Stored Ca\textsuperscript{2+} Depletion-induced Oligomerization of Stromal Interaction Molecule 1 (STIM1) via the EF-SAM Region

AN INITIATION MECHANISM FOR CAPACITIVE Ca\textsuperscript{2+} ENTRY\textsuperscript{[a][b]}

Received for publication, August 29, 2006, and in revised form, October 2, 2006 Published, JBC Papers in Press, October 3, 2006, DOI 10.1074/jbc.M608247200

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Stromal interaction molecule 1 (STIM1) has recently been identified as a key player in store-operated Ca\textsuperscript{2+} entry. Endoplasmic reticulum (ER) luminal Ca\textsuperscript{2+} depletion results in STIM1 redistribution from ER membrane homogeneity to distinctly localized aggregates near the plasma membrane; these changes precede and are linked to cytoplasmic Ca\textsuperscript{2+} influx via Ca\textsuperscript{2+} release-activated channels (CRACs). The molecular mechanisms initiating ER STIM1 redistribution and plasma membrane CRAC activity are not well understood. We recombinitely expressed the Ca\textsuperscript{2+}-sensing region of STIM1 consisting of the EF-hand together with the sterile \(\alpha\)-motif (SAM) domain (EF-SAM) to investigate its Ca\textsuperscript{2+}-related conformational and biochemical features. We demonstrate that Ca\textsuperscript{2+}-loaded EF-SAM (holo) contains high \(\alpha\)-helicity, whereas EF-SAM in the absence of Ca\textsuperscript{2+} (apo) is much less compact. Accordingly, the melting temperature \(T_m\) of the holoform is \(\sim 25^\circ\) C higher than apoform; heat and urea-derived thermodynamic parameters indicate a Ca\textsuperscript{2+}-induced stabilization of 3.2 kcal mol\textsuperscript{-1}. We show that holoEF-SAM exists as a monomer, whereas apoEF-SAM readily forms a dimer and/or oligomer, and that oligomer to monomer transitions and vice versa are at least in part mediated by changes in surface hydrophobicity. Additionally, we find that the Ca\textsuperscript{2+} binding affinity of EF-SAM is relatively low with an apparent dissociation constant \(K_d\) of \(\sim 0.2\)–0.6 mm and a binding stoichiometry of 1. Our results suggest that EF-SAM actively participates in and is the likely molecular trigger initiating STIM1 punctae formation via large conformational changes. The low Ca\textsuperscript{2+} affinity of EF-SAM is reconciled with the confirmed role of STIM1 as an ER Ca\textsuperscript{2+} sensor.

Calcium is a fundamental signaling messenger in every eukaryotic cell, regulating a multitude of diverse and kinetically distinct cellular phenomena including gene transcription, protein folding, protein degradation, apoptosis, necrosis, and excitosis, to name a few \cite{1}. The endoplasmic reticulum (ER) \cite{2} is a network of folded membranes that extends through the cytoplasm to the nuclear envelope of eukaryotes. The ER membranes surround an inner cavity, the lumen, that is critical to the function of the ER as a Ca\textsuperscript{2+} signaling organelle \cite{3}. Because vital Ca\textsuperscript{2+}-dependent processes are associated with the ER, it is essential that changes in luminal Ca\textsuperscript{2+} levels do not adversely affect these phenomena. Eukaryotes have evolved store-operated Ca\textsuperscript{2+} entry (SOCE), also termed capacitive Ca\textsuperscript{2+} entry, as a major Ca\textsuperscript{2+} entry pathway in electrochemically non-excitable cells \cite{4}. SOCE is the process whereby modest ER Ca\textsuperscript{2+} store depletion leads to plasma membrane (PM) Ca\textsuperscript{2+} release-activated channel (CRAC) activation, providing a sustained Ca\textsuperscript{2+} elevation in the cytoplasm from extracellular sources and ultimately refilling the ER luminal Ca\textsuperscript{2+} stores \cite{5}.

