Rapid Reports

Calmodulin Binding to the Polybasic C-Termini of STIM Proteins Involved in Store-Operated Calcium Entry†

Mikael C. Bauer,‡,§ David O'Connell,§,|| Dolores J. Cahill,|| and Sara Linse*,‡,||

Lund University, Biophysical Chemistry, Chemical Centre, P O Box 124, SE221 00 Lund, Sweden, and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

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ABSTRACT: Translocation of STIM1 and STIM2 from the endoplasmic reticulum to the plasma membrane is a key step in store-operated calcium entry in the cell. We show by isothermal titration calorimetry that calmodulin binds in a calcium-dependent manner to the polybasic C-termini of STIM1 and STIM2, a region critical for their translocation to the plasma membrane ($K_D \leq 1$ μM in calcium). HSQC NMR spectroscopy shows this interaction is in the fast exchange regime. By binding STIM1 and STIM2, calmodulin may regulate store refilling, thereby ensuring the maintenance of its own action in intracellular signaling.

Cellular Ca^{2+} signaling in response to extracellular stimuli is dependent on intracellular Ca^{2+} stores. The endoplasmic reticulum (ER)[1] Ca^{2+} store enables rapid Ca^{2+} release throughout the cell. The Ca^{2+} signal is mediated by the ubiquitous protein calmodulin whose affinity for cellular enzymes increases with Ca^{2+} binding. Calmodulin regulates the activity of these enzymes in a calcium-dependent manner. After each signaling event, cytoplasmic Ca^{2+} decreases and calmodulin releases Ca^{2+} that is pumped out of the cell. Endoplasmic reticulum (ER) Ca^{2+} is thus consumed on each signaling event and gradually reaches a critical level where it needs to be refilled from outside the cell. Refilling is controlled by the Ca^{2+} level of the ER store and is mediated by proteins called STIM1 and STIM2 (stromal interaction molecules 1 and 2, respectively) (*1, 3*).

STIM1 and STIM2 are single-pass ER transmembrane proteins. On the luminal (intra-ER) side, each protein comprises two EF-hands and a SAM domain, and on the cytoplasmic (extra-ER) side, the protein comprises a coiled-coil region and a polybasic tail (*4*). The mechanism of STIM1 activation has been investigated in detail (*1, 5, 7*). It is proposed to function as a four-step ER to plasma membrane signaling relay that triggers local store-operated calcium entry (SOC) signals by sequential Ca^{2+} sensing, oligomerization, plasma membrane translocation, and CRAC channel activation. When luminal Ca^{2+} is high, Ca^{2+} is bound to the EF-hand sites and STIM1 is monomeric (*1*). When calcium is depleted from ER stores, calcium dissociates from the EF hand and the protein oligomerizes in the ER membrane. The next event is translocation and docking of STIM1 cytosolic domain(s) to the plasma membrane where puncta form and STIM1 interacts with the Orai1 Ca^{2+} channel of the plasma membrane (*5, 7*). This leads to the influx of Ca^{2+} from the extracellular environment to the ER lumen, thereby refilling the ER. STIM2 seems to operate by a similar mechanism. STIM2 is proposed to be the primary regulator for basal Ca^{2+} influx, while both STIM1 and STIM2 trigger Ca^{2+} influx following receptor-mediated ER store depletion (*3*).

The molecular basis of the translocation event remains elusive. A mutant STIM1 lacking the polybasic tail has been shown to oligomerize but not translocate in response to calcium depletion in the ER (*8*). This process is thought to depend on interactions of the polybasic tail with phosphoinositides in the plasma membrane (*8*). We speculated that the polybasic tail is a signaling domain for proteins involved in the translocation process. The polybasic tails of STIM proteins are rich in basic and hydrophobic residues, which is often seen in calmodulin-binding motifs. Protein array-based screens have identified putative interactions between STIM1 and calmodulin (D. O'Connell et al., unpublished data) and between STIM2 and calmodulin (*9*). While there are no matches of the polybasic tail sequences with the calmodulin target sequence database (http://calcium.uhnres.utoronto.ca/ctdb/pub_pages/resources/index.htm), the lack of any strong consensus sequence and the diversity of identified calmodulin binding regions prompted the question of whether calmodulin interacts with the polybasic tail. Therefore, the polybasic tails of the STIM proteins were evaluated as potential calmodulin-binding regions.

In this study, we show that linear peptides comprising the polybasic tails of human STIM1 (residues 667−685) and STIM2 (residues 730−746) bind to calmodulin with high affinity in a calcium-dependent manner. Experimental evidence comes from isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy.

The binding to calmodulin of peptides corresponding to the polybasic tails of STIM1 and STIM2 was studied at a

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* To whom correspondence should be addressed. E-mail: Sara.linse@bpc.lu.se. Telephone: 0046462228246. Fax: 0046462224543.

‡ Lund University.

§ These authors contributed equally to this work.

|| University College Dublin.

