Published in final edited form as:
Biochem Soc Trans. 2006 November ; 34(Pt 5): 887–893. doi:10.1042/BST0340887.

The structural biology of voltage-gated calcium channel function and regulation

F. Van Petegem and **D.L. Minor Jr**[1]
Departments of Biochemistry and Biophysics, and Cellular and Molecular Pharmacology, Cardiovascular Research Institute, California Institute of Quantitative Biomedical Research, University of California San Francisco, San Francisco, CA 94158-2330, U.S.A.

Abstract

Voltage-gated calcium channels (Ca_Vs) are large (~0.5 MDa), multisubunit, macromolecular machines that control calcium entry into cells in response to membrane potential changes. These molecular switches play pivotal roles in cardiac action potentials, neurotransmitter release, muscle contraction, calcium-dependent gene transcription and synaptic transmission. Ca_Vs possess self-regulatory mechanisms that permit them to change their behaviour in response to activity, including voltage-dependent inactivation, calcium-dependent inactivation and calcium-dependent facilitation. These processes arise from the concerted action of different channel domains with Ca_V β-subunits and the soluble calcium sensor calmodulin. Until recently, nothing was known about the Ca_V structure at high resolution. Recent crystallographic work has revealed the first glimpses at the Ca_V molecular framework and set a new direction towards a detailed mechanistic understanding of Ca_V function.

Keywords

calcium-dependent facilitation (CDF); calcium-dependent inactivation (CDI); calmodulin (CaM); IQ domain; structural biology; voltage-gated calcium channel (Ca_V

Introduction

Voltage-gated calcium channels (Ca_Vs) play a unique role in the interplay between electrical and chemical signalling in biological systems [1,2]. Ca_Vs respond to membrane depolarization by opening calcium-selective pores. Calcium ion entry causes further membrane potential changes and, because calcium ions act as chemical messengers, may also ignite intracellular signalling cascades. As one of the principal sources of calcium in excitable cells, Ca_Vs are under intense pressure to control calcium influx and to detect and respond to changes in intracellular calcium concentrations.

Three intrinsic well-characterized processes regulate Ca_Vs: VDI (voltage-dependent inactivation), CDI (calcium-dependent inactivation) and CDF (calcium-dependent facilitation). VDI and CDI aid in calcium influx termination. CDI and CDF both act in a manner that can be thought of as ‘molecular short-term memory’, as channels that have already been opened ‘remember’ this fact and change their inactivation properties or are more readily opened by subsequent stimulation. Despite extensive functional characterization, it is only in the last 3 years that the molecular architecture that underpins

[1]To whom correspondence should be addressed (daniel.minor@ucsf.edu)..

VDI, CDI and CDF has begun to be revealed. As Ca_Vs are major targets for drugs to treat hypertension, arrhythmias and pain [3], understanding channel architecture in atomic detail has implications that reach far beyond basic science.

Ca_V global architecture

Reconstitution of high-voltage-activated Ca_Vs (Ca_V1s and Ca_V2s) requires four basic components: $Ca_V\alpha_1$ (Ca_V α_1-subunit), $Ca_V\beta$, CaM (calmodulin) and $Ca_V\alpha_2\delta$ (Figure 1A) [1,4]. $Ca_V\alpha_1$ is the transmembrane subunit (~1800–2300 amino acids long) that makes the voltage-sensitive, calcium-selective pore [1]. Primary structure analysis indicates that $Ca_V\alpha_1$ encodes four homologous repeats that each bears six transmembrane segments. These individual repeats share a common architecture with other members of the voltage-gated ion channel superfamily [2]. The first four transmembrane segments of each repeat (segments S1–S4) form the voltage-sensing domain, while the remaining two segments (S5 and S6) form the calcium-selective pore. Approximately half of the $Ca_V\alpha_1$ polypeptide forms the intracellular mass of the channel comprising the N- and C-terminal cytoplasmic domains and three interdomain regions (called the I-II, II-III and III-IV loops). These domains are the sites of action for a range of intracellular regulatory and signalling factors.

