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Structure and Function of Voltage-Dependent Ion Channel Regulatory β Subunits†

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ABSTRACT: Voltage-dependent K^+, Ca^{2+}, and Na^+ channels play vital roles in basic physiological processes, including management of the action potential, signal transduction, and secretion. They share the common function of passively transporting ions across cell membranes; thus, it would not be surprising if they should exhibit similarities of both structure and mechanism. Indeed, the principal pore-forming (α) subunits of each show either exact or approximate 4-fold symmetry and share a similar transmembrane topology, and all are gated by changes in membrane potential. Furthermore, these channels all possess an auxiliary polypeptide, designated the β subunit, which plays an important role in their regulation. Despite considerable functional semblences and abilities to interact with structurally similar α subunits, however, there is considerable structural diversity among the β subunits. In this review, we discuss the similarities and differences in the structures and functions of the β subunits of the voltage-dependent K^+, Ca^{2+}, and Na^+ channels.

The action potential of the excitable cell typically consists of a rapid depolarization phase concomitant with the transmembrane influx of Na^+, which may be maintained by the influx of Ca^{2+}, and finally terminated by the repolarizing outflow of K^+. In addition, during the plateau phase associated with Ca^{2+} elevation, a host of Ca^{2+}-dependent biochemical processes may be triggered. The respective Na^+, Ca^{2+}, and K^+ voltage-dependent channels mediate these movements of ions. They are multisubunit complexes (Figure 1), all of which include a pore-forming α subunit and a modulatory β subunit. Channel conductance can be separated into two processes: first, activation which determines the time and voltage dependency of channel opening and second, inactivation which controls channel closure associated with changes in membrane potential and other factors. β subunits play a crucial role in the regulation of these processes. Furthermore, longer timescale channel modulation associated with phosphorylation and guanine nucleotide-binding proteins (G proteins),[1] for example, also appear to be subject to the controlling influences of β subunits. Evidence is also available that additional functions independent of channel modulation exist.

Given the similar roles attributable to the β subunits of the K^+, Ca^{2+}, and Na^+ channels and the structural similarities of the α subunits which they regulate, it would seem reasonable that the β subunits might also share similarities of structure. Surprisingly, however, they appear to have quite diverse structures with which they achieve similar modulatory functions in all the structurally homologous voltage-dependent ion channels. This review focuses on recent developments in understanding the structures and functions of the β regulatory subunits of voltage-dependent ion channels.

ARCHITECTURE OF VOLTAGE-DEPENDENT ION CHANNELS

The voltage-dependent K^+, Ca^{2+}, and Na^+ ion channels all consist of pore-forming α subunits and one or more auxiliary subunits, including a regulatory β subunit. A number of common features exist in the α subunits, including an exact or pseudo 4-fold symmetry and, in general, six transmembrane segments plus a P region which forms the lining of the pore in each subunit or domain. The structures and functions of the pore-forming subunits of the various types of voltage-dependent ion channels have been reviewed recently (e.g., refs *1*−*6*). In this review, we examine the structure/function relationships of the β subunits and their similarities and differences. First, we place the β subunits in the context of the overall architecture of each of the channels (Figure 1).

K^+ Channels. Voltage-dependent potassium channels (K_v) are comprised of a pore-forming tetramer of α subunits, which form a 1:1 association with the accessory (regulatory) β subunits (*7*) (Figure 1A). The α subunits of the mammalian counterparts of the cloned channels of *Drosophila* known as *Shaker*, *Shab*, *Shaw*, and *Shal* are designated K_v1, K_v2, K_v3, and K_v4, respectively (*8*). These families each consist of a number of subfamilies, including $K_v1.1$−1.9, $K_v2.1$−2.2, $K_v3.1$−3.4, and $K_v4.1$−4.3, which display approximately

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[1] Abbreviations: AID, α interaction domain; BID, β interaction domain; GK, guanylate kinase; G protein, guanine nucleotide-binding protein; K_v, voltage-dependent potassium channel; MAGUK, membrane-associated guanylate kinase; TM, transmembrane.