[1] Abbreviations: STIM1, stromal interaction molecule 1; STIM2, stromal interaction molecule 2; ER, endoplasmic reticulum; SAM, sterile α motif; CRAC, calcium release-activated calcium; SOC, store-operated calcium entry; ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance; HSQC, heteronuclear single-quantum coherence.

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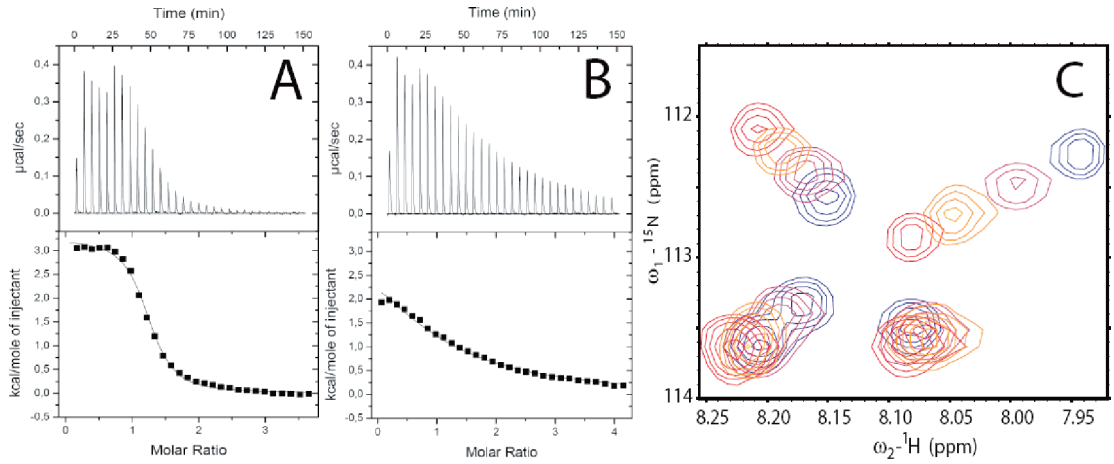

FIGURE 1: Binding of STIM 1 polybasic tail peptide (residues 667−685) to calmodulin. (A and B) ITC data for titrations of the peptide into 10 mM Tris (pH 7.5), 150 mM KCl, and 0.005% Tween 20 containing 30 μM calmodulin and 1 mM $CaCl_2$ (A) or 30 μM calmodulin and 1 mM EDTA (B). In panels A and B, the raw data are shown at the top and the heat per mole of injected peptide is shown at the bottom together with the best fit using a 1:1 binding model. (C) Select region of ^{15}N HSQC spectra of 1 mM [^{15}N]calmodulin in 10 mM Tris (pH 7.5), 150 mM KCl, and 5 mM $CaCl_2$ with 0 (blue), 0.5 (purple), 1.0 (orange), and 1.5 mM (red) peptide.

physiological KCl concentration [10 mM Tris-HCl (pH 7.5), 150 mM KCl, and 0.005% Tween 20] in the presence (1 mM $CaCl_2$) and absence (1 mM EDTA) of Ca^{2+} using isothermal titration calorimetry (ITC) as described in the Supporting Information (SI). The experiment monitors the difference in the amount of heat added to the sample and reference cells to keep them at the same constant temperature of 1 °C above the temperature of an adiabatic shield. For both peptides, binding to calmodulin results in an uptake of heat (positive ΔH) seen as positive signals with each injection. In the presence of Ca^{2+}, similar injection heats are recorded on several injections and then there is an abrupt decay as saturation is approached (Figure 1A and Figure S1A). This shows that the polybasic peptides bind to calmodulin with high affinity in the presence of Ca^{2+}. The more shallow progression of injection heats obtained when the titrations are performed in buffer with EDTA shows that the affinity is greatly reduced in the absence of Ca^{2+} (Figure 1B and Figure S2B). Only frictional heats of injection are observed upon titration of peptide into buffer without protein (Figures S1C and S2C). From fitting to the data in 1 mM Ca^{2+}, we obtain for STIM1 peptide a K_D of 0.8 ± 0.1 μM and for the STIM2 peptide a K_D of 0.9 ± 0.2 μM. In the presence of 1 mM EDTA, the affinity is much lower and we obtain for STIM1 peptide a K_D of 55 ± 3 μM and for the STIM2 peptide a K_D of 150 ± 10 μM.

A titration of ^{15}N-labeled calmodulin with the STIM1 polybasic tail peptide was monitored by ^{1}H−^{15}N HSQC NMR spectroscopy as described in the SI. Addition of peptide causes widespread chemical shift changes over the entire spectrum (Figure S3). This implies that the peptide binds to the protein and affects it globally. The fact that almost every backbone NH signal is shifted upon peptide addition implies that both globular domains are involved in the binding event. Moreover, there are gradual chemical shift changes during the titrations as illustrated by the zoomed-in region of the spectrum (Figure 1C). This indicates that the peptide−protein interaction is in the fast exchange regime of the NMR time scale ($k^{off} > 500$ s^{-1}) and the observed chemical shifts at each point in the titration are population-weighted averages.

