Structure and function of dimeric assembly in voltage-gated H^+ channel

VSOP/Hv1 is a voltage-gated H^+ channel (Hv channel) that is expressed in immunocytes and is involved in countervailing invading bacteria. VSOP/Hv1 has four-transmembrane segments that correspond to the voltage sensor domain (S1-S4) of other voltage-gated channels [1,2]. The composition of the functional unit is uniquely a dimer. Most types of ion channels show oligomeric architecture in the 3-5mer, forming a permeation pathway in the assembly center. In the Hv channel, by contrast, only one subunit of the dimeric unit is sufficient for its channel activity. Thus, the functional role of an assembly that lies outside the forming of the permeation pathway remains unknown.

To identify the assembly domain, we focused on the cytoplasmic C-terminus of mouse Hv1/VSOP (mVSOP/Hv1). Deletion of the C-terminal domain markedly reduced the dimer fraction in a cross-linking western blot. Sedimentation equilibrium for the purified C-terminal protein showed a twofold molecular weight of the monomer, suggesting the assembly domain. Structural analysis of the C-terminal domain was carried out using beamline **BL44XU**. The overall structure was a parallel left-handed, two-stranded coiled-coil (Fig. 1(a)) [3]. The coiled-coil core consists of well-packed hydrophobic residues, showing an I/L core packing pattern, in which Ile/Leu residues are situated and are periodically observed along the entire length of the coiled-coil (layers 1/2, 5/6, and 9/10). Mutation to the I/I or L/L pattern altered the coiled-coil structure to the trimeric one, while the L/I pattern resulted in the tetrameric structure (Fig. 1(b)) [4]. Despite this background, hydrophilic Asn (N231), instead of hydrophobic residues, comprise the core at layer-3 (Fig. 1(c)). They are asymmetrically packed, and notably, the neighboring residues are hydrophobic (I230 and I232) in an irregular gement; suggesting that this layer could be a weakness of core packing. The thermal stability of the mHv1/VSOP coiled-coil was lower (apparent Tm = ~40°C) than those of other naturally occurring coiled-coil proteins (Tm > 65°C), and the unfolding was irreversible (Fig. 1(d)). Thus, the mVSOP/Hv1 assembles into a dimer using a cytoplasmic coiled-coil architecture that is well folded and thermally labile.

The functional significance of the dimeric coiled-coil assembly in mVSOP/Hv1 was assessed using whole-cell patch clamp recordings. Deletion of the C-terminal coiled-coil domain (ΔC) accelerated the channel's activation kinetics (Fig. 2(a)). Given that the assembly of the coiled-coil was thermally labile, the relationship between temperature and activation kinetics was further analyzed. Deletion of the coiled-coil domain shifted the thermosensitivity relationship by 15°C without changing the slope (Fig. 2(b)). We also tested the effects of introducing mutations. The "4R" mutation, where bulky hydrophilic Arg is introduced into the hydrophobic core layers to disrupt the coiled-coil, shifted the thermosensitivity relationship by ~8°C in the direction of the ΔC phenotype (Fig. 2(c)). Assembly was suppressed in the 4R mutant, as shown by size exclusion chromatography. On the other hand, the NIN mutation, where an inverse mutation in hydrophobicity, NIN, is introduced into the lyer-3 to stabilize the coiled-coil, shifted the thermosensitivity relationship by ~5°C away from the ΔC phenotype (Fig. 2(c)). Using the CD spectrum of the coiled-coil protein, the coiled-coil assembly was stabilized by the NIN mutation (Fig. 1(d)). Thus, the stability of the C-terminal domain

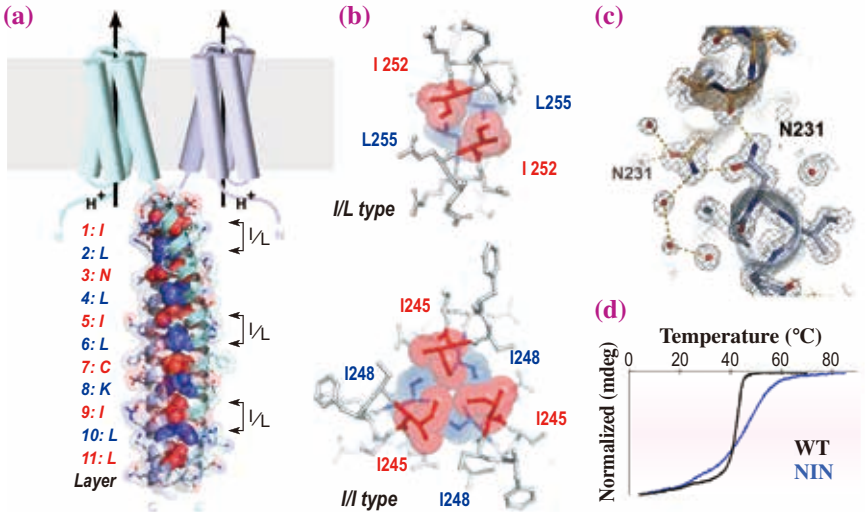

Fig. 1. **(a)** Crystal structure of cytoplasmic dimer coiled-coil. Hydrophobic layers of the coiled-coil core and amino acid residues are indicated. **(b)** Representative geometry of the hydrophobic coiled-coil core (layer-5,6) in the crystal structures: I/L-type dimer (top) and I/I-type trimer (bottom). **(c)** Structure (stick models) and electron density maps in layer-3. **(d)** Temperature dependence of CD signal recorded from WT and the NIN mutant proteins at 222 nm. Signals were recorded while applying a heating temperature gradient.

