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# Modelling of a voltage-dependent $Ca^{2+}$ channel β subunit as a basis for understanding its functional properties

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**Abstract Structure prediction methods have been used to establish a domain structure for the voltage-dependent calcium channel β subunit, β1b. One domain was identified from homology searches as an SH3 domain, whilst another was shown, using threading algorithms, to be similar to yeast guanylate kinase. This domain structure suggested relatedness to the membrane-associated guanylate kinase protein family, and that the N-terminal domain of the β subunit might be similar to a PDZ domain. Three-dimensional model structures have been constructed for these three domains. The extents of the domains are consistent with functional properties and mutational assays of the subunit, and provide a basis for understanding its modulatory function.**

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*Key words:* Calcium channel; Homology model; Structure prediction; Threading; Signal transduction and regulation; Membrane protein

## 1. Introduction

Voltage-dependent calcium channels (VDCCs) are located in the plasma membrane of virtually all excitable cell types and serve to control the influx of $Ca^{2+}$ ions into the cell [1]; the inward $Ca^{2+}$ current influences the intracellular concentration of $Ca^{2+}$. Calcium ions are vital secondary messengers, controlling a wide array of processes including smooth muscle contraction, secretion, transmitter release and pacemaker activity [2]. In addition, calcium channel proteins are likely to become increasingly significant in a clinical context as new research highlights their place in a variety of disease conditions [3].

There is a common arrangement of subunits forming the VDCC complex: the pore-forming α1 subunit is comprised of four homologous domains, each composed of six putative transmembrane spanning regions which are linked by loops on either side of the plasma membrane. The disulphide-linked α2-δ subunit, composed of two distinct peptides, is firmly membrane-anchored by the δ moiety, with the α2 subunit being entirely extracellular. Conversely, the β subunit is entirely cytoplasmic [2]. VDCCs have been classified as L, N, T and P [4] (latterly P/Q [2]), differing in their activation/inactivation properties, localisation and functions, and their susceptibility to calcium channel blocking agents. Although it is clear that $\alpha_1$ subunits that are the molecular counterparts of L ($\alpha_1$C, D, S), N ($\alpha_1$B) and P/Q ($\alpha_1$A) all associate with $\alpha_2\delta$ and β, there is no evidence yet regarding the subunit structure of the recently cloned T type channels [$\alpha_1$G,H] [5].

VDCC activity is modulated by a range of drugs and neurotransmitters [6] many of which act via G protein-coupled receptors. The potential regulatory capacity of the β subunit has long been suspected [7]. Experiments using expressed VDCC subunits suggested that its function might be related to plasma membrane insertion of the α1 subunit [8]. It has also been suggested that this subunit may participate in the gating process of the cardiac L-type channel [9]. Co-expression of the β subunit with α1 has been found to produce significant increases in dihydropyridine (DHP) binding and current density, as well as a dramatic effect on activation and inactivation kinetics.

There are at least nine clonal classes of α1, four classes of β and two α2-δ genes, each containing many splice variants [2]. Such multiplicity may well confer advantage upon the organism, but necessarily generates a highly complex functional picture. It is the goal of this sequence prediction and modelling study to both contribute to understanding this picture in terms of β subunit regulation and to aid in the design of constructs for the cloning and expression of the subunit.

## 2. Materials and methods

Modelling work on the β subunit was carried out using the sequence of the β1b isoform from the brain of *Rattus norvegicus* [10]. The polypeptide is 597 amino acids in length and has the EMBL sequence database identification code RNCCB, ACC X61394. All subsequent references to this sequence will be as β1b.

Three other rat brain β subunit sequences used in multiple sequence alignment were: β3, RNCACH3B/M88751 [11]; β2a, RNCACHBS/M80545 [12]; β4, RNRCACN4A/D38101 [13].

A combination of methods was used including: (1) sequence database searches at the non-redundant GenBank CDS database at the National Centre for Biotechnology Information and the OWL (OWL28 2, 168,636 sequences) composite database, using the BLAST search algorithm [14]; (2) multiple sequence alignment using the ClustalW program [15]; (3) secondary structure prediction using three different methods: PHD [16]; SOPM [17]; DSC [18]; (4) protein domain families analyses using the Prodom database [19] and associated tools and the Pfam database [20]; (5) a consensus approach to fold recognition using the threading algorithms 123D [21], UCLA-DOE [22] and TOPITS [23]; and (6) visual inspection.

