sensory proteins that are found in plants and certain protozoa, including the causative agent of malaria, *Plasmodium falciparum*. CDPKs have diverse regulatory functions, including involvement in the triggering of the lytic cycle of malarial infection. CDPKs contain an autoinhibitory junction (J) region whose calcium-dependent interaction with the tethered regulatory calmodulin-like domain (CaM-LD) activates the catalytic kinase domain. We report here the X-ray crystal structure of the J-CaM-LD region of CDPK from *Arabidopsis thaliana* (AtCPK1), determined to 2.0 Å resolution using multiple-wavelength anomalous dispersion (MAD). The structure reveals a symmetric dimer of calcium-bound J-CaM-LD with domain-swap interactions, in which the J region of one protomer interacts extensively with the carboxy-terminal EF-hand domain (C-lobe) of the partner protomer. However, as the J-CaM-LD is monomeric in solution, the activated monomer was modelled to account for the intra-molecular recognition of the two domains. While the J-CaM-LD segment mimics certain aspects of target motif recognition by CaM other features are specific to CDPKs, in particular the combination of the strong interaction between the N and C-lobes of the CaM-LD and the exclusive use of only the C-lobe in the recognition of the covalently tethered target region. Combined with our previous observations showing that there is likely to be strong interactions between this tethered J region and the CaM-LD even at basal Ca^{2+} concentrations, the new structural data indicate that the response to calcium of CDPKs is clearly unique among the CaM family.

Keywords: calcium-dependent protein kinase; CDPK; calmodulin-like; X-ray structure

Introduction

Calcium-dependant protein kinases (CDPKs) are widely distributed in plants and diverse roles are emerging for these proteins in gene expression, metabolism, signalling pathways and ion transport.[1–4] CDPKs are also found in protozoa, including the causative agent of malaria, *Plasmodium falciparum*.[5] Recent evidence has shown for example that CDPK plays a critical role in the reproductive cycle of *Plasmodium berghei*[6] with the transgenic ablation of a CDPK gene resulting in the complete disruption of parasite transmission. The understanding of the structure and function of this family

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Abbreviations used: CDPK, calcium-dependent protein kinase; CaM, calmodulin; J, junction region; CaM-LD, calmodulin-like domain; CaMK, calmodulin-dependent protein kinase.

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of proteins is therefore of significant potential utility to agriculture and human health.[7]

CDPKs differ from homologous calmodulin-dependent protein kinases (CaMKs)[8] in having a calmodulin-like regulatory apparatus (CaM-LD) fused to the CaM-binding region of the catalytic kinase domain (K). Similar to CaM, the CaM-LD consists of two structural domains (termed the N and C "lobes"), each containing two EF-hand helix-loop-helix Ca^{2+}-binding motifs. The CaM-LD in CDPKs and CaM typically share about 40% sequence identity; indeed the CaM-LD region can be replaced by a CaM sequence to produce a chimeric CDPK in which activity is preserved[9] and exogenous CaM or a CaM-LD can both also partially activate a truncated CDPK.[10]

While there is no clearly established universal mechanism of activation even among the CaMKs, both CaMKs and CDPKs are regulated by an autoinhibitory region located immediately C-terminal to the kinase domain. In both types of kinase, auto-inhibition is relieved through the Ca^{2+}-dependent interaction with CaM or the CaM-LD, respectively. In CDPKs, the 31-residue regulatory junction (J) region (Ala414-Ile444), joins the kinase and the CaM-LD (Figure 1(a)). The C-terminal part of the J region encompasses a pseudosubstrate autoinhibitor and a CaM-LD binding site.[10] The covalent tethering of the CaM-LD to its regulatory J region in CDPKs is a unique feature within the CaM superfamily.

Recently, our biophysical analyses of CDPK[11] revealed that the Ca^{2+}-affinity of the C-lobe in CDPK is significantly greater than that of the N-lobe. This finding suggested that there are differential roles of the two lobes in the activation of CDPKs, with the calcium-loading of the N-lobe as the likely trigger for physiological CPDK activation. The structural details of the interactions of the CaM-LD with the J region are elucidated here using X-ray crystallography, allowing us to develop a structural model for an intramolecular complex of J-CaM-LD that leads to kinase activation. In contrast to the canonical wrap-around binding mode that has been most frequently observed in CaM structures with target segments, the CDPK structure reveals that the binding of the C-terminal segment of the J region to the CaM-LD occurs exclusively by interactions with residues in the C-terminal lobe of the CaM-LD. Structural and sequence comparisons between the CaM-LD and CaM are used to rationalize this finding and to provide mechanistic insight into the physiological activation of CDPKs.

Results

Overview of the J-CaM-LD structure

The crystal structure reveals two molecules of Ca^{2+}-loaded J-CaM-LD protein in the asymmetric unit, associated in a 2-fold symmetric homodimer (Figure 1(b)). The structure shows the expected four-helix bundle EF-hand domains with the J region forming an amphipathic α-helix that docks into the CaM-LD. The individual lobes of the CaM-LD overlay well with each other (RMSD = 0.9 Å for backbone C^{α} atoms, Figure 1(c)) as well with the individual lobes in a number of other CaM-target structures (RMSD typically in the range of 0.9 Å to 2.4 Å, this is examined further below). The inter-domain linker appears to be in a coil conformation in one protomer but partially helical in the other.

The principle interactions observed between the two molecules in the CDPK homodimer are between the J region from one molecule and the hydrophobic pocket in the C-lobe of the CaM-LD from the partner molecule. Our observations from analytical ultracentrifugation, pulsed-field gradient diffusion NMR and non-dissociating mass spectrometry (data not shown) indicate that the protein does not self-associate to form such a dimer in solution even at concentrations as high as 2 mM. Hence, the dimer observed in the crystal structure (Figure 1(b)) represents a "domain swapped" version of the solution structure of the protein.

Interactions and relative orientation of the CaM-LD lobes

One striking feature of the structure is the interaction between the two lobes of the CaM-LD. The interface between the C-lobe and the N-lobe is shown in detail in Figure 2(a). The lobes interact predominantly *via* residues in helices h1 (N-lobe) and h5 (C-lobe) with additional contributions from residues in h2 and h8. The interface is composed of both hydrophobic interactions (e.g. Met459 and Met462 in h1 with the aromatic ring of Phe529 in h5), and electrostatic interactions (e.g. the carboxylate of Glu458 (h1) H-bonding with Thr533 (h5)). Also, Val483 in h2 contributes significant contacts to the aromatic rings of both Phe529 and Tyr579 (in h5 and h8, respectively).

The residues that form the inter-lobe interface in the CaM-LD are not conserved in CaM, in accord with the absence of interactions between the C and N-lobes in CaM (Figure 2(b)). The CaM-LD residues corresponding to the key contact sites, Met459 and Phe529, are Ala and Arg residues, respectively, in CaM. Met462 and V483 are also important contributors to the interface, and both of these residues correspond to Leu residues in CaM. Interestingly, the NMR model of the CaM-LD of soybean CDPKα also reveals an inter-domain contact, but surprisingly, this is a different interface from that described here for the *Arabidopsis* isoform CPK1.[12]

Recognition of the J region

The amphipathic α-helical (h0) segment of the J region (Met433-Ile444), contacts the CaM-LD primarily through residues on its hydrophobic face, which engage the hydrophobic pocket in the C-lobe. Electrostatic interactions also contribute to the interface between the J region and the C-lobe. The