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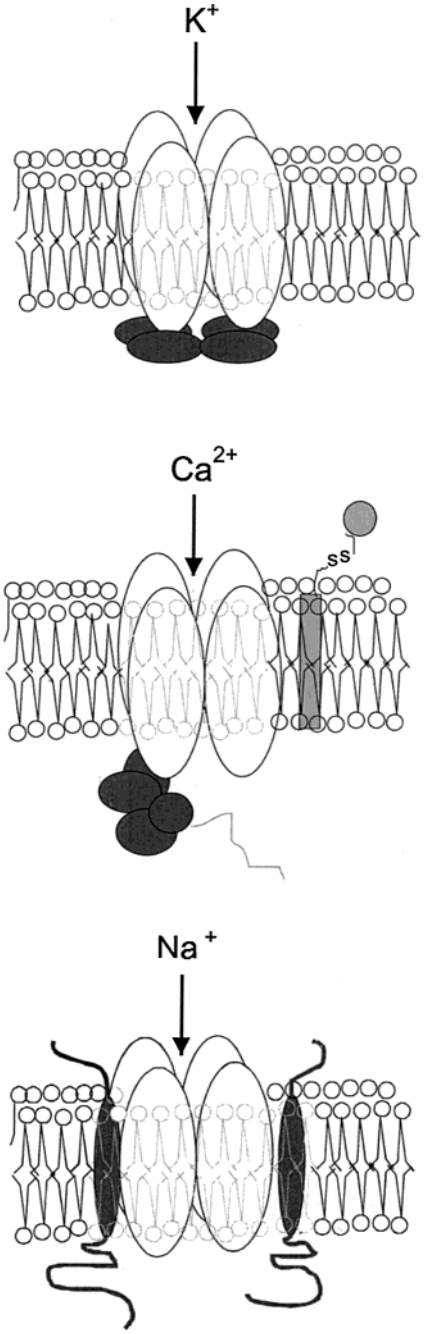

FIGURE 1: Schematic representations of the subunit architectures of the voltage-dependent ion channels: (The β subunits are shaded in dark gray.) (A) K_v channel: the β subunits form a 1:1 complex with the tetrameric α subunits; (B) Ca^{2+} channel: one β subunit per pseudo 4-fold monomeric α1 subunit is shown. Each β subunit is proposed to consist of five domains, the C-terminal domain being largely unstructured. The disulfide-linked α2δ subunit is shown in light gray; (C) Na^+ channel: one or more transmembane β subunits form a complex with the pseudo 4-fold monomeric α subunit.

40% homology between them. Each α subunit contains six transmembrane (TM) segments, designated S1−S6, with intracellular N and C termini (*9*). Tetramerization appears to be controlled to some extent by the N-terminal T1 domain (*8*), a feature not found in either Ca^{2+} or Na^+ channels. Between transmembrane segments S5 and S6 is the highly conserved H5 region (also designated the P region) which appears to be involved in conferring the K^+ selectivity of the channel (*10*, *11*). Toxins used to probe the architecture of the mouth of the pore indicated that the H5 region extends

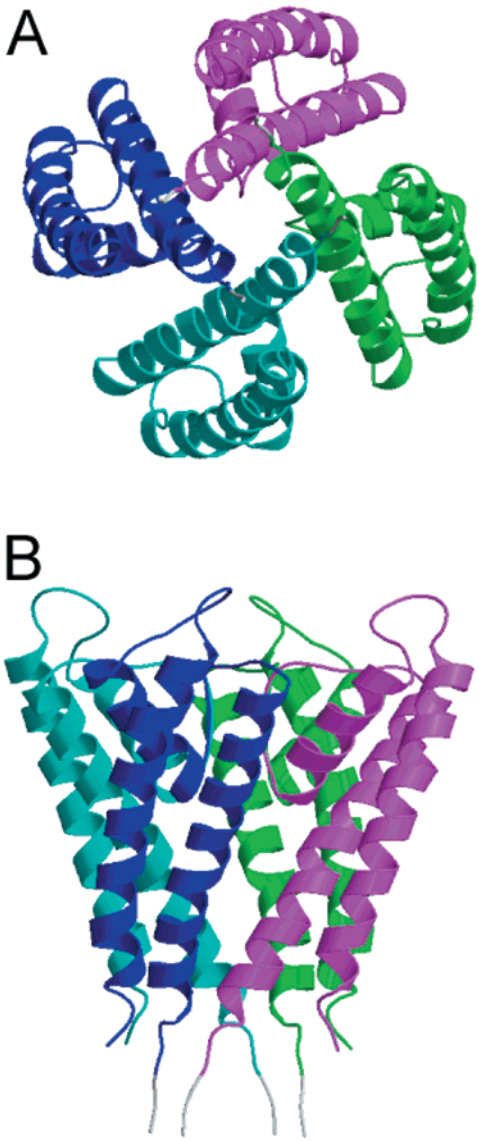

FIGURE 2: Crystal structure of the potassium channel (KcsA) of *S. lividans* [Protein Data Bank (PDB) ID 1BL8] (*15*) formed from four identical α subunits: (A) viewed from the cytoplasmic side, perpendicular to the 4-fold axis which lies along the central pore; (B) viewed from the side. The four subunits are depicted in different colors using Raster3D software (*125*).

about 5 Å into the mouth, forming a narrow cone (*12*, *13*). In addition, mutation studies provided evidence that parts of the S4−S5 cytoplasmic linker and the S6 region are located in the inner mouth of the pore (*14*).