Quanta software (Molecular Simulations Inc.) was used to build homology models of the individual domains of the β1b sequence, and additional display of these models was accomplished with Rasmol, V2.6 [24]. Validation of the models was carried out using: (1) protein health checks in the Quanta software, (2) Procheck, V3.0 [25], and (3) WhatIf, V4.99 software [26].

## 3. Results and discussion

### 3.1. Structure prediction

The length of the β1b sequence at 597 amino acids indicates that this protein is probably multi-domain in nature. Secondary structure prediction suggests a total of six or seven α

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1        10        20        30        40        50        60        70        80        90
MVQKSGMSRGPYPPSQEIPMEVFDPSPQGKYSKRKGRFKRSDGSTSSDTTSNSFVRQGSAESYTSRPSDSDVSLEEDREALRKEAERQAL
111111111111111111111111111222222222222222222222222222222333333333333333333333333333333333
111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111

         100       110       120       130       140       150       160       170       180
AQLEKAKTKPVAFAVRTNVGYNPSPGDEVPVQGVAITFEPKDFLHIKEKYNNDWWIGRLVKEGCEVGFIPSPVKLDSLRLLQEQTLRQNR
333333333333           44444444444444444444444444444444444444444444444444               55
1111111111111111111222222222222222222222222222222222222222222222222222222222222

         190       200       210       220       230       240       250       260       270
LSSSKSGDNSSSSLGDVVTGTRRPTPPASAKQKQKSTEHVPPYDVVPSMRPIILVGPSLKGYEVTDMMQKALFDFLKHRFDGRISITRVT
5555555555555555555666666666666666666 77777777777777777777777777777777777777777777777777777
444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444

         280       290       300       310       320       330       340       350       360
ADISLAKRSVLNNPSKHIIIERSNTRSSLAEVQSEIERIFELARTLQLVALDADTINHPAQLSKTSLAPIIVYIKITSPKVLQRLIKSRG
777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777
444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444

         370       380       390       400       410       420       430       440       450
KSQSKHLNVQIAASEKLAQCPPEMFDIILDENQLEDACEHLAEYLEAYWKATHPPSRTPPNPLLNRTMATAALAVSPSPVSNLQGPYLVS
77777777777777777777777777777777777777777777777777777777
444444444444444444444444444444444444444444444444444444444444444444444444444444444444555555

         460       470       480       490       500       510       520       530       540
GDQPLDRATGEHASVHEYPGELGQPPGLYPSNHPPGRAGTLWALSRQDTFDADTPGSRNSVYTEPGDSCVDMETDPSEGPGPGDPAGGGT
888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888
555555555555555555566666666666666666666666666666666666666666666666666666666666666666666666

         550       560       570       580       590    597
PPARQGSWEEEEDYEEEMTDNRNRGRNKARYCAEGGGPVLGRNKNELEGWGQGVYIR
888888888888888888888888888888888888888888888888888888888
666666666666666666666666666666666666666666666666666666666
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KEY TO FIGURE

Line 1: sequence numbering
Line 2: sequence
Line 3: PHD secondary structure prediction
Line 4: SOPM secondary structure prediction
Line 5: DSC secondary structure prediction
Line 6: Prodom domain prediction
Line 7: Pfam domain prediction

KEY TO SECONDARY STRUCTURE PREDICTION

Helix
Strand

Fig. 1. Summary of secondary structure and domain predictions for the β1b sequence.

helices plus several β sheets [27]. From the point of view of domain assignment, there are two regions, displaying distinct structural patterns, which suggest the presence of discrete domains: firstly, residues 100–165 show a consensus in the prediction of a series of β strands (Fig. 1). This region, as we shall see, corresponds to a putative SH3 domain, which is typically comprised of a five- or six-stranded β sandwich. Secondly, the region from residues 220–430 shows a clear pattern of structural elements, suggestive of a repeating β-α-β motif (Fig. 1). Other regions of the sequence show a marked absence of such patterns.

