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Biological modelling/Biomodélisation

Study of the residues involved in the binding of β1 to β3 subunits in the sodium channel

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ABSTRACT

The voltage-gated sodium channel (VGSC) is a complex, which is composed of one pore-forming α subunit and at least one β subunit. Up to now, five β subunits are known: β1/β1A, β1B, β2, β3, and β4, encoded by four genes (*SCN1B*~*SCN4B*). It is critical to have a deep understanding of the interaction between β1 and β3 subunits, two subunits which frequently appear in many diseases concurrently. In this study, we had screened out the new template of β1 subunit for homology modelling, which shares higher similarity to β3. Docking studies of the β1 and β3 homology model were conducted, and likely β1 and β3 binding loci were investigated. The results revealed that β1–β3 is more likely to form a di-polymer than β1–β1 based on molecular interaction analysis, including potential energy analysis, Van der Waals (VDW) energy analysis and electrostatic energy analysis, and in addition, consideration of the hydrogen bonds and hydrophobic contacts that are involved. Based on these analyses, the residues His122 and Lys140 of β1 and Glu 66, Asn 131, Asp 118, Glu 120, Glu133, Asn135, Ser 137 of β3 were predicted to play a functional role.
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1. Abbreviations

VGSC	Voltage-gated sodium channels
VDW	Van der Waals
ECD	Extracellular domain
CAMs	Cell adhesion molecules
ZDOCK	Molecular docking software
3MJ7-A	Complex of JAML and Coxsackie and Adenovirus receptor CAR, chain A; 1NEU-A, Myelin Membrane Adhesion Molecule P0, chain A
PDB	Protein Data Bank
ID	Identity
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
GEFS+	Generalized Epilepsy with Febrile Seizures Plus

2. Introduction

In vivo, most Na^+ channel α subunits (Nav1.1–Nav1.9) are associated with one or more β subunits; nowadays, four distinct isoforms (β1~β4) and two splice variants (β1A, β1B) have been discovered [1,2]. Structure/function studies have identified the P loop of domain IV of the Na^+ channel α subunit as a likely interaction point for the extracellular domain of β1, accounting for the fine-tuning of Na^+ channel activation and inactivation gating [3]. More recently, people began to focus on the studies of β subunits and their mutants, as experiments showed that many diseases were associated with β subunits changes, especially in the extracellular domain (ECD), which is in

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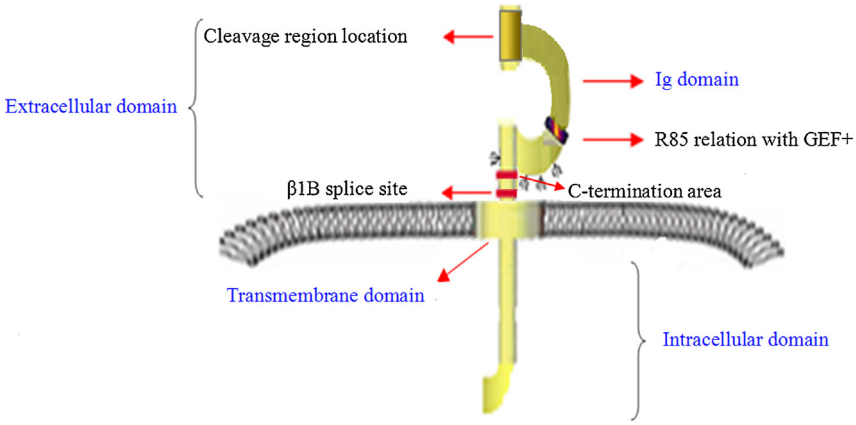

Fig. 1. (Color online.) Basic functional architecture of β1 and with highly conservative structure domain like other β subunits.

charge of the electrophysiological properties of the sodium channel and cell adhesion or migration [4].

As we all know, the β subunits share a common structure: a single membrane domain, an intracellular *C*-terminal domain, and a large extracellular *N*-terminal domain (Fig. 1). The ECD contains four potential *N*-glycosylation sites, which were modified with sialic acid; in this domain, they all have an immunoglobulin-like fold [5,6]. Among these subunits, β1 and β3 are non-covalently linked to VGSC α subunits, while β2 and β4 associate with disulfide connection. Particularly, β1 and β3 have compact relations; they are structurally similar, with 57% sequence identity and related to the same diseases, such as epilepsy and cardiac arrhythmia [4,7,8]. Recent studies reveal that the β1 and β3 subunits are always co-expressed in many tumour cells and involved in epilepsy, cardiac arrhythmia [6,9]. So, we speculate that there must be a special interaction mode between β1 and β3. To prove this inference, we have to know the crystal structure model of β1 and β3; however, there have been so far no published studies about 3-dimensional structure and interaction analysis between β1 and β3.