The K^+ channel is the only ion-selective channel for which an α subunit structure has been determined crystallographically (*15*). The structure (Figure 2) is that of the bacterial channel (KcsA) from *S. lividans*, which is a simplified version of a K_v channel having only two transmembrane domains (equivalent to S5 and S6 in the more complex channels) (*16*). It exhibits considerable homology to the K_v channels, particularly in the pore region. Each of four identical subunits consists of these two TM helices, linked by the ∼30 residue P region, forming a funnel-shaped structure. Channels are produced by the association of a bundle of eight TM helices, two of which are contributed by each monomer. The C-terminal helix lines the pore, while the other TM helix faces the lipid membrane. The P region is a mostly helical structure which extends into the mem-

brane. A narrow selectivity filter (containing the K^+ channel signature sequence TVGYG in the P region) sits above a 10-Å diameter water-filled cavity, which is connected to the intracellular medium via a channel which is 18 Å in length (*15*). The orientation of the four helices toward the center of the cavity (which surrounds the ion with water) enables the channel to overcome the electrostatic constraints which would otherwise pose an energy barrier to the passage of K^+ ions. This simple channel is lacking the voltage-sensitivity function, normally conferred by one of the additional (S4) transmembrane segments present in the larger eukaryotic channels.

While no detailed structure of a full-sized (6 TM) K_v channel has yet been solved, a low-resolution electron microscopic determination (*17*) has shown the relative orientations of the T1 and pore domains and that pore domains are 4-fold symmetric but are larger, as expected, than the KcsA pore, accounting for the four additional TM spans.

Conformational changes play key roles in the activation and inactivation properties of K_v channels. In the former case, for example, it has been shown that depolarization results in translocation of the S4 segment, producing a current which instigates the conformational change that opens the channel pore (*18*). Both N-type and C-type inactivation, so-called for the terminal locations of residues involved, have been detected for the *Shaker* channel. A ball domain model has been proposed for the former type, where N-terminal residues of the α subunit are able, via a flexible linkage, to occlude the channel pore (*19*, *20*). In some cases, it has been suggested that the ball domain resides, instead, on the *β* subunit (*21*) and provides one of the means of regulating the channel function.

Ca^{2+} Channels. Ca^{2+} channels may be broadly divided into low threshold activated type (T) and higher threshold types (L, N, P, Q, R), with this subclassification indicative of their pharmacological properties. As many as five subunits may form the holochannel: α1, *β*, α2*δ*, and *γ* (Figure 1B). The α1 subunit is the pore-forming component and contains the gate and voltage sensor. The *β* subunit is entirely cytoplasmic (*22*) and appears to function in a wide range of regulatory functions. The α2*δ* subunit is the product of cleavage of a single gene product and consists of a cytoplasmic α2 fragment linked to the membrane by a disulfide bond to a transmembrane *δ* fragment. The α2*δ* subunit also appears to be responsible for some modulatory effects on the pore activity, the α2 component having a role in the level of membrane expression with the *δ* component being involved in modulation of the channel gating properties (*23*).

α1 subunits are derived from several different genes. The L-type channel, for example, may incorporate $\alpha1_S$ (*24*), $\alpha1_C$ (*25*), or $\alpha1_D$ (*26*) variants, as they have previously been known. A new nomenclature has been proposed using a systematic naming scheme, analogous to that used for K_v channels, whereby the variants will be designated $Ca_v1.1$, $Ca_v1.2$, and so forth (*27*). Similar diversity is displayed in the auxiliary subunits. The *β* subunits are numbered according to their gene family and given lower case letters for their splice varients (i.e., *β*1a, *β*1b, etc.).

In common with the K_v channel, the pore-forming component of the Ca^{2+} channel is comprised of four similar segments. In this case, however, instead of four separate subunits, they exist in a single polypeptide chain, the α1 subunit, and are designated domains I−IV. Each highly homologous, but nonidentical, domain consists of six transmembrane segments and is linked to the next domain by an intracellular loop region. As with the K_v channel, the pore is comprised of eight TM segments, two (S5 and S6) contributed by each domain. Ca^{2+} selectivity and binding is associated with four conserved glutamate residues in equivalent positions in the pore-lining regions of each of the four domains, with the one in domain III apparently having the greatest effect (*28*). The loop between domains I and II of the α1 subunit is involved in interactions with both the *β* subunit (*29*) and with G proteins (*30*). In the former case, the relevant region is referred to as the α interaction domain (or AID) (*31*). G protein modulation may also be associated with the C-terminal tail in the case of $\alpha1_B$ (*32*).

In contrast to Na^+ channels, the loop between domains III and IV of the Ca^{2+} channel does not appear to have an effect on inactivation (*33*). Instead, residues in the S6 TM segment of domain I appear to be more functionally important in this context (*34*). The mechanism involved remains unclear but does not appear to involve direct channel occlusion, as is the case for both K_v and Na^+ channels. L-type channels are subject to Ca^{2+}-dependent inactivation, which was originally proposed to be related to phosphorylation (*35*). However, later work has indicated the involvement of a specific Ca^{2+} binding motif (an EF hand), present in the C terminus of the $\alpha1_C$ subunit, although the mechanism is not yet understood (*36*).