The results of searches conducted using the Prodom and Pfam protein domain databases are included as lines 5 and 6 in Fig. 1. Clearly the two systems are not in full agreement regarding the domain arrangement. However, the SH3 domain assignment noted above is clearly indicated, numbered 4 by Prodom and 2 by Pfam. The second domain suggested from the secondary structure predictions is also indicated to be a distinct domain, numbered 7 by Prodom and 4 by Pfam. These two regions are the only clear domains, as there is considerable divergence in the domain assignments by the different algorithms for other parts of the sequence. Residues 1–100, for example, are designated a single domain according to the Pfam database, but as three smaller domains (or con-

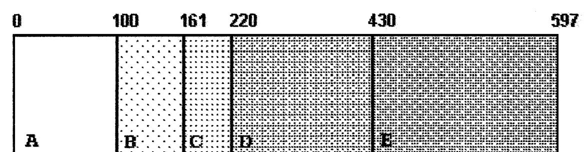


Fig. 2. Domain assignment for β1b, where domain A is the PDZ-like domain, domain B is the proposed SH3 domain, and domain D is the guanylate kinase-like domain.

served subsequences) according to Prodom. Examination of these three conserved subsequences reveals them to be homologous only to other β subunit sequences. Similarly the region around residues 165–220 shows little agreement. A part of this segment is unassigned by both systems (around residues 165–180) and the remainder is a single subsequence according to the Pfam results, but split into two (numbered 5 and 6) by Prodom. Residues 181–193 are dominated by serine residues which could be a linker sequence between two domains. Overall, the analysis of these combined results led us to the assignment of the domains shown in Fig. 2. These domain assignments compare favourably with the functional map produced by Walker and De Waard [2].

Multiple alignment of the four β subunit isoforms, β1b, β3, β2a and β4, further supported this domain assignment. A high degree of conservation was found between residues 57–165 and 216–432. The 57–165 segment of β1b was 67% identical to β3 and 81% to β2, although only 35% to β4. The 226–432 segment was 76% identical to β3, 88% to β2 and 42% to β4. A smaller region around residues 165–210 also displayed a noticeable number of conserved residues.

Initial homology searches using a variety of search and penalty parameters detected only strong homologues for domain B; all of the highly homologous sequences were SH3 domains. Then, three-fold recognition procedures were used, which only produced a positive result for domain D: 1gky, guanylate kinase from yeast [28]. Both methods 123D ($z$-score: 4.45) and UCLA-DOE ($z$-score: 9.37) ranked 1gky as the top hit, whilst TOPITS ($z$-score: 2.08) ranked it fourth. A global binary alignment of the 1gky sequence with domain D revealed a low but significant sequence identity of 17.5%. This is because fold recognition detects structural features using parameters other than sequence identity. It is notable that the fold of 1gky consists of a series of β-α-β units, a motif that was detected for this domain by the secondary structure predictions.

Finally, the membrane-associated guanylate kinase (MAGUK) family of proteins possesses a characteristic pattern in its domain arrangements. A C-terminal guanylate kinase-like domain (assigned to residues 218–430 of β1b) is present which lacks the ATP-binding motif or P-loop associated with kinase activity. In the MAGUKs this domain is preceded by an SH3 domain, as also appears to be the case with the β1b sequence. This domain pattern combined with the knowledge that the MAGUKs play a role in the clustering of $K^+$ ion channels [29] suggested that the β1b isoform could be related to this protein family. The final component of the MAGUK domain structure is a PDZ domain, usually at the N-terminus [29], and hence we examined whether residues 1–100 might be PDZ-like, even though this was not indicated by any of the programs used. The N-terminus of β1b was found to be 17% identical with the third PDZ domain from the human homologue of the discs large protein (Brookhaven Protein Data Bank code 1pdr [30]).

### 3.2. Homology modelling

A model for domain B was constructed from the SH3 template file 1lck [31], which had the highest sequence identity at 26.8% (Fig. 3A). Procheck V3.0 structure validation produced an overall $G$-factor of $-0.71$, suggesting the quality of the structure was well within acceptable limits. The Ramachandran plot showed that only two residues were in disallowed regions, with a Ramachandran $z$-score by WhatIf of $-3.50$. Overall main chain and side chain parameters as evaluated by Procheck were all favourable.