Until recently, the molecular link between ER Ca\textsuperscript{2+} efflux and extracellular influx was not known. However, interfering and small inhibiting RNA studies have independently implicated stromal interaction molecule-1 (STIM1) as the likely Ca\textsuperscript{2+} sensor in the ER \cite{6,7}. This single-pass, type I transmembrane protein of 685 amino acids has been found localized on both the plasma and ER membranes \cite{8,9,10}. The N-terminal regions of STIM1 include a signal peptide, putative EF-hand motif, and predicted sterile \(\alpha\)-motif (SAM) domain. The cytosolic C-terminal region consists of two coiled-coil domains, a Pro/Ser-rich region, and a Lys-rich region \cite{11,12}. The putative EF-hand of STIM1 strongly aligns with the helix-loop-helix consensus sequence of this Ca\textsuperscript{2+} binding motif \cite{13}. The EF-hand in STIM1 is somewhat unorthodox because it is seemingly unpaired, whereas Ca\textsuperscript{2+} sensor proteins regularly have paired or doubly paired EF-hands \cite{14,15}. Therefore, it is plausible that the putative SAM domain of STIM1 may serve to dimerize or oligomerize STIM1 in order to facilitate pairing of the EF-hand \cite{16}.

In the present work, the predicted Ca\textsuperscript{2+}-sensing region of human STIM1 comprising the EF-hand and SAM domain (EF-SAM) was expressed recombinantly in Escherichia coli. The

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\textsuperscript{[a]} This research was made possible through a Canadian Institute of Health Research (CIHR) operating grant (to M. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{[b]} The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. 1.

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\textsuperscript{4} The abbreviations used are: ER, endoplasmic reticulum; PM, plasma membrane; SEC, size exclusion chromatography; C51, chemical shift index; STIM1, stromal interaction molecule-1; SAM, sterile \(\alpha\)-motif; SOCE, store-operated calcium entry; EF-SAM, EF-hand and SAM-containing region of STIM1; CRAC, Ca\textsuperscript{2+} release-activated channel; HSQC, heteronuclear single-quantum correlation; DLS, dynamic light scattering; MALS, multi-angle light scattering; ANS, 1-anilino-8-naphthalenesulfonate.
behavior of EF-SAM was characterized biophysically in the presence and absence of Ca\(^{2+}\). The results demonstrate that EF-SAM binds Ca\(^{2+}\) with an affinity that can be reconciled with physiological ER luminal and extracellular Ca\(^{2+}\) concentrations. The data establish that EF-SAM is monomeric when Ca\(^{2+}\)-loaded but readily forms dimers and oligomers in the Ca\(^{2+}\)-depleted state. This study provides a mechanistic perspective on recent observations made on changes in physical STIM1 distribution associated with Ca\(^{2+}\) fluctuations in cell culture.

**MATERIALS AND METHODS**

**Cloning and Recombinant Expression of EF-SAM**—Human STIM1 cDNA was from Origene Technologies, Inc. The EF-SAM region (Ser-58 to Gly-201) was subcloned into a pET-28a vector (Novagen, Inc.) and expressed with an N-terminal His\(_6\)-tag in BL21(DE3) E. coli cells. EF-SAM was purified under denaturing conditions using nickel-nitrilotriacetic acid resin and refolded according to the accompanying resin protocol (Qiagen, Inc.). After thrombin digestion, the protein was further purified by size exclusion chromatography (SEC). ApoEF-SAM was prepared from the holoform by dialysis versus 100 mM EDTA. Protein concentration was measured using a final concentration of 0.1% (w/v) guanidine-\(\text{HCl}\) containing a volatile Ca\(^{2+}\) source (Ca(CH\(_3\)COO)\(_2\)).

**Titration Experiments**—45Ca\(^{2+}\) binding assays were performed as described previously (21). For CD and fluorescence-based titrations, free Ca\(^{2+}\) was taken to be \(\approx 10^{-5}\) M, as protein concentration in these titrations was \(< 5\times 10^{-5}\) M. All binding data were fit to the Hill equation using nonlinear least squares fitting of data at three different Ca\(^{2+}\) concentrations. The radial absorbance was scanned at 280 nm. Molecular weight determinations were made by global nonlinear least squares fitting of data at three different speeds using the accompanying EQASSOC software (Beckman Coulter). Partial specific volume \(\epsilon = 0.729\) ml g\(^{-1}\) and sample density \(\rho = 1.003\) g ml\(^{-1}\) were used in the calculations.

