Solution Structure of the Na\(_{\text{v}}\)1.2 C-terminal EF-hand Domain*

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Voltage-gated sodium channels initiate the rapid upstroke of action potentials in many excitable tissues. Mutations within intracellular C-terminal sequences of specific channels underlie a diverse set of channelopathies, including cardiac arrhythmias and epilepsy syndromes. The three-dimensional structure of the C-terminal residues 1777–1882 of the human Na\(_{\text{v}}\)1.2 voltage-gated sodium channel has been determined in solution by NMR spectroscopy at pH 7.4 and 290.5 K. The ordered structure extends from residues Leu-1790 to Glu-1868 and is composed of four \(\alpha\)-helices separated by two short anti-parallel \(\beta\)-strands; a less well defined helical region extends from residue Ser-1869 to Arg-1882, and a disordered N-terminal region encompasses residues 1777–1789. Although the structure has the overall architecture of a paired EF-hand domain, the Na\(_{\text{v}}\)1.2 C-terminal domain does not bind Ca\(^{2+}\) through the canonical EF-hand loops, as evidenced by monitoring \(^1\)H,\(^{15}\)N chemical shifts during titration. Backbone chemical shift resonance assignments and Ca\(^{2+}\) titration also were performed for the Na\(_{\text{v}}\)1.5 (1773–1878) isoform, demonstrating similar secondary structure architecture and the absence of Ca\(^{2+}\) binding by the EF-hand loops. Clinically significant mutations identified in the C-terminal region of Na\(_{\text{v}}\)1 sodium channels cluster in the helix I–IV interface and the helix II–III interhelical segment or in helices III and IV of the Na\(_{\text{v}}\)1.2 (1777–1882) structure.

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modulin (33). Nevertheless, whether Ca\(^{2+}\)/H\(^{1+}\) binds specifically to the putative CTD EF-hand and any resultant contribution to channel regulation is controversial (12, 26, 31, 34).

EXPERIMENTAL PROCEDURES

Constructs of the Na\(_{v}\)1.2 CTD were designed by limited proteolysis and H/D exchange experiments. Briefly, the CTD of Na\(_{v}\)1.2, residues 1777–1937 with the amino acid substitutions I1877/Q1878A and an N-terminal His\(_{10}\) tag MSGSHHHHHHHSSGVPGRSHMAS (31), was subjected to proteolytic digestion with proteinase K at 4 °C for 15–60 min using a protein:protease ratio of 50:1–100:1. The termini of the protected proteolytic fragments were mapped by matrix-assisted laser desorption ionization time-of-flight time-of-flight mass spectrometry and N-terminal sequencing. H/D exchange experiments were performed by ExSAR (Monmouth Junction, NJ) and showed protection for proteolytic fragments extending from residues 1789 to 1879. The construct encompassing residues 1777–1882 of the Na\(_{v}\)1.2 CTD defined by the above experiments, including the N-terminal His tag, was used for structure determination by solution NMR spectroscopy.

[U-\(^{13}\)C\(^{15}\)N\(^{15}\)N] Na\(_{v}\)1.2 CTD (1777–1882) was overexpressed in Escherichia coli (BL21 DE3) transformed with a pET28 vector (EMD Biosciences) using M9 minimal media prepared with \(^{15}\)NH\(_{4}\)Cl and \(^{13}\)C\(^{15}\)N\(^{15}\)N-glucose (35). Cultures were grown at 37 °C to \(A_{600}\) = 0.7, induced with 0.5 mM isopropyl \(\beta\)-D-1-thiogalactopyranoside, transferred to 16 °C, and harvested after 72 h. Cells were lysed using a French press, and the Na\(_{v}\)1.2 CTD was purified with Ni\(^{2+}\)-affinity, gel-filtration (Superdex 200), and ion-exchange (Mono Q 5/50 GL) chromatography (GE Healthcare). The N-terminal tag was not removed. Sample buffer consisted of 20 mM d\(_{16}\)-Tris (pH 7.4), 100 mM d\(_{5}\)-glycine, 0.1 mM d\(_{16}\)-EDTA, 1 mM d\(_{10}\)-DTT, 0.02% NaN\(_{3}\), and 10% D\(_{2}\)O. Proteins were exchanged into this buffer using centrifugal concentrators (Amicon Inc.), flash-frozen in liquid N\(_{2}\), and stored at −80 °C. Samples for calcium titrations were subsequently exchanged into 20 mM d\(_{16}\)-Tris (pH 7.4), 0.1 M d\(_{5}\)-glycine, 10 mM d\(_{16}\)-EDTA, 1 mM d\(_{10}\)-DTT, 0.02% NaN\(_{3}\), and 5% D\(_{2}\)O. Protein concentrations of 0.5 and 0.2 mM were used for structural experiments and calcium titrations, respectively. The Na\(_{v}\)1.5 CTD construct, residues 1773–1878, was designed by sequence alignment to Na\(_{v}\)1.2, using bl2seq (36), and protein samples were prepared by the same protocol. Sample temperatures were calibrated using 99.8% MeOD to a splitting of 1.616 ppm for Na\(_{v}\)1.2 (290.5 K) and 1.545 ppm Na\(_{v}\)1.5 (298.0 K) (37).