Na^+ Channels. Substantial sequence similarity is evident between Na^+ α subunits and Ca^{2+} channel α subunits and lead to the proposal that they share a similar transmembrane structure (*37*). In addition to the α subunit, one or two *β* subunits are present in most Na^+ channels (Figure 1C) (*38*). While the *β* subunits modify the properties of the α subunit, they are not essential for functional channel formation (*39*). Multiple types of α subunits have been cloned, and the human brain alone contains at least three variants (*40*, *41*).

In common with the Ca^{2+} channel, the Na^+ channel is comprised of four homologous domains (I−IV), each consisting of six transmembrane segments (S1−S6). S4 in each domain appears to play a pivotal role in channel opening, possessing multiple positively charged residues and moving in response to depolarization (*42*). Like the Ca^{2+} channel, the pore is formed from the four different P regions located between S5 and S6 in each domain (*43*). This is in contrast to the K_v channels where identical pore-forming segments are involved. Again, no molecular structure of a Na^+ channel has yet been observed crystallographically; however, a low resolution cryo-electron microscopy map (*44*) suggests it is a bell-shaped object of dimensions consistent with its proposed TM structure. An apparent 4-fold symmetry is seen, although at this resolution it would be indistinguishable from the actual pseudo-4-fold symmetry present in the molecule; consequently, the structure is consistent with the models for the pore.

Domains III and IV of the Na^+ channel are involved in Na^+ selectivity, with a specific lysine in domain III conferring K^+/Ca^{2+} selectivity and a group of three contiguous residues in domain IV conferring selectivity among monovalent cations (*45*). The high degree of selectivity and ion

throughput displayed by these channels may be the result of specific conformational changes in the pore-forming regions (*46*).

Channel activation, involving the translocation of several charged residues of the S4 segments (*47*), is markedly asymmetrical (*48*). The activation gate has been proposed to involve the S6 segment (*49*). Inactivation appears to be a rather more complex process, involving both slow and fast types. The linker region between domains III and IV is involved in the fast mode (*50*) and has been suggested to function as a hinged lid (*51*). Slow inactivation, by contrast, appears to be linked to a conformational change involving residues in the outer pore region of the channel (*52*).

β SUBUNITS OF VOLTAGE-DEPENDENT ION CHANNELS

K^+ Channel β Subunits. The subfamilies of the aforesaid K_v channels exhibit three distinct classes of β subunits, $K_v\beta1$ (*53*), $K_v\beta2$ (*54*), and $K_v\beta3$ (*55*), together with splice variants of $K_v\beta1$ and $K_v\beta3$ (*56*) which are designated by the nomenclature $K_v\beta1.1$, $K_v\beta1.2$, and so forth. $K_v\beta$ subunits are comprised of approximately 400 residues and have been shown to assemble with α subunits in the endoplasmic reticulum (*57*).

Multiple sequence alignment indicates that these subunits possess a variable N terminus (with identities of ~16% between classes) but an otherwise highly conserved sequence (identities in the range of 62% to 76% between classes). They show considerable homology to the oxidoreductase superfamily, with sequence identities in the range of 15% to 30% which suggested that they would possess a α/β barrel-type fold (*58*). Sequence conservation with the aldo-keto reductases included the catalytic tyrosine and the NADPH cofactor-binding residues.

The β subunits form a tetramer (*59*), which complements the tetrameric nature of the α subunit giving rise to an $\alpha_4\beta_4$ complex. Hydropathy analyses do not suggest the presence of any membrane spanning regions or signal peptides in the β subunit (*53*) and there is no evidence for *N*-glycosylation sites (*60*).

The crystal structure of the N-terminal truncated core (residues 36−360) of β2 (Figure 3A) has been recently determined (*61*). It has been proposed that the N-terminal part of the β subunit not present in the crystal construct may be an unordered structure. The disordered nature of the N terminus was supported by an NMR study on a synthetic peptide corresponding to residues 1−62 (*62*). Although this displayed a largely unstructured form in solution, shifted amide resonances in the regions between residues 2−10, 44−52, and 56−61 did indicate a helical propensity, leading to the conclusion that the peptide existed in an "equilibrium of weakly structured states". The conserved core regions present in the crystal structure form a tetramer, each monomer of which is comprised of a TIM barrel-like fold with approximate dimensions of 90 Å × 90 Å × 40 Å and a central core of eight parallel β strands encircled by intervening α helices. It has a deep cleft that includes the catalytic tyrosine residue found in the reductase structures.