The two Ca_V intracellular subunits, $Ca_V\beta$ and CaM, bind to the I-II loop and the C-terminal cytoplasmic domain respectively and have prominent roles in VDI, CDI and CDF. Despite the fact that CaM is a highly expressed, ubiquitous protein, both forms, apo-CaM [5-7] and Ca^2+/CaM [6-13], bind $Ca_V\alpha_1$. Thus, by virtue of its calcium-independent tethering to $Ca_V\alpha_1$, CaM can be considered an essential Ca_V subunit [14].

Ca_Vs have one other essential protein, $Ca_V\alpha_2\delta$ [15]. $Ca_V\alpha_2\delta$ is made from a single gene product that is proteolytically processed into extracellular (α_2) and membrane-spanning (δ) components that remain covalently associated by a disulfide bond. $Ca_V\alpha_2\delta$ is required for proper cell-surface expression of Ca_V complexes, but whether it also affects the kinetic properties of the channel remains unclear [15]. Perhaps the most interesting $Ca_V\alpha_2\delta$ property is that it is the target for gabapentin, an anti-epileptic and analgesic drug that acts via a yet unknown mechanism [16]. A final subunit, $Ca_V\gamma$, associates with skeletal-muscle calcium channels, but its general importance for other channel types remains uncertain [17].

The quaternary arrangement of Ca_V components remains undefined. Three low-resolution electron microscopy studies of skeletal-muscle Ca_Vs have yielded very different images [18-20]. The limited resolution, together with the lack of a consensus, hinders the mechanistic utility of these studies. The present limitations in the use of native Ca_Vs for high-resolution structural studies has inspired a different approach to obtain insight into the structural basis of Ca_V function. Division of the channel into functional parts has yielded the first two high-resolution structures of intracellular Ca_V components: $Ca_V\beta$, alone and complexed to a part of the I–II loop [21-23], and the Ca^2+/CaM-$Ca_V1.2$ IQ (Ile-Gln) domain complex [24,25]. Like many structural studies, these advances have settled some questions and raised others.

$Ca_V\beta$s

$Ca_V\beta$s are approx. 500-amino-acid cytoplasmic proteins that bind to the Ca_V I–II intracellular loop [26] and affect channel gating properties [27,28], trafficking [29,30], regulation by neurotransmitter receptors through G-protein $\beta\gamma$ subunit activation [31] and sensitivity to drugs [32]. There are four mammalian isoforms and each has separate effects on Ca_V VDI properties [33]. The $Ca_V\beta$ primary sequence encodes five domains, arranged V1-C1-V2-C2-V3. V1, V2 and V3 are variable domains, whereas C1 and C2 are conserved [34]. C1-V2-C2 makes the $Ca_V\beta$ functional core [23,35,36]. The principal $Ca_V\beta$–$Ca_V\alpha_1$

interaction site is a conserved 18-residue sequence in the I–II loop called the AID (*α*-interaction domain) [37,38].

Structural studies reveal that C1 and C2 form an SH3 domain (Src homology 3 domain) and an NK (nucleotide kinase) domain respectively (Figure 1B) [21-23]. The five-stranded SH3 domain has a split architecture in which the last *β*-strand that completes the fold (*β*5) occurs in the primary sequence following the V2 domain (Figure 1B). $Ca_V\beta$s share structural features with a family of scaffolding molecules, the MAGUKs (membrane-associated guanylate kinases) [39], but have some notable differences that include different relative orientation of the SH3 and NK domains as well as the absence of a number of essential MAGUK domains [21-23].

The structural work resolved how $Ca_V\beta$ binds the AID. A contiguous 41-amino-acid region, known as the BID (*β*-interaction domain) [34], which was thought to be the interaction site, was completely buried in the NK domain core and thus could not be the AID interaction site. Co-crystal structures of $Ca_V\beta$–AID complexes [21-23] showed that the AID binds in a conserved, deep groove at the distal end of the $Ca_V\beta$ NK domain [named the ABP (*α*-binding pocket) [22]]. The AID interacts with $Ca_V\beta$ through a set of conserved residues that include two aromatic residues that are deeply buried in the ABP (Figure 1C).

