Solution Structure of the Na\textsubscript{\textalpha} 1.2 C-terminal EF-hand Domain*\textsuperscript{S4}

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Vesselin Z. Miloushev\textsuperscript{5}, Joshua A. Levine\textsuperscript{5}, Mark A. Arbing\textsuperscript{\textasteriskcentered1}, John F. Hunt\textsuperscript{\textasteriskcentered2,3}, Geoffrey S. Pitt\textsuperscript{\textasteriskcentered1,2}, and Arthur G. Palmer III\textsuperscript{\textasteriskcentered1,4}

From the Departments of \textsuperscript{5}Biochemistry and Molecular Biophysics and \textsuperscript{\textasteriskcentered5}Pathology, Columbia University, New York, New York 10032, \textsuperscript{\textasteriskcentered1}Department of Biological Sciences, Columbia University, New York, New York 10027, and \textsuperscript{\textasteriskcentered2}Department of Medicine, Division of Cardiology, Duke University Medical Center, Durham, North Carolina 27710

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\textsubscript{\textalpha} 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 α-helices separated by two short anti-parallel β-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\textsubscript{\textalpha} 1.2 C-terminal domain does not bind Ca\textsuperscript{2+} through the canonical EF-hand loops, as evidenced by monitoring \textsuperscript{1}H,\textsuperscript{15}N chemical shifts during titration. Backbone chemical shift resonance assignments and Ca\textsuperscript{2+} titration also were performed for the Na\textsubscript{\textalpha} 1.5 (1773–1878) isoform, demonstrating similar secondary structure architecture and the absence of Ca\textsuperscript{2+} binding by the EF-hand loops. Clinically significant mutations identified in the C-terminal region of Na\textsubscript{\textalpha} 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\textsubscript{\textalpha} 1.2 (1777–1882) structure.

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\textsuperscript{\textasteriskcentered5} The on-line version of this article (available at http://www.jbc.org) contains supplemental Table I and Figs. S1–S3.

The atomic coordinates and restraints list (code 2kav) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

Complete resonance assignments for Na\textsubscript{\textalpha} 1.2 CTD (BMRB 16032) and backbone resonance assignments for Na\textsubscript{\textalpha} 1.5 CTD (BMRB 16031) have been deposited in the BioMagResBank.

1 A Fellow of the Canadian Cystic Fibrosis Foundation. Present address: UCLA-DOE Institute for Genomics and Proteomics, UCLA, Los Angeles, CA 90095-1570.

2 Established Investigators of the American Heart Association.

3 Supported by National Institutes of Health Protein Structure Initiative Grants P50GM62413 and U54GM074958 to the Northeast Structural Genomics Consortium.

4 A member of the New York Structural Biology Center (supported by National Institutes of Health Grant GM66354). To whom correspondence should be addressed: 701 West 168th St., Box 36, New York, NY 10032-3702. E-mail: agp6@columbia.edu; Tel.: 212-305-8675; Fax: 212-305-6949.

5 The abbreviations used are: VSGC, voltage-gated sodium channel; Na\textsubscript{\textalpha} 1, VSCG type 1; CTD, C-terminal domain; LQT3, Long QT syndrome type 3; CaM, calmodulin; HSQC, heteronuclear single quantum spectroscopy; NOESY, nuclear Overhauser effect (NOE) spectroscopy.
modulin (33). Nevertheless, whether Ca\(^{2+}\) 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 I1877A/Q1878A and an N-terminal His\(_8\) tag MGSSHHHH-HHSSGLPRGSHMAS (31), was subjected to proteolytic digestion with protease 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\(^{13}\)C\(^{15}\)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 [\(^{13}\)C\(^{15}\)N\(^{13}\)C\(^{15}\)N]glucose (35). Cultures were grown at 37 °C to a final OD\(_{600}\) of 0.7, induced with 0.5 mM isopropyl β-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), 100 mM d\(_{5}\)-glycine, 10 µM d\(_{16}\)-EDTA, 1 mM d\(_{10}\)-dithiothreitol, 0.02% NaN\(_3\), and 10% 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 12.8 Hz and Rh = 0.56 ± 0.01, respectively, for the 200 conformers.

Structural quality statistics refer to residues Leu-1790—Glu-1868 of the 15 lowest-energy structures of 200 total structures calculated. NOE completeness was determined with aqua32 (53). The Pearson correlation coefficient (R) and the quality factor (Q) were computed with PALES (54) from C\(^\alpha\)-C\(^\alpha\) dipolar couplings that were not included in the structure calculation. MolProbity scores were calculated for the lowest energy structure (55). Average root mean square deviation values were calculated to the average coordinates with VMD (56). Interhelical distances and angles (rounded to the nearest degree) were computed using interhls. Structural alignments were performed with CE (57), and structure figures were prepared with VMD (56) and MOLMOL (58).