The structure of the complex of β2 (Figure 3B) with the tetramerization (T1) domain of the α subunit has also been determined (*63*). The T1 component of the complex is not thought to be directly involved in inactivation but instead

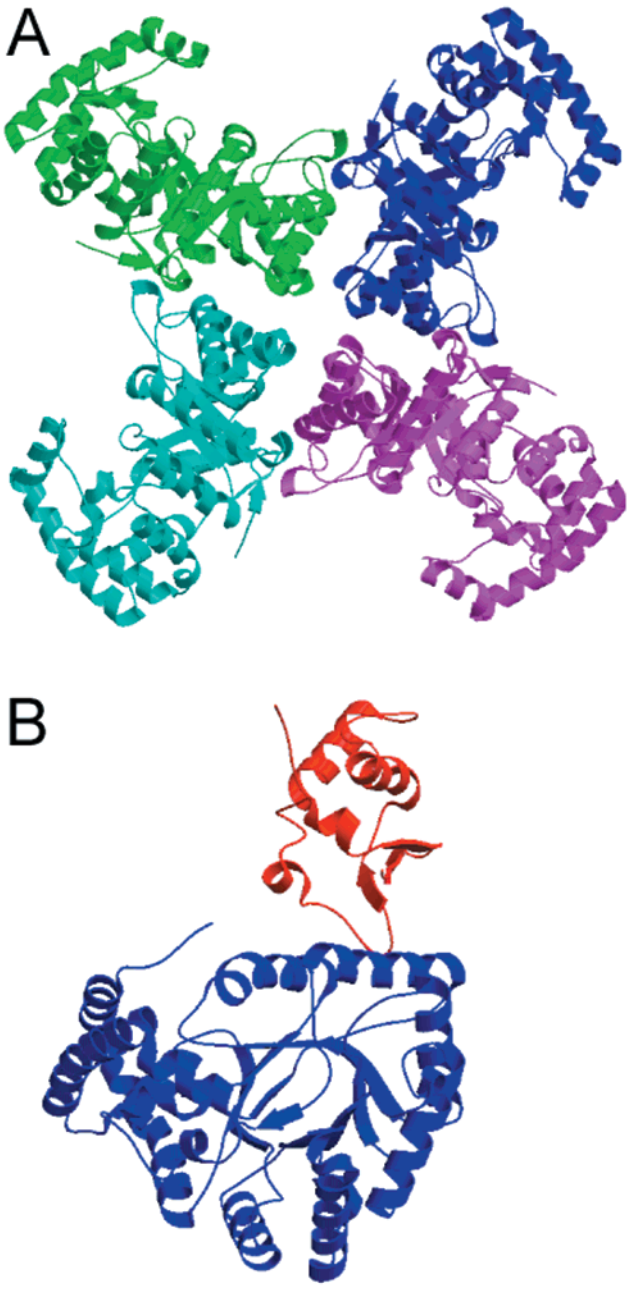

FIGURE 3: Crystal structures of potassium channel β subunits: (A) structure of the conserved core regions of $K_v\beta2$, residues 36−360 [PDB ID 1QRQ] (*61*), showing the 4-fold symmetry of the complex, centered on the pore axis (each subunit forms a TIM barrel-like fold); (B) complex of a single β2 subunit with the α subunit tetramerization (T1) domain (in red) [PDB ID 1EXB] (*63*).

provides a docking platform for the β subunit via four prominent contact loops at the N-terminal side of T1 (*63*). The crystal structure of the complex also suggests that the flat, as opposed to the concave, face of the β subunit is in contact with the α subunit.

The biological functions of the $K_v\beta$ subunits are not well understood; however, roles both in channel gating and cell surface expression have been proposed. These are not universal properties for all $K_v\beta$ subunits, however, as β2 appears to have little effect on channel inactivation although it does have a pronounced role in the kinetics of activation. Such activity seems to be associated with the N terminus of the subunit, and similar variation is apparent in relation to other functions associated with the core domain.

Only $K_v\beta1$ and $K_v\beta3$ induce rapid inactivation of delayed rectifier type K_v channels (*53*, *64*). However, the apparent conversion of the K_v channel from slowly inactivating delayed-rectifier to rapidly inactivating A-type may not be complete. It seems to be subject to further modulation via G protein $\beta\gamma$ subunits, which have been proposed to enhance binding between $K_v\alpha1.1$ and $K_v\beta1.1$ subunits by disrupting channel interactions with microfilaments and thereby increasing the level of inactivation (*65*). It has also been shown that modification of the inactivation response is subject to phosphorylation by protein kinase C or a related enzyme in the case of the $K_v1.5/K_v\beta1.3$ interaction (*66*), possibly in the β C-terminal domain. Protein kinase A-mediated phosphorylation of $K_v\beta1.3$ occurs at Ser24 (*67*) in the N terminus and appears to decrease the modulatory effect of $K_v\beta1.3$.

A hyperpolarizing shift in the midpoint of activation and an increase in the deactivation time constant have been observed in the case of $K_v\beta1.3$ (*68*, *69*). In addition, $K_v\beta3$ has been shown to impede both channel deactivation and recovery from inactivation (*69*). $\beta2$ appears to produce voltage-dependent effects on activation (*64*) and inactivation (*70*). There has been some evidence to indicate that $K_v\beta2$ plays a chaperone function, promoting cell surface expression, glycosylation, and correct folding of nascent α subunits ($K_v1.2$) (*71*). This behavior seems to be specific to the combination of α and β subunits (*57*).