**Ca\(^{2+}\) Titrations**—For CD and fluorescence titration experiments were performed on an XL-A analytical ultracentrifuge (Beckman Coulter). The radial absorbance was scanned at 280 nm. Molecular weight determinations were made by global nonlinear least squares fitting of data at three different speeds using the accompanying EQASSOC software (Beckman Coulter). Partial specific volume \(\epsilon = 0.729\) ml g\(^{-1}\) and sample density \(\rho = 1.003\) g ml\(^{-1}\) were used in the calculations.

**Analytical Ultracentrifugation**—Sedimentation equilibrium experiments were performed on an XL-A analytical ultracentrifuge (Beckman Coulter). The radial absorbance was scanned at 280 nm. Molecular weight determinations were made by global nonlinear least squares fitting of data at three different speeds using the accompanying EQASSOC software (Beckman Coulter). Partial specific volume \(\epsilon = 0.729\) ml g\(^{-1}\) and sample density \(\rho = 1.003\) g ml\(^{-1}\) were used in the calculations.

**RESULTS**

**HoloEF-SAM Is a Well Folded \(\alpha\)-Helical Protein**—The secondary structure of recombinant EF-SAM was assessed using far-UV CD. The spectrum of EF-SAM measured in the presence of Ca\(^{2+}\) (holo) was typical of an \(\alpha\)-helical protein, with two intense minima at \(-208\) and \(225\) nm and a maximum at \(-200\) nm (Fig. 1A). The relatively high intensity of the bands suggests that EF-SAM is well folded in the presence of Ca\(^{2+}\). In the absence of Ca\(^{2+}\) (apo), the minimum at \(225\) nm weakened along with an observed blue shift in the \(208\) nm peak (i.e. new minimum at \(204\) nm) (Fig. 1A).

The considerable conformational differences between holo- and apoEF-SAM were confirmed using NMR. The \(^{1}H(\text{N})\) backbone \(^{15}\)N HSQC spectrum acquired in the presence of Ca\(^{2+}\) was well dispersed (i.e. \(^{1}H(\text{N})\) backbone resonances \(-10.3\) to \(-6.5\) ppm), consistent with a well folded and compact tertiary structure (Fig. 1B). The most downfield-shifted backbone \(^{1}H(\text{N})\) resonance (\(-10.3\) ppm) was assigned to the conserved glycine in the Ca\(^{2+}\)-binding loop (Gly-81), suggesting that the EF-hand chemical shift pattern in STIM1 follows the canonical EF-hand archetype (Fig. 1B; also see supplemental Fig. 1B) (22, 23). Chemical shift index (CSI) calculations (24) from standard triple resonance
based backbone assignments suggest that holoEF-SAM predominantly adopts α-helical secondary structure, which is in agreement with PSIPRED predictions (25) (Fig. 1D). The

\[ 1^H-15N\text{ HSQC spectrum of apoEF-SAM was starkly different; } 1^H(N)\text{ backbone amides had chemical shifts clustered in the } 8.5-7.5\text{-ppm range, and the number of observable peaks decreased significantly (Fig. 1C). The narrow dispersion of } 1^H(N)\text{ backbone chemical shifts indicated that apo- is considerably less folded than holoEF-SAM. The addition of Ca}^{2+}\text{ to the apoprotein restored peak dispersion; however, the intensity of the peaks were weak relative to the holoprotein shown in Fig. 1B, likely because of slow reversibility in the aggregation of apoEF-SAM (data not shown).}

**HoloEF-SAM Is Monomeric**—Because the majority of eukaryotic Ca}^{2+}\text{ sensor proteins have paired or doubly paired EF-hands (12, 13), the molecularity of apo- and holoEF-SAM was assessed. SEC showed a single peak in the presence of excess Ca}^{2+}\text{ with no evidence for changes in elution volume as a function of protein concentration (Fig. 2A, inset). SEC in-line MALS gave a Zimm plot-based molecular mass of } 18\text{ kDa, nearly identical to the expected mass of the monomer (17.4}