Therefore, in the present study, we provide a hypothesis that β1 has certain relationship with β3 subunit, which is different from the results of McEwen et al., who studied the interaction between β subunits and other CAMs in glial cells [10]. Moreover, this paper screened out the homologous mode template of β1 and did the docking studies of β1 and β3 subunits, which provides a strong basis for the further research of β subunits on structure and functional drug targeting.

3. Materials and methods

3.1. Molecular modelling

The amino acid sequences of β1 and β3 were obtained from the database in the National Centre for Biotechnology information. The protein BLAST program against Protein Data Bank available at NCBI was used to select a template structure for homology modelling of β1 and β3. From the selected templates, the three-dimensional models of β1 and β3 were obtained by homology modelling using software package Discovery Studio 2.5. The obtained models were both validated using PROCHEK subsequently [11].

3.2. Dynamics and PROCHECK

In order to demonstrate the more reliability of the newly screened template, we had carried out the dynamic optimization of the β1 modelling structure. The force field was based on FF99SB and the time step was 2 fs. A constant pressure of 1 bar, independently in the *z* direction, was used with a coupling constant $\tau_p = 20$ ps. Water, lipid, and protein were coupled in a temperature bath at 310 K, using a coupling constant $\tau_t = 0.1$ ps. Finally, the stability and reliability of this system were demonstrated.

3.3. Protein–protein docking

To generate the docking model between β1 and β3, the ZDOCK program in the Discovery Studio 2.5, which requires minimal information about the binding sites, was used. Finally, ZDOCK used Pairwise Shape Complementarity, desolvation and electrostatic energy methods to evaluate the proteins interaction [12–14].

4. Results

4.1. Molecular modelling of β1 and β3

Molecular modelling of β1 was carried out based on the crystal structure of the complex of JAML and Coxsackie and Adenovirus receptor, CAR of *Mus musculus* (PDB ID: 3MJ7) chain A [15], which shares 22% sequence identity with β1 (29Glu to 152Arg), while the model of β3 was based on the crystal structure of myelin membrane adhesion molecule P0 (PDB ID: 1NEU) chain A [16], which shares 28% of sequence identity with β3 (25Val to 147Glu), (see Table 1). In addition, two crystal models were obtained by comparing screening, respectively potassium ion and calcium ion channel current β subunits.

Analysis of the Ramachandran plot showed that 73.3% and 80.6% of the residues were in the most favourable regions, with 21.6% and 14.8% in the additional allowed

Table 1
Template screening statistical information of ion channel β1 subunit.

Channel category	PDB ID	Identity ratio	3D structure
Voltage-dependent calcium channel-beta subunit			
Beta subunit isoform 2a[a]	1T0H	12.9%	
Beta subunit functional core[b]	1T3S	12.3%	
Beta-2 subunit in complex with the Cav1.21-lid linker[b]	4DEY	12.2%	
Beta2a subunit and a peptide of the alphalc subunit[a]	1T0 J	11.3%	
Beta4a-A domainc	2D46	11.0%	
Beta-2 subunit in complex with the Cav2.21-lid linker[a]	4DEX	10.9%	
Beta3 subunit[a]	1VYU	9.7%	
BK Beta subunit Kcmnb 2[c]	1JO6	9.6%	
Beta3 subunit complex with aid[a]	1VYT	4.8%	
Voltage-dependent potassium channel-beta subunit			
Beta subunit (I211r) IN Complex with cortisone[a]	3EB4	15.9%	
Kv1.2 potassium channel-beta subunit complex[a]	2A79	15.4%	
Full-length shaker potassium channel Kv1.2[a]	3LUT	15.4%	
Structure of the cytoplasmic beta subunit-tl[a]	1EXB	15.4%	
Beta subunit IN complex with cortisone[a]	3EAU	15.3%	
Beta subunit (W121a) IN Complex with cortisone[a]	3EB3	15.3%	
Paddle chimera channel IN association with beta subunit §	2R9R	6.3%	
Structure of a votage-dependent^{+} channel-beta subunit[a]	1QRQ	4.6%	
Ig superfamily			
Crystal structure of the complex of Jaml and CAR[d]	3MJ7	22.2%	
Crystal structure of the extracellular domain from P0[a]	1NEU	28%	

[a] *Rattus norvegicus.*
[b] *Oryctolagus cuniculus.*
[c] *Homo spaiens.*
[d] *Mus musculus.*