On the basis of the similarity of the K_v β subunit structure to the aldo-keto reductases, two functional roles have been proposed for it, although no actual enzymatic substrate has been identified (*61*). The first is the "voltage-dependent enzyme hypothesis", whereby gating-associated changes of the α subunit could induce a β subunit conformational change, leading to exposure of the active site, thus linking membrane electrical excitability to catalysis. The other mechanism involves a conformational change of the β subunit occurring when the NADPH cofactor is oxidized to $NADP^+$, thus mediating the gating of the K_v channel (*61*).

The $K_v\beta2$ variant has a shorter N terminus as compared to $K_v\beta1$ and $K_v\beta3$, and this may well be related to its different functional behavior. The relevant mechanism is thought to be analogous to that of the ball-like domain model proposed for the K_v channel α subunit (*20*, *72*) that serves to induce inactivation via occlusion of the channel pore when in the open state. Alignment of the $K_v\beta1$ N-terminus sequence with the sequences of the inactivation gates of the α subunits does indeed reveal a similar serine-cysteine motif preceding a region of positively charged residues (*53*).

Ca^{2+} Channel β Subunits. The β subunits of the voltage-dependent calcium channel are larger (by up to ~70 kDa) than the β subunits of the other channels. Four clonal classes ($\beta1-\beta4$) have been identified plus a number of splice variants, giving rise to specific patterns of association. All four may combine in neuronal channels (*73*−*78*), though the principal component of brain N- and P-type channels is $\beta4$ (*78*). Skeletal muscle and cardiac channels, on the other hand, seem to be exclusively associated with $\beta1a$ and $\beta2a$ subunits (*75*, *79*, *80*), whereas the $\beta1b$ and $\beta1c$ isoforms have only been identified in brain and spleen channels (*81*). $\beta2a$ has also been detected in brain (*82*), as has $\beta3$, which has also been detected in smooth muscle tissues (*75*).

Multiple sequence alignment reveals a very highly conserved core in two segments (~65% and ~78% sequence identity) flanking a more variable region, together with variable N and C termini. Preliminary sequence analysis predicted there would be a series of helices, none of which appeared to be transmembrane (*79*). More detailed structural modeling of the $\beta1b$ subunit has proposed five discrete domains (Figure 4), homologous to the membrane-associated guanylate kinase (MAGUK) protein family (*83*). Homology models have been produced for three of the domains, corresponding to the putative PDZ-like, SH3, and guanylate kinase (GK)-like domains. Sequence homology in all cases was low (~17% for PDZ-like, ~27% for SH3, and ~18% for GK-like); however, secondary structure prediction methods provided additional support for these structures. Furthermore, circular dichroism spectroscopic studies of cloned domains (*84*) support these fold assignments. It may be significant that, in the case of the GK-like domain, alignment to the model template structure (yeast guanylate kinase) was restricted to the phosphate-binding region of the latter, indicating a possible site of phosphorylation of the β subunit. The SH3 domain, by contrast, yielded a good alignment, producing a model comprised of the antiparallel β sandwich structure typical of SH3 domains, together with appropriate residue orientations in the active site region. Although the PDZ-like domain is the most speculative of the three models and would only be possible in the longer isoforms, it was noted that the appropriate binding motifs for a PDZ-binding domain are found in the $\alpha1$ subunit of the Ca^{2+} channel. Of the two other domains, one represents a short region separating the SH3 and GK-like domains and the other the C-terminal ~150 residues of the subunit. The latter domain appears to be an unordered structure, as the modeling study (*83*) found no basis for the assignment of any ordered structure and circular dichroism spectroscopic results were consistent with a stable but unordered structure (*84*). The homologous MAGUK protein SAP97 (*85*) has also been modeled. That study pointed to the potential importance of intramolecular domain interactions which may prove relevant to the Ca^{2+} channel β subunit. It was suggested, for example, that both closed and open conformations could exist in vivo, with the intermolecular domain interaction sites masked in the closed form. Mutant structures associated with disease states may be present mostly in the open state.

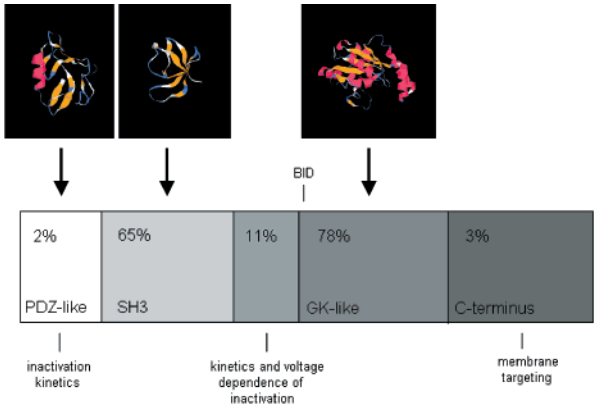

FIGURE 4: Schematic map summarizing key domain structural and functional features of the calcium channel β subunit, numbered according to the $\beta1b$ sequence. Percentage identities between $\beta1$ isoforms are indicated, together with putative domain assignments. "BID" is the β interaction domain. Homology models (*83*) for three of the domains are shown above the appropriate regions of the map.