\begin{figure}[h]
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\caption{Ca}^{2+}\text{-related structural changes in EF-SAM. A, far-UV CD spectra in the presence (●) and absence (○) of Ca}^{2+}\text{ at } 20^\circ\text{C. Protein at } 0.15\text{ mg ml}^{-1}\text{ was incubated with } 0.5\text{ mM EDTA for apo. The holocurve was measured after adding } 10\text{ mM CaCl}_2\text{ to the aposample. B and C, } 1^H-15N\text{ HSQC spectra of Ca}^{2+}\text{-loaded (●) and -depleted (○) EF-SAM at } 20^\circ\text{C. Solution conditions were } 10\text{ mg ml}^{-1}\text{ protein with } 2\text{ mM CaCl}_2\text{ for holoEF-SAM and no added } \text{CaCl}_2\text{ for the apoform. The conserved glycine (gly81) in the EF-hand binding loop is indicated with a circle. D, } 1^3C\text{-13C CSI versus residue number. Four or more consecutive positive CSI values indicate α-helix (cylinders); four or more negative values indicate a β-strand (block arrows/arrowheads).}
\end{figure}

\begin{figure}[h]
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\caption{Ca}^{2+}\text{-dependent monomerization of EF-SAM. A, SEC with in-line MALS in the presence (●) and absence (○) of Ca}^{2+}\text{ at } 4^\circ\text{C. Elution buffers were } 2\text{ mM CaCl}_2\text{ and } 10\text{ mM EDTA for holo- and apoEF-SAM, respectively. The MALS molecular weights of the holoprotein (●) were consistent with a monomer, whereas those of the apoprotein (○) were dimeric. The inset shows a concentration-dependent Ve for apoEF-SAM (○), which is absent for the holoform (●). B, sedimentation equilibrium ultracentrifugation of holo- (left) and apoEF-SAM (right) at } 20^\circ\text{C. Buffers contained } 1\text{ mM CaCl}_2\text{ and } 1\text{ mM EDTA for holo- and apoEF-SAM, respectively. Protein was at } 0.32, 0.22, \text{ and } 0.15\text{ mg ml}^{-1}\text{ for apo- and } 0.72, 0.53, \text{ and } 0.36\text{ mg ml}^{-1}\text{ for holoEF-SAM. C, glutaraldehyde cross-linking as a function of Ca}^{2+}. \text{ Reactions were performed at } 0.8\text{ mg ml}^{-1}\text{ protein, and } 4^\circ\text{C. 0.01 mg protein was loaded per lane.}
\end{figure}
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kDa) (Fig. 2A) (20). In the presence of excess EDTA, a concentration-dependent elution volume was observed (Fig. 2A, inset), and the MALS-determined molecular mass (~35 kDa) was indicative of a dimer (Fig. 2A). The apochromatogram showed evidence for the presence of higher order oligomeric species with considerable protein eluting in the void volume (data not shown). Ultracentrifugation sedimentation equilibrium experiments of holoEF-SAM demonstrated linear plots of In(abs) versus radius² for all centrifugation speeds, suggesting that the sample was composed primarily of one species (Fig. 2B). The molecular mass, determined using global self-association analysis, was 14.8 kDa, consistent with a monomer (Fig. 2B). Apoprotein gave a nonlinear plot of In(abs) versus radius² only at the highest centrifugation speed, suggesting that the sample was composed of more than one species (Fig. 2B). Global self-association analysis of all apoform data sets gave a molecular mass of 33.8 kDa, consistent with a dimer. The best data fits (i.e. lowest variance) were with the monomer-dimer-tetramer model for apoform data. Glutaraldehyde-mediated cross-linking of EF-SAM as a function of Ca²⁺ concentration was visualized by SDS-PAGE and Coomassie staining. The intensity of the monomer band increased with Ca²⁺ levels, whereas the highest dimer population was observed in the absence of Ca²⁺ (Fig. 2C). The molecular experiments demonstrated that the SAM domain does not function as a dimerization motif when EF-SAM is Ca²⁺ loaded; at the same time, the apoform displays a clear tendency to form dimers and/or oligomers.

