

Oligomerization of the voltage-gated proton channel

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Abbreviations: g_H , proton conductance; g_H - V , proton conductance-voltage relationship; H_V1 , human proton channel protein; $H_V1\Delta C$, C-terminal truncation of H_V1 ; mVSOP, mouse proton channel; pH_i , intracellular pH; pH_o , extracellular pH; τ_{act} , activation time constant; τ_{tail} , deactivation (tail current) time constant; V_{rev} , reversal potential; VSD, voltage-sensing domain; WT, wild-type

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The voltage-gated proton channel exists as a dimer, although each protomer has a separate conduction pathway, and when forced to exist as a monomer, most major functions are retained. However, the proton channel protomers appear to interact during gating. Proton channel dimerization is thought to result mainly from coiled-coil interaction of the intracellular C-termini. Several types of evidence are discussed that suggest that the dimer conformation may not be static, but is dynamic and can sample different orientations. Zn^{2+} appears to link the protomers in an orientation from which the channel(s) cannot open. A tandem WT-WT dimer exhibits signs of cooperative gating, indicating that despite the abnormal linkage, the correct orientation for opening can occur. We propose that C-terminal interaction functions mainly to tether the protomers together. Comparison of the properties of monomeric and dimeric proton channels speaks against the hypothesis that enhanced gating reflects monomer-dimer interconversion.

Introduction

The voltage-gated proton channel, H_V1 , appears to exist mainly as a dimer.¹⁻³ Although each protomer contains its own conduction pathway, the protomers comprising the dimer interact during channel opening.⁴⁻⁶ In our recent study of Zn^{2+} interaction with human H_V1 , the data supported the surprising hypothesis that high affinity binding of Zn^{2+} occurs at the interface between monomers (or protomers) in the dimer, rather than within each individual monomer. Beyond this,

several observations provided suggestive clues to the assembly and stability of the dimer and the role of the C-terminus. These observations raise questions that provide a basis for further experiments.

Results and Discussion

Protomers in the dimer are not strongly attached. Evidence that the dimer is loosely attached can be deduced from cross-linking studies. Lee et al.² studied Cys residues at 15 different locations in the human H_V1 molecule, and observed cross-linking under several conditions. The cross-links were mainly consistent with a dimer interface occurring at the outer end of the two S1 domains, illustrated in the cartoon in Figure 1A. The proximity of the inner ends of S4 in this orientation might facilitate the C-terminal coiled-coil interaction that is thought to be the main determinant of dimerization.^{1,3} In contrast, in the model suggested by our Zn^{2+} studies (Fig. 1B), the S1 domains are far apart. However, in the Lee et al.² study, cross-linking induced either by CuP (CuSO₄ and *o*-phenanthroline) or by M3M (1,3-propanediyl bismethanethiosulfonate) revealed distinct cross-linking of Cys at position 194, which is immediately adjacent to His¹⁹³, and thus is inconsistent with the dimer conformation in Figure 1A. Lee et al.² proposed that this result might indicate that proximity of these residues occurs, albeit rarely. Based on evidence from mutations involving His¹⁹³ and His¹⁴⁰ in various combinations,⁶ we concluded that Zn^{2+} can bind simultaneously to the His¹⁹³ residues from both monomers, and that when Zn^{2+} binds at this location, the proton channel