The structure consists six antiparallel β strands forming two sheets packed at right angles to each other. The sheets contain strands a–c and d–f, respectively. Strands b and c are particularly short and may be two sections of the same strand, as is the case in the SH3 domain of spectrin, for example [32]. In the model, these strands are connected by a long loop strongly reminiscent of an irregular antiparallel β hairpin. Strands e and f are themselves connected by the distal loop, so called because of its spatial separation from the putative binding site of this domain [32]. A third large loop connects strands d and e. The binding site for SH3 domains is thought to reside in the region between the two large loops between strands d and e and b and c, respectively. The residues forming this region in the SH3 domain family are well conserved, and being close together in space, form a patch within elements of secondary structure at the surface of the molecule [33]. A manual alignment was employed in an attempt to localise the binding site aromatic residues in the model (Fig. 3B). SH3-containing proteins are frequently located at the inner surface of plasma membranes [34] as is the case with the VDCC β subunit. It is thought that they mediate the assembly of specific protein complexes via binding to proline-rich peptides [35]. The sequence of the VDCC α subunit contains regions rich in proline residues.

Domain D was modelled on the 1gky template structure. The Ramachandran plot indicates only three residues are in disallowed regions, one of which appears to have been inherited from the template structure. The WhatIf validation suite also reports a good Ramachandran $z$-score of $-1.525$. The structure (Fig. 4A) consists of a five-stranded parallel β sheet, surrounded by six α helices. This putative guanylate kinase-like domain would appear not to possess kinase activity as the ATP-binding P-loop motif is absent from the sequence. The guanylate kinase-like domain in synapse associated protein (SAP97) [36] also exhibits a lack of catalytic activity even though the P-loop is present, as it is in members of the MAGUK protein family. This type of domain might be a G-protein-like signaling domain [37], and possibly of functional significance [38], given that an important role is played by G-proteins in the modulation of VDCCs.

Functional studies suggest the N-terminal β interaction domain (BID), is involved in anchoring of the β subunit to the α subunit of the VDCC complex via the α interaction domain (AID) of the latter. Deletion and chimera studies have indicated its role in the voltage-dependence of activation and current increase [2]. In the model structure this important segment is composed of one of the central β strands and one of the surrounding α helices (Fig. 4B).

The model produced for domain A (Fig. 5) is expected to be less precise than those of the other domains, because of the slightly lower sequence identity with the starting model structure. The Ramachandran plot indicated eight residues in disallowed regions, although the overall quality assessment of the plot was within acceptable limits. The $G$-factor is $-1.0$, although other main chain and side chain parameter checks were good. Given the low sequence identity with the template coordinate file and the failure of threading to identify a candidate fold, this model is expected only to represent a general picture of the possible conformation of this domain. It is a