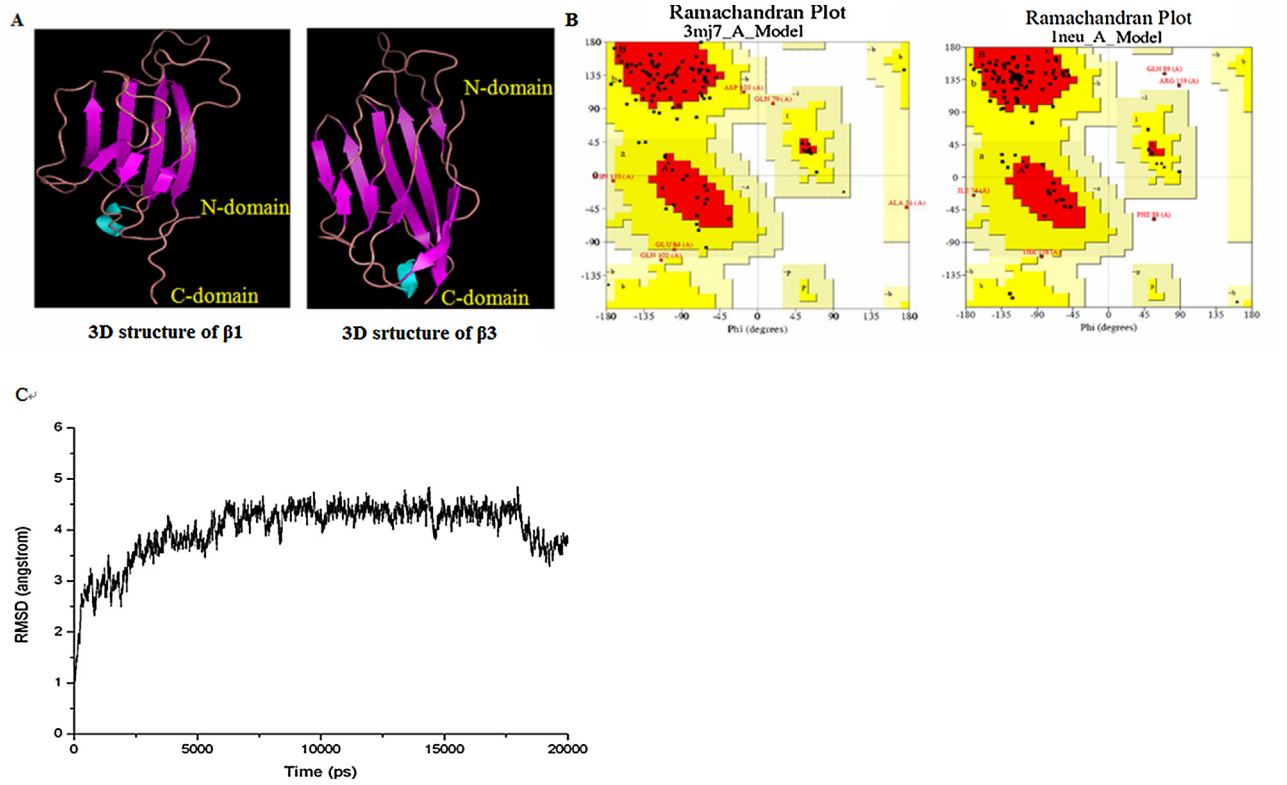

Fig. 2. (Color online.) A. The 3D structures of β1 (left) and β3 (right) were investigated by homology modeling using 3MJ7 and 1NEU as templates. B. Ramachandran plot as the validation for homology modeling using PROCHEK. C. RMSD with respect to the simulation time for a 6-ns molecular dynamics simulation of the β1 model.

regions for β1 and β3, respectively; the dynamic optimization results also indicated that the models are suitable for studies. The 3D structures and the Ramachandran plots of β1 and β3 are shown in Fig. 2A and B, respectively. Fig. 2C shows the evolution of the RMSD during the dynamic process of β1 structure.

4.2. *Docking study between β1 and β3*

Protein–protein dockings of β1–β1 and β1–β3 were carried out (as shown in Fig. 3A and B) for studying the interaction of molecules involved in potential energy, hydrogen bond, hydrophobic contacts. The potential energies were −593.32725 (kcal/mol) and −1068.48017 (kcal/mol), respectively for β1–β1, β1–β3, and showed that the β1–β3 heterogeneous di-polymer form was more stable than the β1–β1 homologous di-polymer form (see Table 2).