While the AID–ABP complex forms the high-affinity $Ca_V\beta$–$Ca_V\alpha_1$ site, there is evidence for lower affinity $Ca_V\beta$–$Ca_V\alpha_1$ interactions [36,40], although recent studies challenge this idea for the I-II loop [41]. By virtue of size ($Ca_V\beta$ ~55 kDa), the varied effects of $Ca_V\beta$ subtypes on channel gating and the sheer amount of $Ca_V\alpha_1$ cytoplasmic mass (~1300 amino acids), it is inescapable that there must be other points of $Ca_V\beta$–$Ca_V\alpha_1$ interaction. Although the exact sites are unknown, the $Ca_V\beta_{2a}$ crystal structure provides clues to candidate sites. One is the fairly deep groove between the SH3 and NK domains on the $Ca_V\beta$ side that is opposite to the ABP [22]. A second is defined by a site where a small portion of the V2 loop of one subunit in the crystal lattice binds to a hydrophobic pocket on the SH3 domain surface of the adjacent $Ca_V\beta$ (Figure 1D) [22]. Such crystallographically defined protein–protein contacts often indicate sites that are in want of a binding partner, even if the observed partner is not the natural one [42]. Finally, the V1, V2 and V3 regions seem likely candidates for elements that alter channel gating through contacts with $Ca_V\alpha_1$.

Despite extensive functional study, the molecular mechanisms by which $Ca_V\beta$s act remain imperfectly understood. In addition to the biophysical changes, $Ca_V\alpha_1$–$Ca_V\beta$ interactions markedly influence cell-surface expression [29,30]. Control of Ca_V trafficking by cellular signalling proteins that regulate $Ca_V\alpha_1$–$Ca_V\beta$ interactions may provide an important means to modulate cellular excitability [29]. There also remains a question surrounding the role of the $Ca_V\beta$–AID interactions in Ca_V inhibition by G-protein *βγ* subunits. Biochemical experiments show that $G_{\beta\gamma}$ binds the AID peptide, albeit with weaker affinity than $Ca_V\beta$ [43]. The relevance of the $G_{\beta\gamma}$ –AID interaction for physiological regulation is unclear and is challenged by the demonstration that key positions required for $G_{\beta\gamma}$ –AID interactions are buried deep in the ABP [22]. Whether the mechanism of $G_{\beta\gamma}$ regulation of Ca_V channels occurs by antagonizing $Ca_V\beta$ binding to the AID or through other mechanisms remains to be determined [31]. Finally, recent studies of $Ca_V\beta_3$ knockout mice have shown in pancreatic *β*-cells that $Ca_V\beta$s have a role in Ca^{2+} oscillation modulation that is independent of $Ca_V\alpha_1$ [44]. Defining such ‘non-channel’ $Ca_V\beta$ functions represents an exciting new direction. Because Ca_V channel subtypes are major targets for drugs for cardiovascular disease, migraine and pain [3], the development of compounds that could interfere with the $Ca_V\alpha_1$–$Ca_V\beta$ interactions based on structural information may provide new ways to modulate Ca_V function in pathological states.

The Ca_V Ca^{2+} /CaM–IQ domain interaction

Association with CaM endows Ca_Vs with CDI and CDF properties [14]. The Ca^{2+}/CaM target site is part of the Ca_V C-terminal cytoplasmic tail known as the IQ domain, a class of domains found in many Ca^{2+}/CaM modulated proteins [45] (Figure 2A). Crystallographic studies have provided the first high-resolution structures of a Ca^{2+}/CaM-IQ domain complex [24,25] and reveal features of the Ca^{2+}/CaM-IQ domain interaction that have important consequences for understanding how CDI and CDF work. CaM has two lobes (N-lobe and C-lobe). Both wrap around a helix formed by the $Ca_V1.2$ IQ domain and each engages a set of three aromatic anchor residues from the IQ domain (Figures 2B and 2C). The complex is unusual in that the relative orientation of Ca^{2+}/CaM and the IQ helix is the opposite to that in most other Ca^{2+}/CaM–peptide complexes. Ca^{2+}/CaM binds to the IQ domain helix in a parallel manner in which the N-lobe binds the N-terminal end of the IQ helix, while the C-lobe binds to the C-terminal end rather than in the usual antiparallel mode.