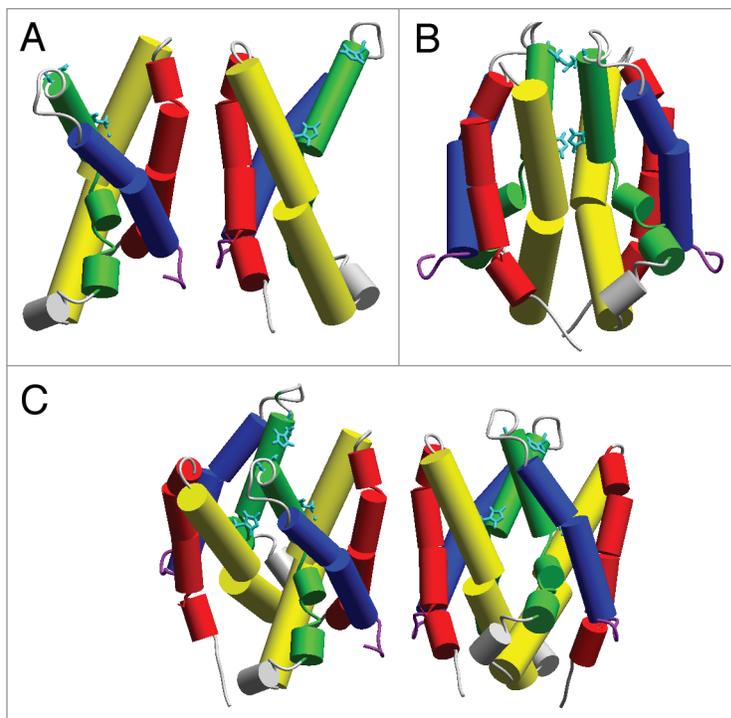


Figure 1. Cartoons showing the general orientation of the dimer proposed by (A) Lee et al.² (B) Musset et al.⁶ and (C) a speculative tetramer. The histidines known to bind Zn^{2+} , His¹⁴⁰ and His¹⁹³ (ref. 32), are shown in turquoise. The domains are color coded: S1 is red, S2 is yellow, S3 is green, S4 is blue. The beginning of the C terminus is shown in pink; neither the N- nor the C-terminus is modeled. Lee et al.² proposed interaction at the top (outer) end of S1 and C terminal coiled-coil interaction. Musset et al.⁶ deduced that His¹⁴⁰ in S2 and His¹⁹³ in the S3-S4 linker can approach closely enough to bind Zn^{2+} in the dimer orientation shown in (B), but not within a single monomeric channel. The tetramer shown in (C) consists of two dimers as in (B), linked through an S1 interaction as shown in (A).

cannot open. This binding requires a quite different dimer interface, as shown in **Figure 1B**. The cross-linking at position 194 observed by Lee et al.² appears to demonstrate that this orientation of the dimer can occur, at least occasionally. This implies that the H_v1 dimer is not tightly attached, and that the two protomers sample different orientations with finite probability. In this context, if the orientation proposed by Lee et al.² were the one from which the channel can open, Zn^{2+} binding between protomers at His¹⁴⁰ or His¹⁹³ when the dimer is in the orientation shown in **Figure 1B** would preclude the cooperative opening transition; unbinding of Zn^{2+} might allow reorientation of the monomers to the position shown in **Figure 1A**, which would permit cooperative opening.

Two dimers of the form shown in **Figure 1A** might be linked together by Zn^{2+} binding to His¹⁹³ or His¹⁴⁰ in each one,

thus forming a Zn^{2+} -induced tetramer. Similarly, two dimers oriented as in **Figure 1B** might be able to interact via the S1 interface (**Fig. 1A**) to form tetramers (**Fig. 1C**), which might even extend to an oligomeric chain. Some evidence indirectly supports the general idea of proton channel clustering. Byerly and Suen⁷ studied proton currents in membrane patches from *Lymnaea stagnalis*, and found that a much smaller fraction of patches exhibited proton currents than K^+ currents; the H^+ currents detected were macroscopic and reflected $>10^3$ channels. This result suggests that H^+ channels are not uniformly distributed in the membrane of snail neurones.

Additional evidence that the dimer is loosely attached comes from the preponderance of monomeric H_v1 protein in western blots.^{1,2,8,9} Koch et al.¹ sometimes observed a weak dimer band for the murine proton channel, but only under non-reducing conditions, that disappeared

on heat treatment, indicating that dimerization most likely does not involve covalent bonds.