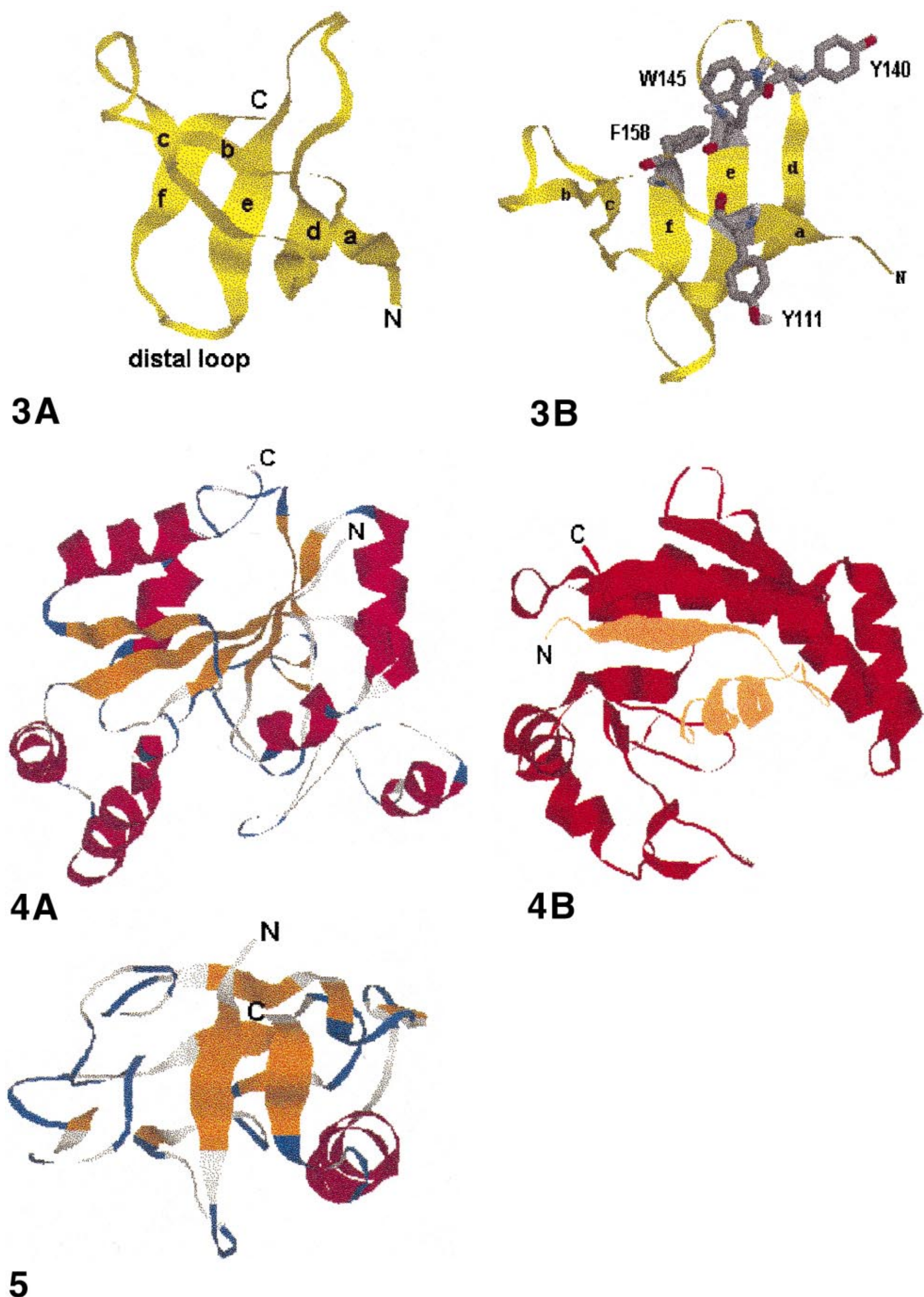


Fig. 3. A: Model of the SH3 domain (residues 107–159 of domain B) of the VDCC β subunit. The strands forming the antiparallel β sandwich tertiary structure are labelled a–f. The distal loop is positioned opposite and well away from the putative active site in this protein family. B: Aromatic residues forming the core of the putative active site of the SH3-like domain. The site is located between the two large loops in the model structure, as is typical for this protein family.

Fig. 4. A: Model (residues 228–409) of domain D of the β subunit, a guanylate kinase like domain. B: A view highlighting the β interaction domain (BID) (in green) at the N-terminal end of the sequence. The model indicates this functionally important segment is composed of one of the central β strands and one of the surrounding α helices

Fig. 5. Model (residues 4–90) for domain A, a PDZ-like domain.

five-stranded antiparallel β barrel flanked by an α helix. The template PDZ domain, by contrast, is flanked by three helices, but the weak alignment produced for this model has resulted in the replacement of two of the helices by loops.

Very little is understood at present of the structure or functioning of the VDCC β subunit, which appears to exert an important modulatory influence on calcium channels. It is likely that the β subunit binding assists the α1 component in adopting a conformation appropriate for a functional calcium channel [39]. More specifically, the role of conformational alteration in regard to specific functions has been investigated, for example, in the modulation of opening

probability [40], or gating characteristics [9]. Observed cooperativity of subunit effects between β, α2δ and γ may further support this mechanism [41]. In this study certain domains of the β subunit sequence have been identified as connected with specific functional types which can be associated with properties found for this protein [2]. It should also be noted that β1b is associated with the plasma membrane when expressed alone in a mammalian cell line, in the absence of α1 subunits [42]. It is possible that one or several of the SH3, PDZ, and GK domains identified in β1b may be involved in this membrane association or in interactions with other proteins involved in calcium channel localisation or function. It will be of importance to identify the target(s) of these domains.

In summary, structure prediction techniques were used to establish a domain structure for this protein and to assist in our understanding of its function. Relatedness between these subunits and the membrane associated guanylate kinases, which play a role in the clustering of certain $K^+$ channels has been suggested, as have the possible functional roles for each of these modelled domains. On the basis of the high degree of residue conservation indicated by the multiple sequence alignment with the other β subunits from rat brain, the models presented here may also apply to those isoforms. Furthermore, the domain assignments described here have assisted in designing the constructs for cloning and expression of hexahistidine tagged domains of β1b (Berrow and Dolphin, unpublished results), since these domains appear to be stable, protease-resistant polypeptides.

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