One puzzle in understanding CDI and CDF has been that the action of opposite CaM lobes on the conserved IQ domain has different roles in the Ca_V1s and Ca_V2s [14]. In Ca_V1s, the Ca^{2+}/C-lobe controls CDI, but CDI is governed by the Ca^{2+}/N-lobe in Ca_V2s. There is a clear role for the Ca^{2+}/C-lobe in Ca_V2 channel CDF but no previous characterization of the origin of Ca_V1 CDF. Titration calorimetry experiments establish two Ca^{2+}/N-lobe binding sites ($K_d \approx 50$ nM and $K_d \approx 20\ \mu$M) on the $Ca_V1.2$ IQ domain and a single high-affinity Ca^{2+}/C-lobe site ($K_d \approx 2$ nM) [24]. The Ca^{2+}/C-lobe site matches well what is known regarding the prominence of the Ca^{2+}/C-lobe in Ca_V1 CDI [9,46]. Disruption of the Ca_V1Ca^{2+}/N-lobe binding site by simultaneous mutation of the three N-lobe aromatic anchors to alanine eliminates CDF and indicates a previously unknown role for the Ca^{2+}/N-lobe interaction site in Ca_V1 CDF [24]. Together with previous work, this observation suggests that there is an exchange of roles for the two CaM lobes between the two channel types.

How might such a role exchange happen? In light of the structural data, comparison of the IQ domains from Ca_V1s and Ca_V2s reveals six amino acids that are conserved within the subtypes but not between the subtypes (Figure 2C). Notably, three positions are aromatic anchor sites. How might these changes cause exchange of N-lobe and C-lobe roles (Figure 2D)? One possibility is that the differences alter the relative affinities of the lobes for their respective IQ domain binding sites (and by linkage the calcium affinities of the individual CaM lobes) and that these changes tip the balance so that N-lobe binds more tightly in Ca_V2 channels and thereby leads to CDI. Another possibility is that the IQ domain residue differences result in an exchange of binding sites so that in Ca_V2 channels the Ca^{2+}/CaM complex binds the IQ domain in the more common antiparallel arrangement (Figure 2D). In this way, the CDF site is in the same relative place (at the N-terminal end of the IQ peptide) but occupied by the other CaM lobe. While such a binding site exchange would provide a simple and elegant explanation for the exchange of lobe-specific roles in CDI and CDF, the situation may be more complicated. There are potential roles for apo-CaM [13,47] and CaMKII [48] in binding to the IQ peptide and in CDI and CDF. There may also be other CaM binding sites on $Ca_{V\alpha_1}$ that affect these processes. Clearly, a Ca^{2+}/CaM–Ca_V2 IQ domain structure and structures of larger portions of the C-terminal domain will help clarify the issue.

Intracellular domains and gating

Given the structures of Ca_V intracellular components, a key question is how these domains influence the opening and closing of the Ca_V pore. The simplest model for understanding $Ca_V\beta$ regulation is that the AID helix is part of a longer helix that continues into the IS6

transmembrane segment (Figure 3A). As IS6 is implicated in channel closing, restriction of the movement of IS6 by $Ca_V\beta_{2a}$, which is anchored to the membrane by a palmitoylation site at the N-terminus of the V1 segment [26], may be an important mechanism for slowing channel inactivation. It is less obvious how the other $Ca_V\beta$s work. $Ca_V\beta_{1b}$ and $Ca_V\beta_3$ speed inactivation and $Ca_V\beta_4$ causes moderate slowing [33]. None of these isoforms has an N-terminal lipid anchor. Because the core SH3 and NK domains are identical, it seems likely that variability in the V1, V2 and V3 segments will be responsible for the other properties via interaction with parts of $Ca_{V\alpha1}$.