Backbone assignments for the Na\(_{v}\)1.2 and Na\(_{v}\)1.5 CTDs were obtained with HNCO, HNCA, HNCA/CB, HNCCACB, and CBCA(CO)NH experiments; side-chain assignments for Na\(_{v}\)1.2 CTD were obtained with HBQA(CB-CACO)/NH and HCCH-two-dimensional total correlation spectroscopy (TOCSY) experiments (38). A 10% \(^{13}\)C sample was used for stereospecific assignment of Leu and Val methyl groups (39). NOE connectivities were obtained with \(^{15}\)N/NOESY-HSQC (80-ms mixing time), \(^{13}\)C\(_{\text{car}}\)/siphatic-NOESY-HSQC (100 ms), and \(^{13}\)C\(_{\text{car}}\)/NOESY-HSQC (80 ms). Residual dipolar coupling constants were measured in a sample containing 15 mg/ml PF\(_{1}\) phage (Asla Biotech) using two-di-
motif. Assignments of \(^1\)H,\(^{15}\)N resonances for the Na\(_V\)\(_{1.2}\) CTD and the Na\(_V\)\(_{1.5}\) CTD are, respectively, 99 and 97% complete. Notably, Asn-1835 could not be assigned in the \(^1\)H,\(^{15}\)N HSQC of Na\(_V\)\(_{1.2}\). The resonances for Asn-1831 (the homologue of Asn-1835) and Gln-1832 were not assigned, and the resonance for Ile-1833 appears broadened in \(^1\)H,\(^{15}\)N HSQC of Na\(_V\)\(_{1.5}\). Moreover, homologous resonances Leu-1855 in Na\(_V\)\(_{1.2}\) and Met-1851 in Na\(_V\)\(_{1.5}\) have liminal intensities in \(^1\)H,\(^{15}\)N HSQC spectra. These observations suggest conserved dynamics between isoforms. For the Nav1.2 CTD (1777–1882), \(^{13}\)C and \(^{13}\)C\(^\circ\) assignments are 100% complete, \(^{13}\)C assignments are 97.1% complete, \(^1\)H aromatic assignments are 89.1% complete, and non-aromatic \(^1\)H assignments are 97.7% complete. The Na\(_V\)\(_{1.2}\) CTD construct contains six proline residues, of which Pro-1789, Pro-1807, Pro-1827, and Pro-1845 are in a \emph{trans} conformation, whereas Pro-1828 and Pro-1834 are in a \emph{cis} conformation. The \emph{cis} conformation is evidenced by stronger X-Pro \(^{1}H^{\alpha}-H^{\alpha}\) than X-Pro \(^{1}H^{\alpha}-H^{\alpha}\) NOE contacts and differences of \(^{13}\)C\(^\circ\)-\(^{13}\)C\(^\circ\) chemical shifts of 9.4 and 8.5 ppm, respectively (59, 60).