**RESULTS**

The isolated Na\(_v\)1.2 CTD (1777–1882) and Na\(_v\)1.5 CTD (1773–1878) constructs each contain the region just after their respective predicted IVS6 transmembrane helix and extend to a region highly conserved among all VGSCs just before the IQ
motif. Assignments of $^1$H, $^{15}$N resonances for the NaV1.2 CTD and the NaV1.5 CTD are, respectively, 99 and 97% complete. Notably, Asn-1835 could not be assigned in the $^1$H, $^{15}$N HSQC of NaV1.2. 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 NaV1.5. Moreover, homologous resonances Leu-1855 in NaV1.2 and Met-1851 in NaV1.5 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$^\alpha$ assignments are 100% complete, $^{13}$C$^\beta$ assignments are 97.1% complete, $^1$H aromatic assignments are 89.1% complete, and non-aromatic $^1$H assignments are 97.7% complete. The NaV1.2 CTD construct contains six proline residues, of which Pro-1789, Pro-1807, Pro-1827, and Pro-1845 are in a trans conformation, whereas Pro-1828 and Pro-1834 are in a cis conformation. The cis conformation is evidenced by stronger X-Pro H$^\alpha$-H$^\alpha$ than X-Pro H$^\alpha$-H$^\delta$ NOE contacts and differences of $^{13}$C$^\beta$-$^{13}$C$^\alpha$ 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 NOE, and long-range NOEs (Fig. 1) are observed for residues in helices I to IV and the partially structured C terminus of helix IV. 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 NaV1.2 CTD. Respective values $<0.02$ ppm were obtained for corresponding residues 1802–1813 and 1832–1849 in the NaV1.5 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 carbonyl atoms of Phe-1812 in NaV1.2 and Phe-1808 in NaV1.5, 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 NaV1.2 and NaV1.5, respectively (Fig. 3). A structure-based sequence alignment of calmodulin and NaV1.2 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 NaV1.2 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 motifs characteristic of paired EF-hand domains.
Structural alignment of the Na V1.2 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 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 NaV1.2 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 \textit{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...
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

### Table 1: Structural statistics for Na$_{v}$1.2 CTD

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Unique NOE distance restraints</td>
<td>1772</td>
</tr>
<tr>
<td>Intra-residual</td>
<td>699</td>
</tr>
<tr>
<td>Sequential</td>
<td>442</td>
</tr>
<tr>
<td>Medium range (2 ≤ i ≤ 5)</td>
<td>321</td>
</tr>
<tr>
<td>Long range (i &gt; 5)</td>
<td>310</td>
</tr>
<tr>
<td>Residual violations &gt;0.3 Å per structure (n = 15)</td>
<td>1.2 ± 1.1</td>
</tr>
<tr>
<td>Maximum violation (Å)</td>
<td>0.27 ± 0.2</td>
</tr>
</tbody>
</table>

**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

**Residual dipolar coupling restraints**

| H-N                             | 41          |
| N-C                             | 64          |
| H$^\alpha$-C$^\alpha$           | 44          |

**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 (percentiles) 3.09 (20th); 20.33 (31th)
- 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 shift-
ing 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 syn-
drome, 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
1/H9252-C
1/H9252
distance of the
corresponding residues in the NaV1.2 CTD structural ensemble
is 9.6 ± 0.4 Å. The C
1/H9252-C
1/H9252
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) if
it is formed. Furthermore, although Tyr-1795 in NaV1.5 was
predicted to contribute to the hydrophobic interface between
helices I and IV (27), the corresponding residue Tyr-1799 in