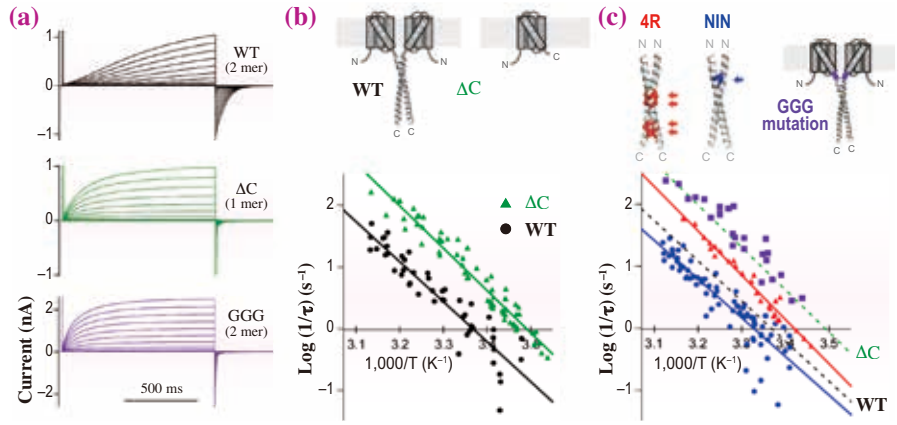

Fig. 2. **(a)** Representative currents through WT and the mutant channels recorded at 25°C from HEK293 cells. **(b, c)** Activation kinetics (Y-axis) of WT and the mutants were analyzed while varying temperature (X-axis). Accumulated data and the linear regression line are shown.

determined the channel activation kinetics in a temperature-dependent manner such that the coiled-coil dimer assembly inhibited channel activation.

The coiled-coil structure we characterized began only five residues downstream of the last transmembrane helix (S4), which is considered to move substantially when the channel opens. Increasing the linker flexibility by Gly-Gly-Gly mutation altered the thermosensitivity relationship to the monomeric ΔC phenotype (Fig. 2), suggesting that a positional proximity of two channels is not sufficient to produce the thermal stability seen in the WT phenotype. The value of the effective gating charge (Zδ) was also decreased to half by the GGG mutation, as shown in the monomeric ΔC channel. This half-Zδ value suggests that two channel subunits gate independently. Thus, the cytoplasmic coiled-coil not only regulates the thermosensitivity of the channel, but also mediates the cooperative gating between the two subunits. These functional regulations are considered to be established by a continuous a-helix consisting of the S4 voltage sensor helix and the coiled-coil strands (Fig. 3).

Ion channel subunits assemble into multimers, sometimes in heteromultimeric, to realize adequate functionality. This study shows that the dimeric coiled-coil assembly regulated the channel activation kinetics in a temperature-dependent manner, such that depolarization-induced increases in H^+ conductance appear to be optimized in the range of body temperature; this may be one of the functional significances of the Hv channel structure. In fact, an Hv channel exhibiting high-temperature dependence is expressed in phagocytes [5] and contributes to immunological responses under conditions of local and whole-body fever. The strong voltage dependence, that is, high Zδ value, in the activation gating underpinned by the dimeric coiled-coil structure also plays an essential role in sustaining the production of reactive oxygen species in the mammalian immune system [5]. In addition, our experimental approaches and findings in this study should bring about appreciable understanding of concepts generalizable to other larger and more complex ion channels.

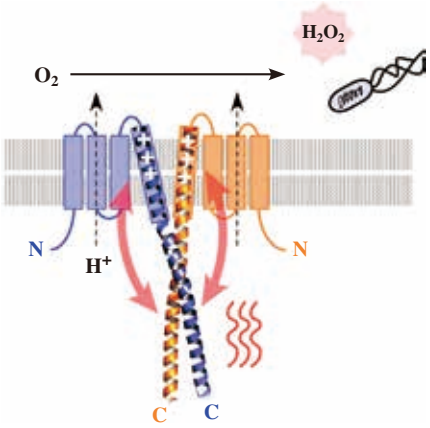

Fig. 3. Hv channel is a key regulator in the production of reactive oxygen species for digestion of invading bacteria. Cytoplasmic coiled-coil mediates the cooperative gating and adjusts the range of temperature over which VSOP/Hv1 operates. Two channel subunits interact via the cytoplasmic coiled-coil assembly. Gating movement in the transmembrane region is physically restricted by the cytoplasmic coiled-coil domain and the rigid linker region, which enables structural change of one subunit to affect the other.

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