The IQ domain is too far in primary sequence from IVS6 to postulate a simple model for how Ca^{2+}/CaM effects gating. One interesting question is whether $Ca_V\beta$ and the CaM domain collaborate in some way to affect the movement of a common element in the pore (Figure 3B). The possibility that such a linkage happens via the isoleucine residue of the IQ domain [49] is unlikely as this residue is completely buried by the Ca^{2+}/C-lobe [24].

There are other large Ca_V cytoplasmic regions. Defining interactions between these domains, their structures, their relationships to $Ca_V\beta$ and the CaM/IQ domain and their conformational changes are at the heart of determining how Ca_Vs work. Eventually, we will need to see the architecture of the entire approx. 0.5 MDa complex and how Ca_Vsintegrate into signalling networks [50]. Until then, pairing the piece-by-piece dissection of Ca_V structure with biochemical and functional studies is likely to be fruitful for understanding Ca_V design, function and regulation.

Acknowledgments

F.V.P. is an American Heart Association Postdoctoral Fellow. D.L.M. acknowledges support from the McKnight Foundation for Neuroscience, Rita Allen Foundation, Searle Scholars Award, Beckman Young Investigator Award and the National Institutes of Health.

Abbreviations used

ABP	α-binding pocket
AID	α-interaction domain
Ca	voltage-gated calcium channel
CDF	calcium-dependent facilitation
CDI	calcium-dependent inactivation
CaM	calmodulin
MAGUK	membrane-associated guanylate kinase
NK	nucleotide kinase
SH3 domain	Src homology 3 domain
VDI	voltage-dependent inactivation

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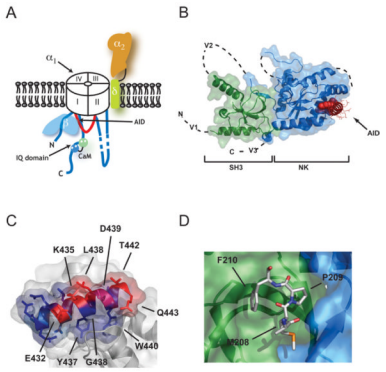

Figure 1. Voltage-gated calcium channel: $Ca_V\beta$ interactions

(**A**) Schematic cartoon of a voltage-gated calcium channel. Ca_Vs are composed of five polypeptides. The pore-forming $Ca_V\alpha_1$ subunit (white) is approx. 1800–2300 amino acids long, depending on the subtype, and is composed of four homologous membrane-spanning domains (I–IV) that are bridged intracellular loops. The transmembrane domains are indicated. For clarity, the shortest intracellular loop, the III–IV loop, is not depicted. Although the cartoon shows an anticlockwise transmembrane domain arrangement, the pore-forming subunit handedness is not known. $Ca_V\alpha_2\delta$ (orange, α_2, and light green, δ) is a single pass transmembrane subunit formed from two disulfide-linked, glycosylated polypeptides: the extracellular α_2 subunit (~930 amino acids) and the transmembrane δ subunit (~150 amino acids). Two intracellular proteins complete the complex. $Ca_V\beta$ (light blue) is an approx. 480–600-amino-acid subunit that binds with high affinity to the I-II intracellular loop (shown in red) at a binding domain known as the AID. Ca_Vs also bind CaM. One of the sites of action of CaM is a conserved domain, known as an IQ domain, that is located on the C-terminal cytoplasmic segment of the α_1 subunit. The N- and C-terminal lobes of CaM are shown in green and blue respectively. (**B**) The $Ca_V\beta$–AID complex structure. Co-ordinates are for the $Ca_V\beta_{2a}$–$Ca_V1.2$ AID complex [22]. SH3 (green) and NK (blue) domains are indicated. V1, V2 and V3 show the locations of the three variable domains that are absent from the structure. The AID (red) binds to a deep groove (the ABP) in the NK domain. AID residues tyrosine, tryptophan and isoleucine are shown as CPK. The remaining residues are shown as lines. (**C**) The $Ca_V\beta$–AID interaction uses conserved interactions. The degree of conservation among the $Ca_V\alpha_1$ subunits is shown. Blue, invariant; magenta, conserved; red, variable. Selective $Ca_V1.2$ AID residues are indicated. (**D**) Protein–protein interaction observed in the $Ca_V\beta_{2a}$ structure between a portion of the V2 loop (residues 208–210) and a hydrophobic pocket on the SH3 surface. The binding site is on the opposite side of the SH3 domain from that shown in (**B**).