EF-SAM Binds Ca²⁺ with Low Affinity—⁴⁵Ca²⁺ binding to EF-SAM was assessed at ambient temperature using an equilibrium ultrafiltration procedure (21). At saturation, the molar ratio of bound Ca²⁺ to EF-SAM was ~1 (Fig. 3A). Positive electrospray ionization mass spectrometry under native buffering conditions and in the presence of excess Ca²⁺ showed a single Ca²⁺-related adduct attributed to Ca²⁺- and Na⁺-bound EF-SAM (i.e. +40(Ca²⁺), +23(Na⁺), −3(H⁺)), confirming the notion of a single Ca²⁺ binding site (Fig. 3A, inset). The⁴⁵Ca²⁺ data analyzed with the Hill equation showed that binding was of low affinity (Kₐ = 0.6 mM) (Fig. 3A). The low affinity resulted in substantial data scatter and great uncertainty in the Ca²⁺-ligated Kₐ (Fig. 3A; Table 1). At 20 °C, the intrinsic fluorescence emission spectra of apo- and holoEF-SAM (λₑₓ = 280 nm) were distinct; the Ca²⁺-loaded form had a higher fluorescence intensity and was red-shifted by ~2 nm compared with the apoform (Fig. 3B, inset). Change in fluorescence intensity as a function of Ca²⁺ levels was employed as a further probe to assessing binding affinity. The Kₐ derived from the Hill-fitted data was ~0.6 mm (Table 1; Fig. 3B). At 10 °C, where the fraction of folded apoEF-SAM was shown to be higher (see below), apparent Kₐ values were ~0.2 mm (Table 1; Fig. 3B). CD ellipticity at 208, 222, and 230 nm as a function of Ca²⁺ concentration was also used to probe affinity. Hill-based Kₐ values were consistent with other measurements at 20 °C (~0.5 mm) (Table 1; Fig. 3C). Overall, the data show that Ca²⁺ binds to EF-SAM with low affinity (sub-mm), consistent with STIM1 functioning as an ER luminal Ca²⁺ sensor.

Ca²⁺ Drastically Increases the Thermodynamic Stability of EF-SAM—The thermal stability of EF-SAM was assessed in the presence and absence of Ca²⁺ by following changes in CD ellipticity at 225 nm as a function of temperature (Fig. 4A). Revers-

| TABLE 1 |
| --- |
| Apparent Ca²⁺ dissociation constants derived from the Hill equation |
| Probe | Temperature | Kₐ |
| --- | --- | --- |
| ⁴⁵Ca²⁺ | ~22°C | 0.55 ± 0.12 |
| Fluorescence | 10°C | 0.17 ± 0.04 |
| Fluorescence | 10°C | 0.19 ± 0.11 |
| Fluorescence | 10°C | 0.49 ± 0.06 |
| Fluorescence | 20°C | 0.60 ± 0.05 |
| CD(208 nm) | 20°C | 0.52 ± 0.03 |
| CD(222 nm) | 20°C | 0.51 ± 0.02 |
| CD(230 nm) | 20°C | 0.52 ± 0.02 |

* Performed at ambient temperature.  
* Derived from 200–240-nm spectra of one titration.
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Unlike thermal denaturation, chemical denaturation was completely reversible. Urea denaturation was monitored by following the changes in intrinsic fluorescence. Urea unfolding induced a red shift and a decrease in the intensity of the emission spectrum ($\lambda_{ex} = 280$ nm) (Fig. 4B, inset). The fluorescence of the fully folded apoprotein was confirmed by using 10% (v/v) glycerol. The glycerol-stabilized curve had a folded base line with emission spectra identical to apoprotein in the absence of urea and glycerol. Data were fit to the $N \leftrightarrow U$, scheme and the linear extrapolation method was used to calculate $\Delta G$ (Table 2) (15). The concentration of urea, where the fraction of unfolded macromolecules $= 0.5$ ($C_{mol}$), was significantly higher for the holo- versus apocurves (0.23 and 2.02 M, respectively) (Fig. 4B). The cooperativity of unfolding, reflected in the fitted $m$-values, was higher for holo, consistent with the $\Delta C_p$ trend (26). $\Delta G$ values were somewhat lower than those determined by thermal analysis, but both analyses (thermal and chemical) were in excellent agreement regarding the Ca$^{2+}$-induced stabilization ($= +3.2$ kcal mol$^{-1}$ monomer).