Medium range \(^1\)H-\(^1\)H NOEs, steady-state \(^1\)H-\(^{15}\)N NOEs, and \(^1\)H-\(^{15}\)N NOE contacts for residues Ser-1848 and Gly-1849 is represented by two short anti-parallel \(^\beta\)-strands, consistent with homology models based on structures of paired EF-hand domains (25, 26). Comparison of interhelical angles of helices I and II of Na\(_V\)\(_{1.2}\) CTD and the N-terminal lobe of the prototypical EF-hand protein calmodulin suggests that the isolated Na\(_V\)\(_{1.2}\) CTD most closely resembles the canonical apoEF-hand conformation (Fig. 1 and supplemental Fig. S1). Secondary chemical shifts indicate that the Na\(_V\)\(_{1.2}\) CTD has a similar secondary structural architecture as Na\(_V\)\(_{1.2}\) CTD (Fig. 1C).

The structure of Na\(_V\)\(_{1.2}\) CTD is presented in Fig. 2 and supplemental Fig. S1 with statistical details of the calculation presented in Table 1. The structure contains four \(\alpha\)-helices and two short anti-parallel \(\beta\)-strands, consistent with homology models based on structures of paired EF-hand domains (25, 26). Comparison of interhelical angles of helices I and II of Na\(_V\)\(_{1.2}\) CTD and the N-terminal lobe of the prototypical EF-hand protein calmodulin suggests that the isolated Na\(_V\)\(_{1.2}\) CTD most closely resembles the canonical apoEF-hand conformation (Fig. 3D and Table 2). The hydrophobic interface between helices I and IV predicted through mutational analysis (27) is observed with direct NOE contacts between residues Phe-1795, Phe-1798, and Tyr-1799 in helix I and Leu-1855, Ile-1857, and Leu-1858 in helix IV.

Helices I and IV contribute to the hydrophobic core of the protein, with a majority of aromatic side chains contributed from helix I. The segments between Gln-1811—Glu-1814 and Arg-1851—His-1853 participate in an anti-parallel \(\beta\)-sheet. An additional anti-parallel \(\beta\)-sheet contribution from residues Met-1846—Val-1847 is not present in all conformers of the structural ensemble. The helix II-III interhelical segment, delimited by two \emph{cis} proline residues, Pro-1828 and Pro-1834, is well ordered in the structural ensemble. The conformation of residues Asp-1826—Leu-1829 is consistent with a type VI tight-turn (61), also called a \(\beta\_6\)-turn (62). The unique di-proline-leucine motif, Pro-1827—Leu-1829 extends the helix II-III interhelical segment by forming a small handle at the base of helix II (Figs. 3, D and E). The absence of long-range NOE contacts for residues Ser-1848 and Gly-1849 is represented by disorder of this region in the ensemble.

The segment from residues Ser-1869 to Arg-1882 is predicted to have residual helical content based on secondary \(^{13}\)C chemical shifts and characteristic \(d_{\alpha\alpha}(i, i+3)\) and \(d_{\beta\beta}(i, i+3)\) NOEs (Fig. 1). A short helix V is observed in the final ensemble extending from Gly-1870 to Arg-1876 with a backbone root mean square deviation of 0.59 Å when superposed on itself (supplemental Fig. S1). However, the reduced magnitudes of the secondary \(^{13}\)C chemical shifts and the \(^1\)H-\(^{15}\)N NOEs for helix V compared with helices I to IV, suggest that the helical conformation is not fully populated in solution. Furthermore, helix V does not exhibit residual dipolar couplings or long-range NOE contacts and, hence, is not well defined relative to the core EF-hand domain structure (supplemental Fig. S1).

Additional interactions present in longer constructs of the CTD or in complexes with other components of the VGSC may stabilize helix V.