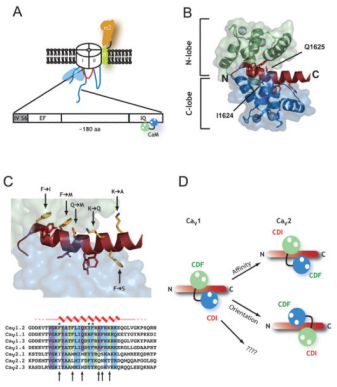

Figure 2. Structural insights into Ca^{2+}-CaM regulation of Ca_Vs

(**A**) Schematic diagram of the conserved Ca_V C-terminal domain involved in calcium-dependent Ca_V regulation. The domain begins immediately after the last transmembrane segment of transmembrane domain IV (IVS6) and contains an EF-hand motif, a conserved intervening region and the IQ domain. Ca^{2+}/CaM binds tightly (K_d in nM) to the IQ domain. (**B**) High-resolution structure of the Ca^{2+}/CaM–$Ca_V1.2$ IQ domain complex [24]. Ca^{2+}/CaM N-lobe (green) and C-lobe (blue) and IQ domain (dark red) are shown. Aromatic anchor positions on the IQ domain are shown in stick representation. The positions of Ile^{1624} and Gln^{1625} that define the IQ domain are also shown. Ca^{2+} ions are shown as spheres. (**C**) Six key Ca^{2+}/N-lobe–IQ domain and Ca^{2+}/C-lobe–IQ domain contact positions are conserved within Ca_V1s and Ca_V2s, but differ between the two subfamilies. Top: structure from (**B**) in which $Ca_V1.2$ residues are shown as sticks with the amino acid differences between $Ca_V1.2$ and $Ca_V2.1$ indicated. Bottom: sequence alignment of Ca_V1 and Ca_V2 IQ domains. Colours indicate residues that contact the Ca^{2+}/N-lobe (green), Ca^{2+}/C-lobe (blue) and both lobes (purple). Arrows indicate positions of the amino acid differences between Ca_V1s and Ca_V2s. (**D**) Hypotheses for the swapping of lobe-specific roles for Ca^{2+}/CaM in CDF and CDI of Ca_V1s and Ca_V2s. The Ca_V1 schematic diagram shows the parallel orientation of the Ca^{2+}/CaM-$Ca_V1.2$ IQ domain complex. The changes in key positions indicated in (**C**) may cause the swapping of CDF and CDI roles by causing affinity changes (right-hand side top), a reversal of Ca^{2+}/N-lobe and Ca^{2+}/C-lobe binding positions to an antiparallel orientation (bottom), or interactions with yet to be characterized structural elements.

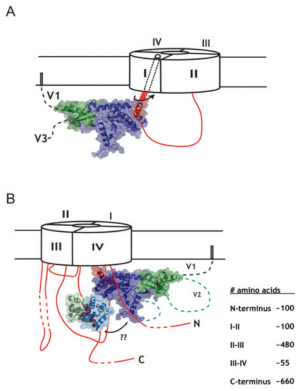

Figure 3. Structural insights into Ca_V gating

(**A**) Cartoon model of how $Ca_V\beta$ affects $Ca_{V\alpha 1}$ gating. For simplicity, only the I–II loop is shown. $Ca_V\beta$ influences the movement of IS6. (**B**) Potential interplay between Ca^{2+}/CaM regulation and $Ca_V\beta$ regulation of Ca_V channel gating. How such interactions occur remains to be revealed. The lengths of the intracellular domains are indicated.