Once the C-terminus tethers the dimer together, its job is done. C-terminal coiled-coil interaction is thought to maintain the proton channel in a dimeric state,¹⁻³ although a contribution from the N-terminus was seen for the murine proton channel.¹ Coiled-coils classically form from α -helices with amino acid sequences in which hydrophobic (H) and polar (P) residues are interspersed in the pattern $(HPPHPPP)_{n \geq 3}$ (reviewed in ref. 10). Truncation of the C-terminus results in the channel expressing mainly as a monomer, which retains channel functionality.^{1,3} Not only do protein structural prediction programs (e.g., PredictProtein) indicate that the C-terminus ought to form coiled-coil associations, a recent crystal structure shows that isolated C-termini of the human proton channel, hH_v1 , assemble as a dimer in coiled-coil formation.¹¹ In this study, Li and colleagues also examined localization of hH_v1 when the N- or C-termini were truncated. They concluded that the C-terminus was critical for trafficking to the plasma membrane; without it, most of the channels appeared to localize in unspecified intracellular compartments. This group also noted a pH-dependent structural change of the C-terminus, which is intriguing in light of the profound effects of pH_i on proton channel gating.¹²⁻¹⁵

The two dimer models in **Figure 1** differ markedly in the proximity of the S4 ends (the beginning of the C-termini). Are both consistent with coiled-coil interaction? Coiled-coil is predicted to extend from K221 to L262 (PredictProtein), with probability increasing from position 228 to 235. The crystal structure of the C-terminus construct exhibits coiled-coil interaction between residues 226–266.¹¹ The dimer orientation shown in **Figure 1A** allows coiled-coil interaction starting within 2–3 residues after emergence from the membrane. In our model (**Fig. 1B**), coiled-coil interaction would necessarily begin some distance from the end of S4. Based on the distances observed in the model, the first 7 amino acids of the C-terminus could bridge the gap between S4 domains, allowing