Binding of Ca\(^2+\) by the Na\(_V\)\(_{1.2}\) (1777–1882) and Na\(_V\)\(_{1.5}\) (1773–1878) CTDs was assessed by monitoring \(^1\)H,\(^{15}\)N chemical shifts as a function of Ca\(^2+\) concentration (0–4.5 mM). Chemical shift perturbations exhibit titration behavior suggesting that the interaction occurs on a fast-exchange timescale with equilibrium constants of 1.65 ± 0.03 mM for Na\(_V\)\(_{1.2}\) CTD and 3.28 ± 0.13 mM for Na\(_V\)\(_{1.5}\) CTD (Fig. 3 and supplemental Fig. S2), consistent with a previous report for the Na\(_V\)\(_{1.5}\) CTD (33). However, resonance assignments were not obtained previously, and the structure of Na\(_V\)\(_{1.2}\) CTD now reveals that chemical shift perturbations >0.05 ppm are localized to residues in the N terminus of helix I, the linker between helices II and III, the C terminus of helix IV and the partially structured helix V. Thus, this weak Ca\(^2+\) binding site is distal to the canonical EF-hand loop motifs. In contrast, the average chemical shift change between the end points of the titration is <0.01 ppm in the N-terminal EF-hand loop (residues 1806–1817) and in the C-terminal EF-hand loop (residues 1842–1853) for the Na\(_V\)\(_{1.2}\) CTD. Respective values <0.02 ppm were obtained for corresponding residues 1802–1813 and 1832–1849 in the Na\(_V\)\(_{1.5}\) CTD. In comparison, the average chemical shift changes of the N-terminal EF-hand loop between apoCa\(^2+\) and Ca\(^2+\)-loaded calmodulin are 0.59 and 0.65 ppm in the N-terminal and C-terminal domains, respectively (63, 64). In particular, canonical Ca\(^2+\) binding by an EF-hand would require coordination of a Ca\(^2+\) atom by the backbone carboxyl atoms of Phe-1812 in Na\(_V\)\(_{1.2}\) and Phe-1808 in Na\(_V\)\(_{1.5}\), leading to significant chemical shift changes for inter-residual and sequential amide resonances (65, 66). In opposition, chemical shift changes less than 0.02 ppm were observed for backbone amide resonances for residues Phe-1812—Ile-1813 and Phe-1808—Ile-1809 of Na\(_V\)\(_{1.2}\) and Na\(_V\)\(_{1.5}\), respectively (Fig. 3). A structure-based sequence alignment of calmodulin and Na\(_V\)\(_{1.2}\) and a comparison of Ca\(^2+\)-induced chemical shift changes are shown in supplemental Fig. S3.

**DISCUSSION**

The solution structure determined by NMR spectroscopy for the Na\(_V\)\(_{1.2}\) CTD (1777–1882) exhibits a core-ordered domain from residues Leu-1790 to Glu-1868, with four \(\alpha\)-helices and two short anti-parallel \(\beta\)-strands arranged in tandem helix-sheet-helix motifs characteristic of paired EF-hand domains.
Structural alignment of the NaV1.2 CTD and calmodulin reveals that the structure is more similar to apo-Ca\(^{2+}\)/H11001 calmodulin than to peptide target and/or Ca\(^{2+}\)/H11001-loaded calmodulin. The NaV1.5 CTD (1773–1878), which shares 83% identity with the NaV1.2 CTD, adopts a similar secondary structure and, likely, tertiary structure.

Titrations monitored by NMR chemical shift perturbations demonstrate that the canonical EF-hand loops of the NaV1.2 CTD (1777–1882) and NaV1.5 (1773–1879) CTDs, with 83% identity and 93% similarity. Non-conservative substitutions are shown in bold type. Secondary structure elements predicted from chemical shifts using TALOS (49) are shown as bars for \(\alpha\)-helices and arrows for \(\beta\)-strands. 

\(\Delta^{13}C\) chemical shifts for NaV1.2 CTD (1777–1882) indicate a well folded domain encompassing residues Leu-1790—Glu-1868. The W1802 resonance is aliased in the \(^{15}N\) dimension from 131.5 ppm.

Structural alignment of the Na\(\alpha\)1.2 CTD and calmodulin reveals that the structure is more similar to apo-Ca\(^{2+}\) calmodulin than to peptide target and/or Ca\(^{2+}\)-loaded calmodulin. The Na\(\alpha\)1.5 CTD (1773–1878), which shares 83% identity with the Na\(\alpha\)1.2 CTD, adopts a similar secondary structure and, likely, tertiary structure.

Titrations monitored by NMR chemical shift perturbations demonstrate that the canonical EF-hand loops of the Na\(\alpha\)1.2 CTD (1777–1882) and Na\(\alpha\)1.5 CTD (1773–1878) do not bind Ca\(^{2+}\); rather, Ca\(^{2+}\) binds weakly at a site distal to the canonical loops near the N terminus of helix I, the linker between helices II and III, the C terminus of helix IV, and the partially structured helix V. The high resolution crystal structure of calmodulin identified an additional Ca\(^{2+}\) binding site in the homologous region corresponding to the helix II-III linker, but the authors judged this site to be non-physiological (67).