### TABLE 2
Comparison of helix orientations in EF-hand proteins

| Interhelical angles are shown in degrees with interhelical distances shown in Å in parentheses. Calculations refer to the following structures. Ca-CaM is Ca
1/H11001-loaded target-free calmodulin (PDB code 1EXR, 1.00 Å) (67), IQ-Ca-CaM is Ca
1/H11001-loaded calmodulin bound to the voltage-gated Ca
1/H11001 channel CaV1.2 IQ-motif (PDB code 2F3Y, 1.45 Å) (81), and apoCaM is apoCa
1/H11001 target-free calmodulin (PDB code 1CFD, NMR) (82), with helix I defined as residues 6–18, helix II as residues 29–38, helix III as residues 45–54, and helix IV as residues 65–74. Interhelical angles for the NaV1.2 CTD are the averages with S.D. for the structural ensemble.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Helix II</th>
<th>Helix III</th>
<th>Helix IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-CaM</td>
<td>85 (20.4)</td>
<td>−134 (25.5)</td>
<td>91 (14.6)</td>
</tr>
<tr>
<td>IQ-Ca-CaM</td>
<td>92 (18.3)</td>
<td>−161 (21.9)</td>
<td>117 (10.1)</td>
</tr>
<tr>
<td>ApoCaM</td>
<td>136 (12.9)</td>
<td>−93 (21.2)</td>
<td>126 (11.9)</td>
</tr>
<tr>
<td>NaV1.2 CTD</td>
<td>152 ± 2 (10.9 ± 0.1)</td>
<td>−103 ± 6 (20.9 ± 0.3)</td>
<td>143 ± 1 (15.7 ± 0.2)</td>
</tr>
<tr>
<td>Ca-CaM</td>
<td>83 (10.1)</td>
<td>−20 (16.7)</td>
<td></td>
</tr>
<tr>
<td>IQ-Ca-CaM</td>
<td>107 (11.2)</td>
<td>−41 (18.6)</td>
<td></td>
</tr>
<tr>
<td>ApoCaM</td>
<td>125 (11.9)</td>
<td>−49 (12.9)</td>
<td></td>
</tr>
<tr>
<td>NaV1.2 CTD</td>
<td>100 ± 5 (10.8 ± 0.4)</td>
<td>−46 ± 2 (13.4 ± 0.1)</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3
Structure-based sequence alignment

N-terminal calcium binding loop of CaM aligned with the respective N-terminal EF-hand loop sequences from NaV1.2 and NaV1.5 CTD sequences, based on a structural alignment between apo-CaM and the NaV1.2 CTD.

| Ca
1/H11545 coordination | X | Y | Z | −Y | −X | −Z |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Position 1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>NaV1.2</td>
<td>D</td>
<td>P</td>
<td>D</td>
<td>A</td>
<td>T</td>
<td>Q</td>
</tr>
<tr>
<td>NaV1.5</td>
<td>D</td>
<td>P</td>
<td>E</td>
<td>A</td>
<td>T</td>
<td>Q</td>
</tr>
<tr>
<td>CaM</td>
<td>D</td>
<td>K</td>
<td>D</td>
<td>G</td>
<td>T</td>
<td>I</td>
</tr>
</tbody>
</table>

FIGURE 4. NMR structure of NaV1.2 (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), 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.
NaV1.2 is found in a position closer to the surface; the total side-chain exposed surface area is 103 ± 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 ± 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 NaV1.5 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 NaV1.5 introduces a di-proline motif, as is observed in wild type NaV1.1, NaV1.2, NaV1.3, and NaV1.7, but shifted by one residue. The residue corresponding to Arg-1826 in NaV1.2 is Leu-1830, and some local difference in conformation probably exists. Like L1825P, the R1826H mutation leads to persistent current in NaV1.5, 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 NaV1.1, 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 NaV1.2 is at the start of helix IV and may disturb a putative surface for interaction with the β subunit, as interaction with the β1 subunit and the CTD is suggested to occur through the second helix-sheet-helix motif by yeast-two-hybrid analysis of residues Lys-1846—Arg-1886 in NaV1.1 (78). Additionally, the M1852T mutation in NaV1.1, also associated with generalized epilepsy and febrile seizures plus, results in decreased current (loss of function). This phenotype can be rescued by co-expression and trafficking defect, this mutation may destabilize helix III, further suggesting that the second helix-sheet-helix motif may be important for interaction with other components of the sodium channel.

The notable exception to the above patterns is the LQT3 mutation D1790G in NaV1.5, resulting in a relative negative shift in the voltage dependence of inactivation in the presence of the β subunit (19, 80). D1790G corresponds to position D1794 in helix I of Nav1.2 and may disrupt the helix by introduction of a glycine residue, with the effect of propagating to helices III and IV.

The mechanisms and extent of NaV1 CTD function in binding the IQ motif and the specific role of calmodulin as well as Ca$^{2+}$ in multiple phases of inactivation remains to be elaborated. Interactions with the IQ motif may be more complicated than present models and may involve additional components (33). Previous evidence shows that Ca$^{2+}$-dependent regulation of VGSC is mediated by calmodulin (31), with the exact mode of interaction yet to be determined. The solution structure of the NaV1.2 C-terminal domain and chemical shift assignments of NaV1.5 (1773–1878) are initial steps in elucidating the mechanism of inactivation, extended to other isoforms by virtue of high degrees of homology. The current work provides a template 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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**REFERENCES**

Structure of the Na\textsubscript{v}1.2 C-terminal EF-hand