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The International Journal of Biochemistry & Cell Biology 38 (2006) 209–221
IJBCB
www.elsevier.com/locate/biocel

Troponin C/calmodulin chimeras as erythrocyte plasma membrane Ca^{2+}-ATPase activators

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Received 29 April 2005; received in revised form 2 August 2005; accepted 23 August 2005

Abstract

Calmodulin (CaM) and troponin C (TnC) are EF-hand proteins that play fundamentally different roles in animal physiology. TnC has a very low affinity for the plasma membrane Ca^{2+}-ATPase and is a poor substitute for CaM in increasing the enzyme's affinity for Ca^{2+} and the rate of ATP hydrolysis. We use a series of recombinant TnC (rTnC)/CaM chimeras to clarify the importance of the CaM carboxyl-terminal domain in the activation of the plasma membrane Ca^{2+}-ATPase. The rTnC/CaM chimera, in which the carboxyl-terminal domain of TnC is replaced by that of CaM, has the same ability as CaM to bind and transmit the signal to Ca^{2+} sites on the enzyme. There is no further functional gain when the amino-terminal domain is modified to make the rTnC/CaM chimera more CaM-like. To identify which regions of the carboxyl-terminal domain of CaM are responsible for these effects, we constructed the chimeras rTnC/3CaM and rTnC/4CaM, where only one-half of the C-terminal domain of CaM (residues 85–112 or residues 113–148) replaces the corresponding region in rTnC. Neither rTnC/3CaM nor rTnC/4CaM can mimic CaM in its affinity for the enzyme. Nevertheless, with respect to the signal transduction process, rTnC/4CaM, but not rTnC/3CaM, shows the same behaviour as CaM. We conclude that the whole C-terminal domain is required for binding to the enzyme while Ca^{2+}-binding site 4 of CaM bears all the requirements to increase Ca^{2+} binding at PMCA sites. Such mechanism of binding and activation is distinct from that proposed for most other CaM targets. Furthermore, we suggest that Ala_{128} and Met_{124} from CaM site 4 may play a crucial role in discriminating CaM from TnC.

Keywords: Calmodulin; Troponin C; PMCA; Plasma membrane Ca^{2+}-ATPase; Chimeras; EF-hand proteins; Calcium

Abbreviations: CaM, calmodulin; TnC, troponin C; sTnC, fast skeletal muscle TnC; rTnC, recombinant TnC; TNS, 2-(*p*-toluidino) naphthalene-6-sulfonic acid

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doi:10.1016/j.biocel.2005.08.016

1. Introduction

CaM represents the central Ca^{2+} sensor in non-muscle cells, where it functions to recognize the Ca^{2+} signal and to coordinate changes in cellular energy metabolism involving the mobilization of energy reserves and the transcriptional regulation of genes (Chin & Means, 2000). CaM interacts with a variety of different target enzymes. Understanding the structural basis of CaM's interaction with its target enzymes is important in understanding how CaM accomplishes its diverse regulatory functions.

The three-dimensional structures of CaM complexed to various model CaM-binding peptides have already been determined (Ikura et al., 1992; Meador, Means, & Quiocho, 1992, 1993). These structures show that CaM envelops the model peptide in a hydrophobic tunnel by bending and twisting sharply about its central helix. The protein is known to bind to its targets in a sequential manner, encompassing: binding of the carboxyl-terminal domain, binding of the amino-terminal domain, and collapse of CaM around the CaM-binding peptide (Bayley, Findlay, & Martin, 1996; Brown, Martin, & Bayley, 1997; Krueger et al., 1998; Peersen, Madsen, & Falke, 1997; Sun & Squier, 2000).

CaM-peptide structures show a great deal of similarity despite considerable divergence in the sequences of the peptides. The affinity of CaM for model peptides is fairly constant, with typical K_d values of about 0.5–3 nM (Blumenthal et al., 1985; Hanley et al., 1987; Lukas, Burgess, Prendergast, Lau, & Watterson, 1986; Vorherr et al., 1990, 1993; Zhang & Vogel, 1994). These observations suggest that CaM binding to model peptides follows a general mechanism, driven primarily by strong hydrophobic interactions that are relatively independent of the peptide's exact primary sequence. Backbone folds of the amino- and carboxyl-terminal domains in CaM are structurally similar, and in many instances one domain or the other has been reported to partially activate a range of different target proteins to varying extents (Newton, Oldewurtel, Krinks, Shiloach, & Klee, 1984; Newton, Klee, Woodgett, & Cohen, 1985; Persechini, McMillan, & Leakey, 1994). Despite the general tertiary structural homology among individual CaM-binding domains, however, target protein activation usually involves the association of specific elements in CaM with specific sequences within the CaM-binding region of target proteins (Barth, Martin, & Bayley, 1998; Bayley et al., 1996; Chapman, Alexander, Vorherr, Carafoli, & Storm, 1992; Ikura et al., 1992; Meador et al., 1992, 1993; Newton et al., 1984; Persechini et al., 1994; Sun & Squier, 2000).

The plasma membrane Ca^{2+}-ATPase, a CaM target protein, is one of the key enzymes controlling the Ca^{2+} level in cells. The minimum CaM-binding domain includes residues 1091–1110 of hPMCA4b (Penheiter, Filoteo, Penniston, & Caride, 2005; Verma et al., 1988), and has the expected propensity for forming an amphiphilic helix of basic and apolar amino acids. It functions as an autoinhibitory domain of the pump, binding to two "receptor" sites near the active center (Falchetto, Vorherr, Brunner, & Carafoli, 1991; Falchetto, Vorherr, & Carafoli, 1992) of the Ca^{2+}-ATPase. The amino-terminal region of the CaM-binding domain interacts with a site located between the phosphorylatable aspartate (D^{475}) and the FITC- (ATP-) binding site (K^{609}), whereas the carboxyl-terminal region interacts with the cytoplasmic loop between transmembrane domains 2 and 3 corresponding to the "transduction unit" of the P-type ATPases (Carafoli, 1994).

The relative importance of carboxyl- and amino-terminal domains in activating the plasma membrane Ca^{2+}-ATPase is controversial and previous results have been interpreted in different ways. Based on experiments with mutant CaMs and CaM fragments it was suggested that the carboxyl-terminal domain of CaM is indispensable for interaction with the Ca^{2+}-ATPase (Gao et al., 1993; Kosk-Kosicka & Bzdega, 1991, 1992), while its amino-terminal domain has been suggested to lack the ability to activate this enzyme without prior association of the carboxyl-terminal domain (Guerini, Krebs, & Carafoli, 1984). In agreement, an NMR study showed that the complex formed between CaM and the autoinhibitory peptide of PMCA has an unusual global structure in which, in contrast with other peptide/CaM complexes, only the carboxyl-terminal domain of CaM envelops the autoinhibitory domain of PMCA, and no evidence for a collapsed structure was found (Elshorst et al., 1999). On the other hand, Sun and Squier (2000) showed that the isolated amino- and carboxyl-terminal domains of CaM are both able to fully activate the plasma membrane Ca^{2+}-ATPase, but the affinity of the amino-terminal domain in binding to the enzyme is 10,000-fold less than that of the carboxyl-terminal domain. Based on known structures, molecular models showing collapsed structures for the complex formed by CaM/inhibitory-domain-of-PMCA have been proposed (Penheiter et al., 2005).

Recently, Bartlett et al. (2003) reported that the inability of oxidized CaM to fully activate the plasma membrane Ca^{2+}-ATPase is the result of the site-specific oxidation of Met_{144} and Met_{145}, located in the carboxyl terminus of CaM. This observation is consistent with earlier suggestions that these methionines function as