A structure-based sequence alignment with calmodulin also suggests that the canonical EF-hand loops of Na\(\alpha\)1.2 CTD do not bind Ca\(^{2+}\) (Table 3, Figs. 2, D and E, and supplemental Fig. S3). Chelation of Ca\(^{2+}\) requires an acidic residue, such as Glu or Asp, at sequence position 1817, corresponding to position 12 in a canonical EF-hand calcium binding motif (68), rather than the
Lys residue present in Na\textsubscript{v}1.2. Mutation of the corresponding residue, Glu to Lys, in Drosophila melanogaster calmodulin abolishes Ca\textsuperscript{2+} binding, although this mutation may mimic a Ca\textsuperscript{2+}-bound state in the context of certain targets (69, 70). Lys is found at position 12 in the non-canonical Ca\textsuperscript{2+} binding loop of scallop myosin essential light chain; however, coordination of Ca\textsuperscript{2+} is accomplished by an acidic residue at position −2, the backbone carbonyl group at position +2, and a water molecule (71). In Na\textsubscript{v}1.2 the residue at position +2 is Pro, and the residues at positions −3 and −2 are Glu and Lys. The latter two residues have chemical shift changes less than 0.05 ppm after the addition of 4.5 mM Ca\textsuperscript{2+}.

Higher affinity Ca\textsuperscript{2+} binding has been reported for longer constructs of Na\textsubscript{v}1.5 CTD, residues 1773–1920 and residues

FIGURE 2. Solution structure of the ordered core EF-hand motif of the Na\textsubscript{v}1.2 CTD, residues Leu-1790–Glu-1868. Structural statistics are presented in Table 1; the structure of the construct from residues Gly-1777 to Arg-1882, including the N- and C-terminal regions, is shown in supplemental Fig. S1. Traces through the backbone heavy atoms of the 15 lowest energy conformers described in Table 1 are superposed in panel A. Phenylalanine side chains are superposed in panel B. Phe-1859 is not shown because the aromatic chain is not well ordered in the ensemble. A ribbon diagram of the lowest-energy structure is presented in panel C. The structural alignment of the N-terminal EF-hand motifs of Na\textsubscript{v}1.2 (blue ribbon) and apoCa\textsuperscript{2+} calmodulin PDB code 1CFD (green ribbon) is shown in panel D. The structural alignments to Ca\textsuperscript{2+}-bound calmodulin PDB code 1EXR (orange ribbon) is shown in panel E. Pentagonal bipyrimidal coordination (dashed lines) of calcium by the N-terminal calmodulin (PDB code 1EXR) EF-Hand (orange) is shown with the corresponding residues of Nav1.2 in panel F. Coordination by T28 (green dashed line) occurs through a water molecule.
1773–1925 that include the IQ motif, and binding is abolished by mutation of the IQ motif (33). However, the resonance assignments obtained for NaV1.5 indicate that chemical shift perturbations for key EF-hand canonical loop residues Phe-1808—Ile-1809 are not larger in these longer constructs (comparing the inset of Fig. 3B with supplemental Fig. 5D of Ref. 33), suggesting that higher affinity binding of Ca\(^{2+}\) also does not involve the canonical EF-hand loops.

The solution structure of NaV1.2 CTD can be used to predict the effect(s) of clinical mutations in VGSCs (Fig. 4) because of the high degree of homology between VGSC CTDs. Generally, clinically significant mutations that map in the CTD can be divided into two classes, with some overlap for several sites (supplemental Table SI). Mutations in Nav1.5 associated with the Long QT variant 3 (LQT3) cardiac arrhythmia phenotype and a subset of mutations in Nav1.1 associated with certain epilepsy syndromes lead to persistent current during maintained depolarization. A second set of mutations in Nav1.1 associated with multiple epilepsy syndromes and mutations in Nav1.5 associated with the Brugada syndrome cardiac arrhythmia led to decreased current, resulting from loss of function or enhanced inactivation kinetics.