The hydrogen bond of β1–β1 is located at 122His–140Lys, while in the case of β1–β3 ten pairs of hydrogen bonds exist: Glu 66–Arg 132; Glu 66–His 131; Asn 131–Pro 66; Asn 131–Arg 65; Glu 133–Arg 65; Asp 118–Arg 51; Glu 120–Val 25; Ser 137–Thr 138 and Asn135–Thr 138.

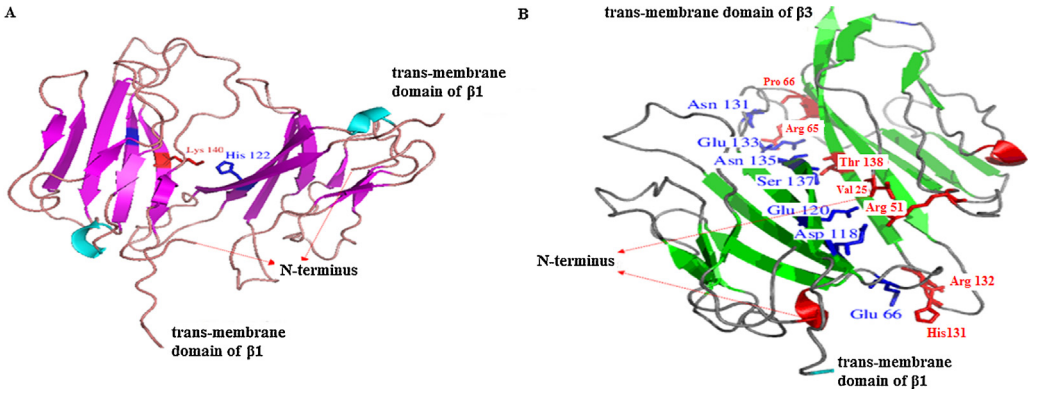

Fig. 3. A. (Color online.) The docking model of β1–β1 was shown with one pair of hydrogen bond (His 122 and Lys 140). B. The docking model of β1–β3 was shown with 10 pairs of hydrogen bonds (red for β1, blue for β3). (Glu 66——Arg 132; Glu 66——His 131; Asn 131——Pro 66; Asn 131——Arg 65; Glu 133——Arg 65; Asp 118 = ===Arg 51; Glu 120——Val 25; Ser 137——Thr 138; Asn135——Thr 138). (For interpretation of references to color, see the online version of this article.).

Table 2
Molecular interaction parameters between β1 and β3 subunit.

Items	β1–β1	β1–β3
Force field	CHARMm	CHARMm
Potential energy (kcal/mol)	−593.32725	−1068.48017
VDW energy (kcal/mol)	−134.90669	−402.55395
Electrostatic energy (kcal/mol)	−458.42056	−665.92622
Hydrogen bonding (pairs)	1	10
Residues involved in hydrophobic contacts	a: Ser137 Thr64 Thr136 Cys43 Asn135	Thr64 Thr136
	b: Leu39 Leu127 Phe67 Phe128 Val139	
	c: Glu29 Glu66 Glu133 Glu145	Leu117 Leu127 Leu140 Pro133
	d: Lys44 Lys62 Lys141 Arg45 Arg46 His143	Pro142 Phe126 Phe129
		Glu65 Glu67 Asp130
		Arg144

a: Polar amino acid; b: non-polar amino acid; c: acidic amino acids; d: alkaline amino acid.

5. Discussion

Currently, at least four VGSC β subunits have been cloned and characterized, but little research has been done about their structure and function. Therein most of the studies focused on β1, since many mutations involving β1 have been reported in neurological diseases, such as epilepsy, EGFS+ [17].

So far, only a model of β3 structure has been elaborated by homology modelling. However, our research brought in another hypothesis using a protein–protein model for docking β1 and β3, respectively, which is more likely to form a heterodimer (β1–β3). The results based on the pairs of hydrogen bonds between β subunits and including numbers of hydrophobic groups and potential energy as an important index of the investigation were considered as well. Meaningfully, the position of the hydrogen bond (122His–140Lys) between β1 subunits was just located in disulfide linkage area in the topological structure where the GEFS + mutations sites are centralized. We can conclude that His122 and Lys140 may play an important role in the function of β1 subunit.

From the potential energy results (−593.32725 kcal/mol and −1068.48017 kcal/mol) respectively for β1–β1, β1–β3, and the number of hydrogen bonds and hydrophobic groups (between β1 and β3, there exist 9 more additional hydrogen bonds than in the β1–β1 case), we can clearly prove that the combination β1–β3 is more stable than β1–β1. Also, we can predict many additional functions for the amino acid within the binding areas for β1 or β3, for example, cell surface migration and adhesion or regulation of Na^+ current.

Disclosure of interest

The authors have not supplied their declaration of conflict of interest.

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