A range of functional effects has been identified for the Ca^{2+} channel β subunit, including ligand binding (*82*, *86*), modulation of inactivation (increasing the rate of both voltage and Ca^{2+} inactivation) and activation rates (*82*, *86*, *87*), hyperpolarizing shifts in the rates, and peak amplitude increases (*82*, *88*). Some of these functions may be associated with the specific domains of the subunit, and the domain map resulting from modeling work (*83*) exhibited good correlation with the functional map of a typical β subunit (Figure 4) (*89*). A key interaction site comprised of a sequence with the consensus K—E- - -PYDVVPSMRP—LVGPSLKGYEVTDMMKQALFDF is to be found at the N-terminal end of the second highly conserved (GK-like) domain (Figure 4). This is designated the β interaction domain (BID) and has been shown to be capable of modulating current increase and voltage-dependence of activation when coexpressed with the α1 subunit. The latter contains the complementary α interaction domain (AID) located in the I—II linker region between S6 of domain I and S1 of domain II. This region appears to be essential in the case of β1b for the kinetics and voltage-dependence of inactivation (*90*). The BID interaction site does not, however, appear to be required for localization or membrane targeting of the subunit (*91*). This seems, instead, to be associated with the C terminus of the β1b and β2a subunits (*92*). Deletion studies localized this to an acidic motif between residues 547 and 556 of β1b (*93*). The C terminus has also been identified as an additional α1 subunit interaction site in the case of the β2a/α1e (*94*) and β4/α1a complexes (*95*). The N terminus of β2 also appears to have a distinct role, involving the regulation of channel inactivation (*96*). Identification of β2a palmitoylation sites (Cys3 and Cys4) (*97*) and subsequent analysis suggested that dynamic palmitoylation might be a part of the mechanism of regulation (*98*). Further post-translational modification in the form of phosphorylation has also been implicated in Ca^{2+} channel regulation (*99*). All isoforms of β2, for example, are phosphorylated by protein kinase A in the C-terminal region (*100*). Cardiac channel regulation by β subunits has been shown by the loss of channel stimulation in the absence of protein kinase A-mediated phosphorylation sites at Ser478 and Ser479 (*101*).

Certain functional features may be associated with specific domains of the Ca^{2+} channel β subunit. A PDZ-like domain could be involved in inactivation kinetics, for example, yet its more established role, like that of the SH3 domain seems to be in protein assembly. A hinged-lid model has been proposed for the observed fast inactivation properties in the case of the α1e Ca^{2+} channel (*102*), although this might be explained in terms of the BID interaction modulatory effect, perhaps the result of conformational change, and there is evidence that β subunits are concerned with the induction of such change (*92*, *103*). Likewise, the apparently unordered C terminus is associated with membrane targeting, but this function has only been associated with a small local motif in this relatively large domain (*93*).

Intramolecular interactions may be a key feature of the functioning of the Ca^{2+} channel β subunit, as appears to be the case for the MAGUK protein SAP97 (*85*). Another example is the SH3 domain of SAP90/PSD95 which interacts with its GK-like domain (*104*). These proteins tend to form multimers (*105*). It has been shown that ion channels are not only bound by MAGUK proteins such as SAP90 but that their function is also affected (*106*) and that they play a role in the clustering of *Shaker*-type K_v channels (*107*). It would appear, then, that the mediation of protein complex assembly associated with members of the MAGUK family might well prove to be a role associated with the Ca^{2+} channel β subunit. Thus, distinct from regulation by conformational change or G protein antagonism, regulation may be mediated via interactions with wider regulatory and cytoskeletal networks.

Na^+ Channel β Subunits. Unlike the K_v and Ca^{2+} β subunits, the β subunits of the voltage-dependent Na^+ channels are transmembrane glycoproteins. Indeed, if anything, they are more similar in structure to the Ca^{2+} channel $\alpha 2\delta$ auxiliary subunit, which also appears to possess a single transmembrane span.

β subunit family members include β1, β2 (*108*), β3 (*109*), and a splice variant of β1 designated β1a (*110*). β1 and β3 are more closely related (~45% sequence identity) to each other than either is to β2 (β1 identity of ~18%; β3, ~13%). They exhibit type I topology incorporating a single transmembrane region flanked by a large extracellular N-terminal domain and smaller intracellular C-terminal domain. β1a has an additional 66 residue region inserted between the transmembrane and N-terminal domains (*109*—*111*). The N-terminal domain may include a V-like immunoglobulin fold which, in the case of β2, shows homology to the cell adhesion molecule contactin (*111*). The other Na^+ β subunits have homology to the cell adhesion molecule myelin P_o (*63*). The N-terminal domains of β1 (residues 1—120) (*112*) and β3 (residues 1—123) (*109*) have been modeled on rat myelin P_o as having a structure comprised of 10 β strands in two antiparallel sheets.