Multiple mutations in NaV1.1 and NaV1.5 associated with an increased persistent current are observed at positions clustering in the corresponding helix I of the NaV1.2 CTD. The F1808L mutation

### TABLE 1

| Quantity                                | Value |
|-----------------------------------------|-------|
| Unique NOE distance restraints          | 1772  |
| Intra-residual                          | 699   |
| Sequential                              | 442   |
| Medium range (2 ≤ i ≤ 5)                | 321   |
| Long range (i > 5)                      | 310   |
| Residual violations >0.3 Å per structure (n = 15) | 1.2 ± 1.1 |
| Maximum violation (Å)                   | 0.27 ± 0.2 |
| NOE completeness per shell               |       |
| 2.0-2.5 Å (%)                           | 87    |
| 2.5-3.0 Å (%)                           | 69    |
| 3.0-3.5 Å (%)                           | 57    |
| 3.5-4.0 Å (%)                           | 44    |
| TALOS dihedral restraints (δ, φ)         | 65, 65|
| Residual dipolar coupling restraints    |       |
| H-N                                     | 41    |
| N-C                                     | 64    |
| H\(^{3+}\)/C\(^{3+}\)                   | 44    |
| R-value; Q-factor (64 C\(^{3+}\)–C\(^{3+}\) couplings) | 0.93 ± 0.02; 0.38 ± 0.05 |
| PROCHECK                                |       |
| Most favored (%)                        | 82.2 ± 1.6 |
| Allowed (%)                             | 15.8 ± 1.7 |
| Generously allowed (%)                  | 1.41 ± 0.8 |
| Disallowed (%)                          | 0.5 ± 0.9 |
| MolProbity score; all-atom clash score  | 3.09 (20th); 20.33 (31th) |
| (percentiles)                           |       |
| Average backbone r.m.s.d. (Å)           | 0.80  |
| Average all atom r.m.s.d. (Å)           | 1.29  |

**FIGURE 3.** Ca\(^{2+}\) titration of Na\(_V\)1.2 (1777–1882) (panel A) and Na\(_V\)1.5 (1773–1878) (panel B). The plots show joint \(^1\)H,\(^15\)N chemical shift deviations from resonance assignments in 0 mM Ca\(^{2+}\). The titration was performed by serial addition of Ca\(^{2+}\) obtaining the following concentrations: 0 (red), 0.1 (orange), 0.5 (maroon), 1.5 (magenta), 2.5 (cyan), 3.5 (blue), and 4.5 mM (green) for Na\(_V\)1.2 (panel A) and (0 (red), 0.1 (orange), 0.5 (maroon), 2.5 (magenta), 3.5 (cyan), 4.5 (blue), and 5.5 mM (green) for Na\(_V\)1.5. Insets show resonances Phe-1812—Ile-1813 and Phe-1808—Ile-1809 for Na\(_V\)1.2 and Na\(_V\)1.5, respectively. Titration curves are shown in supplemental Fig. S2. In panel C the joint \(^1\)H,\(^15\)N chemical shift changes for Na\(_V\)1.2 (1777–1882) at 4.5 mM Ca\(^{2+}\) are mapped onto the lowest energy structure, interpolated between 0 ppm (blue) and 0.1 ppm (red).
mutation associated with intractable childhood epilepsy with generalized tonic clonic seizures in NaV1.1 may destabilize the protein core because the aromatic ring of Phe-1798 in NaV1.2 contacts residues in helix IV and the helix II-III interhelical segment (4, 72). The insertion of an Asp residue at position 1795, Y1795insD, leads to both LQT3 and Brugada syndrome phenotypes in NaV1.5 and potentially disrupts helix I by shifting the register of helical interactions (73).

Substitution at position Tyr-1795 in NaV1.5 differentially leads to decreased inactivation for Y1795C in LQT3 or enhanced inactivation kinetics for Y1795H in Brugada syndrome, whereas both substitutions lead to sustained current during maintained depolarization and negative shift of voltage dependence of inactivation (27, 74). The Y1795C mutation has been suggested to form an intra-molecular disulfide bond with Cys-1850 in NaV1.5 (32). The average C$_\text{H}$-C$_\text{H}$ distance of the corresponding residues in the NaV1.2 CTD structural ensemble is 9.6 ± 0.4 Å. The C$_\text{H}$-C$_\text{H}$ distance in cysteine disulfide bonds ranges from 3.4 to 4 Å (75); thus, the proposed disulfide bond may be intermolecular or require structural rearrangement on the order of several angstroms between helix I and IV (Fig. 4).