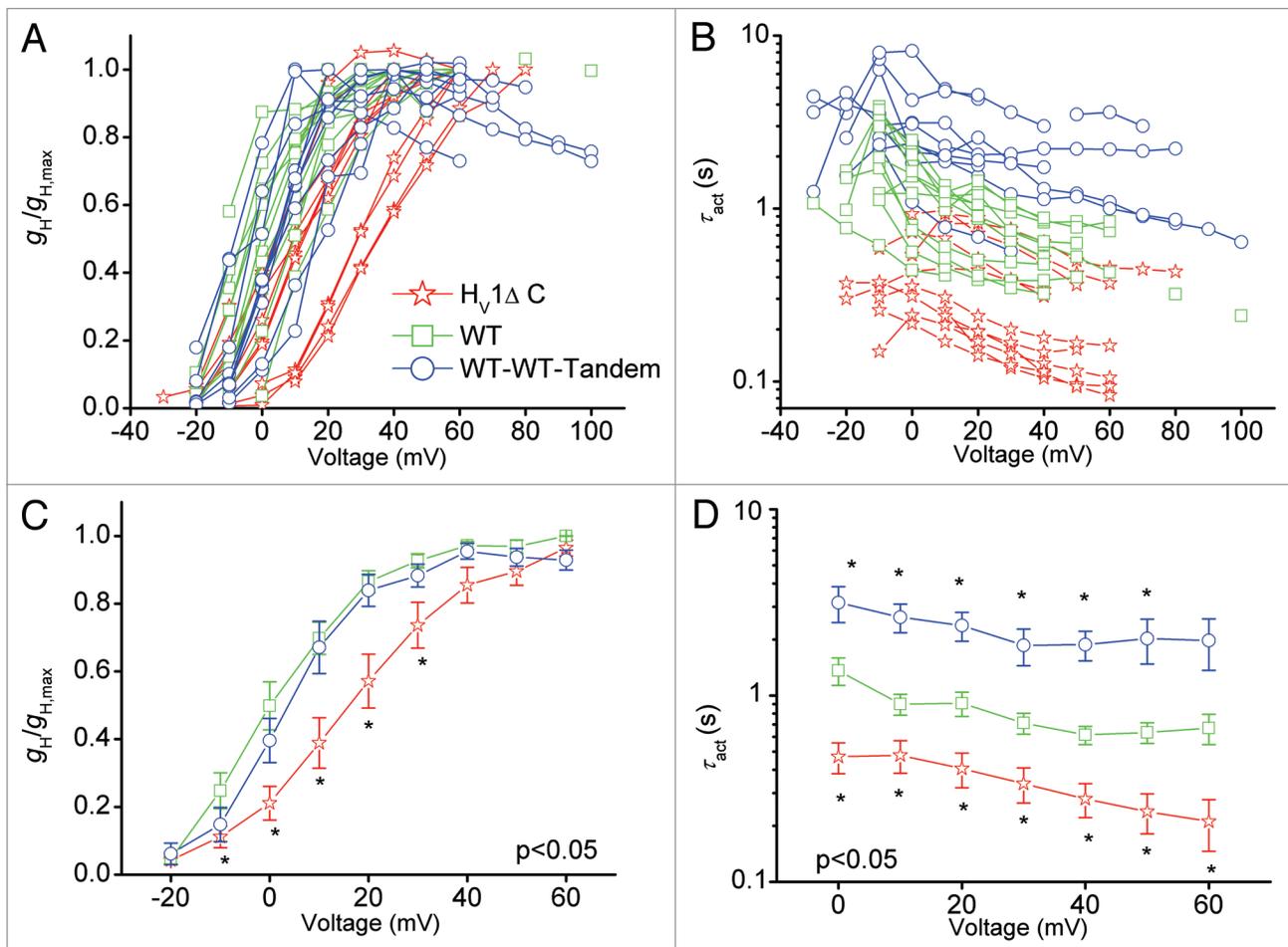


Figure 2. Comparison of voltage dependence and gating kinetics of WT (□), WT-WT tandem dimer (○) and $H_v1\Delta C$ (monomeric, ☆) channels expressed in HEK-293 cells. (A) Normalized g_H - V relationships are plotted for individual cells, all studied in whole-cell configuration at pH_o 7.0, pH_i 6.5 in solutions containing EGTA. Each g_H value was obtained by fitting a single rising exponential, and taking the extrapolated steady-state value, using reversal potentials measured in each solution. (B) The time constant of current activation (τ_{act}) is plotted for the cells in (A). (C) Mean \pm SEM g_H - V relationships for the 11 WT, 10 WT-WT tandem dimer and 11 $H_v1\Delta C$ expressing cells in (A), with $*p < 0.05$ vs. WT. The slope of the g_H - V relationship in the monomeric construct ($H_v1\Delta C$) appears to be shallower than that of WT H_v1 , consistent with the report by Gonzalez et al.⁴ that the apparent gating charge of the monomer, determined from the limiting slope of the g_H - V relationship, was only half that of the WT dimer. (D) Mean \pm SEM τ_{act} for the same cells as (C), with $*p < 0.05$ vs. WT. It should be noted that these whole-cell measurements are subject to depletion effects (e.g., droop of the g_H - V relationship at large depolarizations); previously reported comparisons of τ_{act} in WT vs. $H_v1\Delta C$ expressing cells⁶ were based on excised patch measurements to minimize these effects, and at $pH_o = pH_i = 7.5$.

coiled-coil to begin at L228 and continue through five remaining heptads. Because stable coiled-coils generally require 3–4 heptads,¹⁶ coiled-coil interaction of the C-terminus in our dimer model orientation is plausible. Perhaps C-terminal interactions preserve the dimeric state, while allowing some freedom of rotation of each protomer. In a WT-WT tandem dimer evaluated by Musset et al.⁶ the six amino acid linker would obligatorily constrain the channel to dimeric status, obviating the necessity of coiled-coil interaction. Evidence for cooperative gating of the WT dimer⁴⁻⁶ suggests that when

the dimeric channel does open, the two protomers interact physically, which in turn suggests that they adopt a particular preferred orientation with respect to each other. Because the WT-WT tandem dimer exhibited signs of cooperative gating like that of the WT dimer (see below), it seems clear that different intermonomer tethering devices permit the channel to adopt the dimeric conformation from which it preferentially opens.