**ApoEF-SAM Reversibly Forms Oligomers**—The solvent-accessible hydrophobicity of EF-SAM in the presence and absence of Ca$^{2+}$ was probed by monitoring the extrinsic fluorescent properties of ANS (27). The emission spectrum of ANS incubated with apoEF-SAM was blue-shifted and demonstrated increased intensity compared with ANS with no protein added (Fig. 5A). Upon the addition of saturating concentrations of Ca$^{2+}$, the spectrum shifted back to longer wavelengths and displayed a decreased intensity compared with the apoprotein; but it remained blue-shifted and more intense than ANS alone (Fig. 5A). The ANS data suggest that the conformational transition that accompanies Ca$^{2+}$ depletion includes an increase in solvent-accessible hydrophobic area.

Dynamic light scattering was employed to determine differences in propensity to oligomerization as a function of Ca$^{2+}$. Upon the addition of Ca$^{2+}$, the decays in the apofor correlation function shifted to shorter delay times, indicative of smaller hydrodynamic radii (Fig. 5B, inset). Mass-weighted regularization deconvolution results indicated that the majority of protein in the holosample had a radius of hydration consistent with monomer (i.e. <1.5 nm), whereas the distribution of radii in the apoprotein was indicative of larger dimeric/oligomeric species (Fig. 5B). The DLS data suggest that Ca$^{2+}$ can induce the dissociation of oligomeric apoEF-SAM into smaller subunits.

**TABLE 2**

| Protein$^{a}$ | Probe$^{b}$ | $T_m$ (°C) | $\Delta H (T_m)$ (kcal mol$^{-1}$) | $\Delta C_p (T_m)$ (kcal mol$^{-1}$ K$^{-1}$) | $C_{mol}$ | m-value | $\Delta G (10 {°C})$ kcal mol$^{-1}$ | $\Delta \Delta G (10 {°C})$ kcal mol$^{-1}$ |
|---------------|-------------|------------|---------------------------------|---------------------------------|---------|---------|----------------------------------|------------------|
| + Ca$^{2+}$   | Thermal     | 45.2 ± 0.0 | 122.6 ± 2.0                     | 4.5 ± 0.3                       | 0.8     | 1.5     | 4.5 ± 0.3                        | +3.2              |
| + Ca$^{2+}$   | Urea        | 19.2 ± 0.2 | 43.0 ± 2.0                      | 0.7 ± 0.8                       | 0.3     | 1.0     | 1.3 ± 0.8                        | +0.4              |
| − Ca$^{2+}$   | Thermal     | 18.1 ± 0.1 | 21.2 ± 0.2                      | 2.1 ± 0.2                       | 1.7     | 1.4     | 3.7 ± 0.4                        | +3.2              |
| − Ca$^{2+}$   | Urea        | 0.3 ± 0.8  | 1.7 ± 0.4                       | 0.5 ± 0.4                       | 0.3     | 1.1     | 1.3 ± 0.8                        | +0.4              |

$^{a}$ + Ca$^{2+}$ = excess CaCl$_2$; − Ca$^{2+}$ = excess EDTA.

$^{b}$ Thermal = CD melting curve; Urea = intrinsic fluorescence chemical denaturation curve.

$^{c}$ $\Delta H (T_m)$ = $\Delta H$ at the melting temperature.

$^{d}$ $\Delta C_p (T_m)$ = $\Delta C_p$ at the melting temperature.

$^{e}$ $\Delta G (10 {°C})$ = $\Delta G$ at 10°C.

$^{f}$ $\Delta \Delta G (10 {°C})$ = $\Delta G_{holo} - \Delta G_{apo}$ at 10°C.
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Previous experiments have shown that removing anionic residues in the predicted Ca$^{2+}$ binding loop result in constitutive STIM1 puncta formation that is independent of ER Ca$^{2+}$ store depletion (7, 28, 29). More recent experiments have identified other essential elements in these processes. For example, Orai1 is an important CRAC component that works synergistically with STIM1 to conduct Ca$^{2+}$ into the cytoplasm from extracellular provisions (30–35). It is now thought that the conformational changes accompanying Ca$^{2+}$ binding or dissociation of Ca$^{2+}$-binding proteins (i.e. calreticulin) have comparable affinity (i.e. $K_d = 10^{-8} - 10^{-9} \text{ M}$) (44). Free Ca$^{2+}$ concentrations in the ER lumen of eukaryotes have been estimated at $\sim 0.2$–2 mM ($44$–$46$). Low affinity can be reconciled with these high Ca$^{2+}$ levels and is amenable with the function of STIM1 as an ER sensor, given the myriad of Ca$^{2+}$-dependent processes occurring in the ER (1). Highly efficient Ca$^{2+}$ chelation would be detrimental to these processes by requiring extremely low Ca$^{2+}$ concentrations for STIM1 puncta formation. PM-associated STIM1 has an extracellularly directed EF-SAM region, where Ca$^{2+}$ levels are maintained at $\sim 1$–2 mM ($47$). Although there is no evidence that STIM1 senses changes in extracellular Ca$^{2+}$, the low Ca$^{2+}$ affinity of EF-SAM is congruent with its PM orientation.