**TABLE 2**

Comparison of helix orientations in EF-hand proteins

| Molecule       | Helix II | Helix III | Helix IV |
|----------------|----------|-----------|----------|
| Ca-CaM         | 85 (20.4)| −134 (25.5)| 91 (14.6) |
| IQ-Ca-CaM      | 92 (18.3)| −161 (21.9)| 117 (10.1) |
| ApoCaM         | 136 (12.9)| −93 (21.2)| 126 (11.9) |
| Na$_v$.1.2 CTD | 152 ± 2 (10.9 ± 0.1)| −103 ± 6 (20.9 ± 0.3)| 143 ± 1 (15.7 ± 0.2) |

| Molecule       | Helix II | Helix III | Helix IV |
|----------------|----------|-----------|----------|
| Ca-CaM         | 83 (10.1)| −20 (16.7)| 65 (15.3) |
| IQ-Ca-CaM      | 107 (11.2)| −41 (18.6)| 80 (16.5) |
| ApoCaM         | 125 (11.9)| −49 (12.9)| 129 (14.1) |
| Na$_v$.1.2 CTD | 100 ± 5 (10.8 ± 0.4)| −46 ± 2 (13.4 ± 0.1)| 98 ± 6 (14.4 ± 0.4) |

**TABLE 3**

Structure-based sequence alignment

| Ca$^{2+}$ coordination | X | Y | Z | −Y | −X | −Z |
|------------------------|---|---|---|----|----|----|
| Position               | 1 | 2 | 3 | 4  | 5  | 6  |
| Na$_v$.1.2            | D | P | D | A  | T  | Q  |
| Na$_v$.1.5            | D | P | E | A  | T  | Q  |
| CaM                   | D | K | D | G  | D  | G  |

FIGURE 4. NMR structure of Na$_v$.1.2 (1777–1882) CTD with functionally significant mutations observed in Nav1.1 and Nav1.5 channels. The lowest energy structure of the calculated ensemble is shown. Mutations leading to persistent current cluster in helices I and IV (shown in red) and the helix II-III segment (shown in orange), whereas a position (1842) at which mutation (M1852T) leads to decreased current is shown in blue. Position 1799 at which substitutions lead to increased or decreased inactivation is shown in violet, and residue Cys-1854 is shown in green. The putative subunit interaction site is shown in pink.
Na$_v$1.2 is found in a position closer to the surface; the total side-chain exposed surface area is $103 \pm 10$ Å$^2$ for the conformers in Table 1. Hence, mutations at position Tyr-1799 may also affect interactions with other components of the intact channel. On the other hand, the conserved Trp-1802, corresponding to Trp-1798 in Nav1.5, is not completely accessible as observed previously (27); the total side-chain exposed surface area is $9 \pm 5$ Å$^2$ for the conformers in Table 1.

The L1825P mutation associated with LQT3 and the R1826H mutation associated with sudden infant death syndrome in Na$_v$1.5 occurs in the helix II-III interhelical segment (76, 77). The L1825P mutation results in significant persistent current and slows kinetics of inactivation. Interestingly, the L1825P mutation in Na$_v$1.5 introduces a di-proline motif, as is observed in wild type Na$_v$1.1, Na$_v$1.2, Na$_v$1.3, and Na$_v$1.7, but shifted by one residue. The residue corresponding to Arg-1826 in Na$_v$1.2 is Leu-1830, and some local difference in conformation probably exists. Like L1825P, the R1826H mutation leads to persistent current in Na$_v$1.5, further suggesting that the helix II-III interhelical segment is critical to channel inactivation.

Two mutations implicated in interactions with other components of the sodium channel cluster in helices III and IV. The D1866Y mutation in Na$_v$1.1, associated with generalized epilepsy and febrile seizures plus, leads to persistent current and decreased fast inactivation kinetics in the presence of the β subunit (78). The corresponding position Asp-1856 in Na$_v$1.2 is replaced to begin probing specific interactions between the C-terminal domain and other components that play a role in inactivation of voltage-gated sodium channels.

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