The results of this study thus provide strong evidence that STIM1 and STIM2 bind to calmodulin in a Ca^{2+}-dependent manner. We have localized the binding site on the STIM proteins to the polybasic tail using linear peptides comprising residues 667−685 of STIM1 and residues 730−746 of STIM2. We show that the STIM peptides bind to calmodulin with high affinity (K_D = 0.8 and 0.9 μM, respectively) in the presence of Ca^{2+} but with much lower affinity in the absence of Ca^{2+} (K_D = 55 and 150 μM, respectively).

Calmodulin is a key mediator of the cytosolic Ca^{2+} signaling events that depend on the ER store as one important source of quickly mobilized Ca^{2+}. It is intriguing that calmodulin, being dependent on store refilling for this role as the major cytosolic mediator of Ca^{2+} signaling events, binds to STIM1 and STIM2 which are major players in this refilling event. We speculate that calmodulin thereby is directly involved in regulating this fundamentally important function to ensure the continuation of its own action in intracellular signaling.

Calmodulin is a promiscuous regulator that binds to a very large number of intracellular proteins with high affinity in a Ca^{2+}-dependent manner (*9, 10, 12*). Complex formation often involves and depends on both globular domains of calmodulin (*13, 16*), but the flexible linkage between these domains allows for considerable variation in the structures of the complexes (*10*). Several binding motifs are known which are comprised of basic and hydrophobic residues (*11, 17*). Intriguingly, the positive charge of the motifs seems to have a relatively weak influence on the calmodulin binding affinity (*18, 19*), and the motifs are categorized by the spacing of large hydrophobic groups (*17*). We performed homology searches with the polybasic tail sequences against the calmodulin target database (http://calcium.uhnres.utoronto.ca/ctdb/pub_pages/resources/index.htm) but did not find any match. Therefore, the polybasic tails of STIM proteins comprise novel calmodulin binding motifs. The Ca^{2+} dependence of the affinity, the 1:1 stoichiometry, and the widespread changes in the HSQC spectrum upon peptide binding suggest possible modes of interaction between calmodulin and the STIM polybasic tails. Ca^{2+} binding to calmodulin favors the exposure of hydrophobic surfaces

on both globular domains. It is likely that the polybasic tails bind via their hydrophobic residues to these hydrophobic sites and become more or less engulfed between the two calmodulin domains in accordance with many other Ca^{2+}-dependent calmodulin targets (*10, 11, 14, 15*).

In many calmodulin-regulated enzymes, the active site is blocked by an autoinhibitory segment that upon binding of Ca^{2+}-loaded calmodulin is released and the enzyme activated (*20*). In the proposed model of STIM1 action, dissociation of Ca^{2+} from the EF hands on the luminal side of the ER membrane lowers the affinity of the polybasic tail for other cytosolic domain(s) of STIM1 (*8*). The interaction of the polybasic tail with calmodulin would thereby be facilitated.

Previous studies on the role of different parts of STIM have suggested that the polybasic tail might be important in gating of the formed Orai1 channels in the plasma membrane, and in translocation (*3, 8, 21, 22*). With our finding that both STIM1 and STIM2 bind calmodulin with high affinity, we speculate that calmodulin takes part in store-operated calcium entry either by helping to translocate STIM proteins to the plasma membrane or by disassembling the STIM−Orai1 complexes. The calcium-dependent binding of calmodulin to the polybasic tail suggests that store-operated calcium entry is under the control of both luminal and cytosolic calcium. When the channels are formed, the cytosolic Ca^{2+} concentration is increased, and calmodulin is in its calcium-bound state with a higher affinity for STIM. In the event that the binding leads to disassembly of the STIM−Orai1 complexes, this interaction could act as a molecular switch to terminate the Ca^{2+} uptake. Indeed, a recent study has shown that Ca^{2+}-bound (but not Ca^{2+}-free) calmodulin has an inhibitory role in store-operated Ca^{2+} entry and makes the event transient (*23*). Although the binding site for calmodulin was not determined, it was concluded from cellular studies with and without different components that calmodulin acts at the level of STIM2−Orai1 complexes (*23*). Thus, we propose that the calmodulin−STIM2 interaction acts as a molecular switch that terminates store-operated calcium entry. The process is initiated at low Ca^{2+} concentrations when calmodulin is Ca^{2+}-free and cannot compete with Orai1 for binding to the polybasic tail of STIM2. But with an increase in cytosolic Ca^{2+}, calmodulin becomes a potent competitor that terminates the event by binding the polybasic tail, thus disassembling the STIM2−Orai1 complex.

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SUPPORTING INFORMATION AVAILABLE

Additional data and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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