In polypeptide terms, Ca$^{2+}$ sensors can be defined as proteins that undergo a conformational response to the binding or dissociation of Ca$^{2+}$, ultimately regulating some specific function (12). The predisposition of apoEF-SAM to adopt a much less compact tertiary structure, with appreciably less $\alpha$-helicity compared with holo, has been observed with other Ca$^{2+}$ sensor proteins. For example, guanylyl cyclase-activating protein-2 and calcium-binding protein-1 attain a distinctly more compacted fold upon Ca$^{2+}$ binding (48, 49). The Ca$^{2+}$-induced conformational change of EF-SAM is not localized to the EF-hand region. The $^1\text{H}-^1\text{N}$ HSQC spectra indicate a drastic global structural change upon Ca$^{2+}$ binding or dissociation. Also, Ca$^{2+}$ binding to the EF-hand affected the fluorescence of tryptophan residues located solely in the SAM domain, and large differences in the apo- and holoform far-UV CD as well as near-UV CD (data not shown) spectra were observed, consistent with a global change in structure. The Ca$^{2+}$-induced structural change to EF-SAM translates into a 3.2-kcal mol$^{-1}$ augmentation in thermodynamic stability. This extrinsic Ca$^{2+}$ stabilization is in the same range as other EF-hand-containing proteins such as neuronal calcium sensor-1 ($+3.5$ kcal mol$^{-1}$), tryptic C-terminal fragment of calmodulin ($+3.0$ kcal mol$^{-1}$), and tryptic N-terminal fragment of calmodulin ($+3.8$ kcal mol$^{-1}$) ($50$, $51$).

The conformational changes accompanying Ca$^{2+}$ depletion promote a monomer-to-oligomer transition for EF-SAM. Thermal $\Delta C_p$ and denaturant $n$-values suggest that apoEF-SAM undergoes a smaller $\Delta S_{\text{ASA}}$ (change in solvent-accessible surface area) upon unfolding. Assuming that the denatured states of apo- and holoEF-SAM are similar, native apoEF-SAM must be more solvent-accessible and less compact than native
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In conclusion, our data provide new tangible evidence that a low-affinity EF-hand exists in STIM1 and is functional for Ca$^{2+}$ sensing in the ER lumen, where free Ca$^{2+}$ levels are high (sub-mM). Further, the biophysical data presented here support previous single cell imaging observations in which STIM1 displays a pervasive distribution in the Ca$^{2+}$-loaded state, whereas it undergoes a high density, aggregate formation on the ER membrane upon depletion. Our results further demonstrate that EF-

SAM alone can promote a monomer-to-oligomer transition upon Ca$^{2+}$ release from the EF-hand motif and that exposed hydrophobic surfaces in the EF-SAM domain actively participate in self-association of STIM1. These data underscore the significance of EF-SAM in the homotypic association mechanism of STIM1 upon Ca$^{2+}$ depletion from the ER, which leads to activation of store-operated Ca$^{2+}$ channels on the PM. Our data support a conformationally linked model for the control of SOCE (Fig. 6). When Ca$^{2+}$ stores are filled, the majority of STIM1 is monomeric and is evenly distributed throughout the ER and PM. Upon Ca$^{2+}$ store depletion, a large conformational change occurs in EF-SAM, increasing hydrophobicity and promoting ER STIM1 association as a stabilizing and solvation entropy-favoring mechanism. Oligomerized STIM1 ultimately form or add to ER punctae linking STIM1 with PM Orai1, a major component of CRAC activity. Ca$^{2+}$ store depletion results in STIM1 disaggregation and the halting of SOCE. Further structural studies are required to elucidate a more defined picture of STIM1 action in the control of SOCE.

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