Given that H_v1 assembles as a dimer and that coiled-coil interactions of the C-termini are critical to maintaining the dimeric state, what other functions might

the C-terminus play? Several observations indicate that the C-terminus is not essential to many of the characteristic capabilities of the proton channel. The C-terminus can be truncated altogether, and the resulting monomeric channels still exhibit proton selectivity and voltage- and pH-dependent gating.^{1,3,6,17} On the other hand, the dimer activates more slowly than the monomer^{1,3,6} and with a sigmoid time course, as opposed to the exponential opening time course of the monomer.^{4,6} There clearly are interactions between the protomers during dimer gating,⁴⁻⁶ but do these require specific

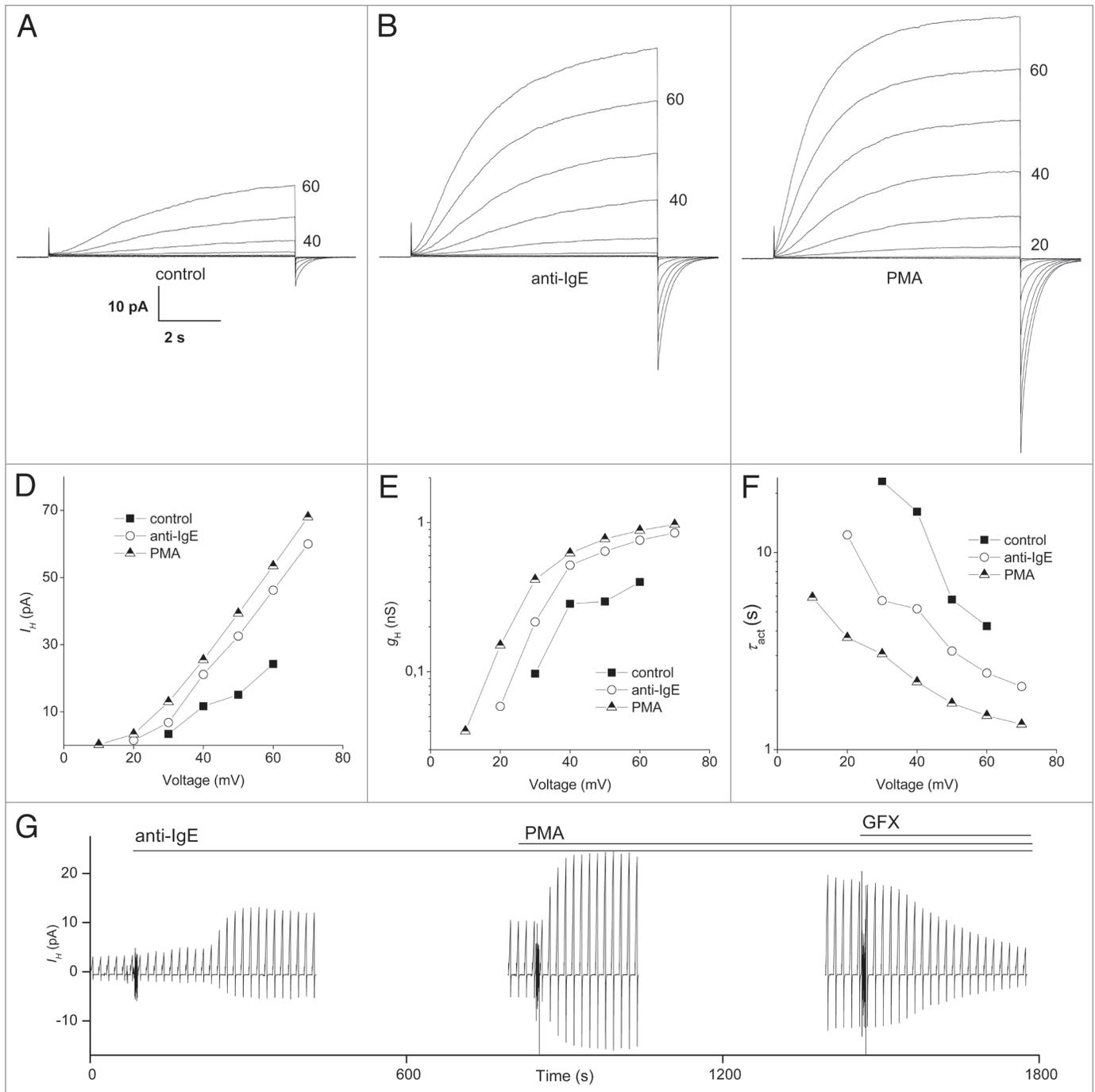


Figure 3. Induction of enhanced gating of proton channels in a human basophil. Families of currents in the same cell are shown before (A) and after anti-IgE (B) and PMA (C). Stimulation increases the proton current, I_H (D) and conductance, g_H (E) and accelerates channel opening, quantified as the activation time constant, τ_{act} (F). The time course of the I_H enhancement is illustrated in (G); at this slow time base, the envelope of peak currents is seen. For further discussion of this system, see ref. 30.

coiled-coil interactions of the C-terminus? Some evidence comes from comparison of the WT-WT tandem dimer with WT channels.

We created a WT-WT tandem dimer by linking the C-terminus of one protomer to the N-terminus of the second

protomer, a topological constraint that might prevent extensive coiled-coil interaction. On the other hand, very little is known about the structure or flexibility of the N-terminus, or the extent to which it might interact with the channel or the C-terminus. Two bits of evidence

indicate that the N-terminus can influence gating. First, phosphorylation of Thr²⁹ induces enhanced gating¹⁸ (see next section). Second, a naturally-occurring mutation of human H_v1, M91T, shifts the g_H -V relationship positively.¹⁹ That the N-terminus distinctly influences gating