In common with the other β subunits, the Na^+ channel β subunits serve to increase the functional expression of ionic currents, and this is particularly significant when both β1 and β2 subunits are coexpressed with the Na^+ channel α subunit (*111*). Saxitonin binding studies have indicated that the β subunit mediates a 4-fold increase in surface expression of channels (*113*) and that a disulfide linkage between β and α subunits has been correlated with the same effect (*114*).

More specifically, both cardiac and brain β1 subunits have been found to affect gating kinetics and to produce an increase in Na^+ current amplitude consistent with an increase in the level of channel expression (*115*, *116*). In contrast to both Ca^{2+} and K^+ channels, it has been shown that the acceleration of inactivation of the Na^+ channel occurs as a result of β1 interactions on the extracellular side of the membrane (*117*). The immunoglobulin domain is implicated as a scaffold for the presentation of charged residues to the type IIa α subunit that is involved in this regulatory mechanism (*112*). The extracellular domain alone is able to modulate gating kinetics, perhaps indirectly through some mechanism involving conformational change (*118*), and may result from interaction with the cytoskeleton (*119*). The β2 subunit, which only occurs in neuronal channels, also exerts similar modulatory effects but is also displays distinct functional behavior (*111*). One example of this is its ability to expand the plasma membrane surface area, leading to increases in cell capacitance (*111*). The recently cloned and expressed β3 subunit has been found to exert a modulatory

function in *Xenopus* oocytes, which operates with quite distinct kinetics (*120*).

Structural homology to the immunoglobulin superfamily prompted investigations into the possible cell adhesion function of Na^+ channel β subunits. Interaction of β2 with specific domains of the extracellular matrix molecules Tenascin-R and Tenascin-C suggests a role in the localization and stabilization of Na^+ channels to specific regions such as the nodes of Ranvier (*121*). Furthermore, such interactions also occur with β1, and the detection of changes in the resultant sodium current has led to the proposal that such binding permits these cell adhesion molecules to regulate Na^+ channel activity (*122*). Cell aggregation and the recruitment of the cytoskeletal protein ankyrin have led to the hypothesis that β1 and β2 subunits provide a communication link between the extracellular and intracellular environments (*122*). It appears that the cytoplasmic domain is essential to this activity, as truncated subunits result in cell adhesion but not ankyrin recruitment (*123*). This domain, in β1 but not in β2, is also the site of binding to receptor tyrosine phosphatase β and may serve to regulate the binding of ankyrin (*124*). It is unclear whether this domain of β1 is involved in interactions with the Na^+ channel α subunits, although such interactions have been demonstrated for the extracellular domain (*112*).

CONCLUSIONS

Much has been achieved in elucidating the ways in which the voltage-dependent ion channel β subunits serve to modulate channel behavior. In all cases, a role is apparent in channel gating and expression levels, and there is evidence that the mechanisms may include the induction of conformational change. The Na^+ channel β stands out in possessing a unique cell adhesion function, which would appear to help regulate channel activity in a novel fashion. It is also apparent that other molecules, possibly in concert with the β subunits, can modify channel activity. The $\beta\gamma$ moiety of G proteins is receiving much attention in relation to the Ca^{2+} channel and is also implicated in the case of K^+ channels.

Clearly, the β subunits discussed here share the common function of modulating the activities of their respective ion channels. However, despite the fact that the only experimentally determined structure for any of the β subunits is that of the K^+ channel, no significant structural similarities are likely to exist between the β subunits of the different ion channel types. They exhibit considerable variation in amino acid sequence, size, and post-translation modifications. Some similarities are evident in the effects of modifications on the β subunits; phosphorylation, for example, is implicated in the cases of both Ca^{2+} and K^+ subunits. However, other forms of post-translational modifications, such as glycosylation and palmitoylation, show considerable variation among the β subunits. Furthermore, the Na^+ β subunit is unique in being an integral membrane protein.

Actually, what may be most notable feature of the β subunit regulation is how structurally diverse all of the β subunits are, given that they all function as modulators of structurally very similar pore-forming α subunits. For the various types of channels, the β and α subunits appear to utilize very different structural motifs for their macromolecular interactions in order to produce functionally similar effects. Of additional interest is the great diversity displayed within β subunit families and the reasons behind often subtle functional variation as well as the diverse subunit combinations that are capable of assembling into functional membrane pores. Given this diversity, plus the roles ion channels play in a wide range of diseases and the contributions that the β subunits make to their regulation, these subunits may ultimately prove to be particularly good targets for specific rational drug design and diagnostic screening.

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SUPPORTING INFORMATION AVAILABLE

Figures of the K^+, Ca^{2+}, and Na^+ channel β subunits from various sources and a table of the physical characteristics of voltage-dependent ion channel β subunits. This material is available free of charge via the Internet at http://pubs.acs.org.

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