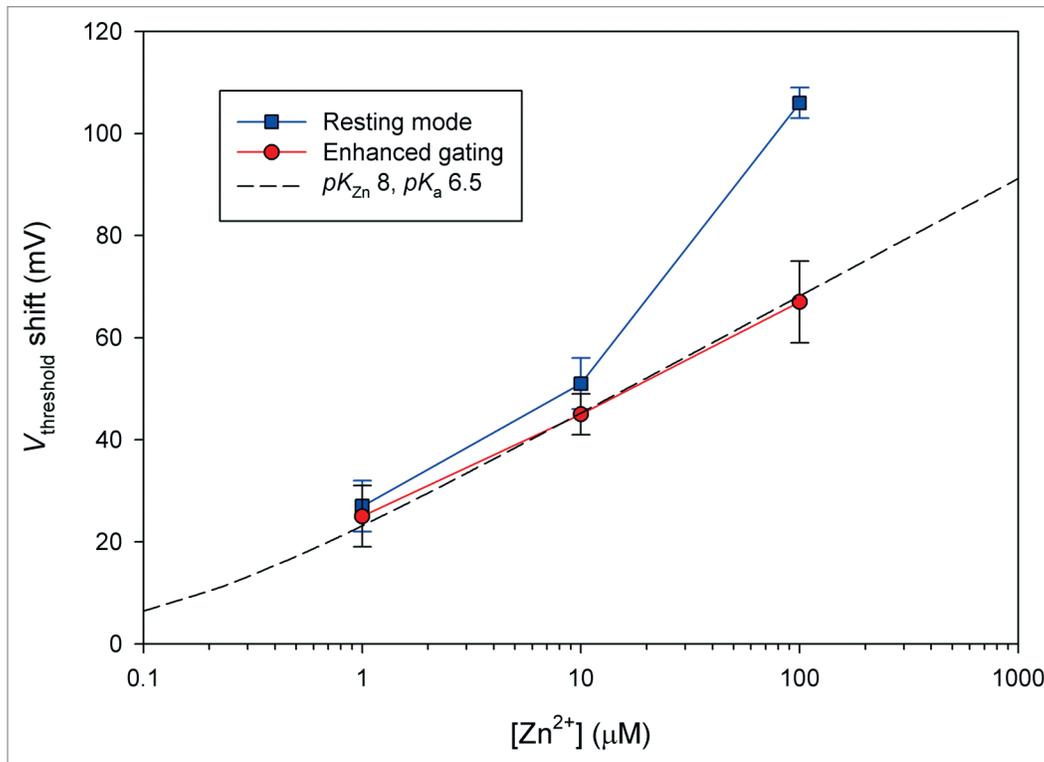


Figure 4. The Zn²⁺ sensitivity of resting (●) and PMA-activated (■) proton currents in human basophils is similar. The mean ± SEM shift of the g_H - V relationship by the indicated concentration of Zn²⁺ is plotted ($n = 3-5$ for all points). These data were previously reported in ref. 30. Dashed line shows the predicted shift of $V_{\text{threshold}}$ assuming that the channels cannot open when Zn²⁺ is bound, according to Model 6 from ref. 33, in which $pK_a = 6.5$, $pK_M = 8.0$ and the cooperativity factor $a = 0.01$ and $V_{\text{threshold}} \text{ shift} = \ln(1/(1 - P_{\text{Zn}})) \times 10 \text{ mV}$, where P_{Zn} is the probability that Zn²⁺ is bound.

seems consistent with proximity to the membrane-spanning domains.

In view of the possibility that the WT-WT tandem dimer might not be capable of normal C-terminal interactions, it seemed possible that in this construct, the two monomers might be functionally uncoupled and cooperative gating might be disrupted. However, the WT-WT tandem dimer functionally resembled the native dimer more closely than the H_v1ΔC monomer (C-truncated H_v1). Both exhibited a sigmoid activation time course, suggestive of cooperative gating, and their Zn²⁺ sensitivity, quantified either as slowing of τ_{act} or as the shift of the g_H - V relationship, was identical.⁶ These results indicate that the interactions between protomers that occur in the dimer during channel opening also occur in the tandem dimer construct. In Figure 2, gating of proton currents in WT H_v1 and WT-WT tandem dimers is compared in more detail. There was no discernible difference in the position or steepness of the g_H - V relationship (Fig.

2A and C). However, Figure 2B and D illustrate that the opening kinetics (τ_{act}) of the tandem WT-WT dimer tended to be somewhat slower than the WT channel, although both varied substantially among cells. In contrast, activation of the monomer is several times faster than the WT channel^{1,6} (Fig. 2B and D). Thus, the only difference detected between WT channels and the WT-WT tandem dimer was that the tandem dimer activated more slowly. Evidently, the interactions between protomers that result in slow and sigmoidal opening of H_v1 do not require coiled-coil interaction of the C-terminus (assuming that this does not occur in the WT-WT tandem dimer).

In summary, the proton channel dimer appears to be loosely attached, with the ability to sample various orientations with finite probability. From multiple initial orientations, the protomers can rotate to reach a conformation from which cooperative opening can occur. Once the channel is localized in the membrane, the main function of coiled-coil interactions

of the C-terminus may be to tether the two protomers together. Because S4 is thought to move substantially during gating, at least in K⁺ channels,^{20,21} but likely also in proton channels,⁴ any C-terminal interactions must still allow S4 adequate freedom of movement. Perhaps monomeric H_v1 constructs that lack the C-terminus open more rapidly^{1,6} (Fig. 2B and D) because they are liberated from C-terminal constraints.

Dimer-monomer interconversion does not explain the enhanced gating mode. In neutrophils, eosinophils, basophils, osteoclasts, monocytes and related cell lines, stimulation by a variety of agonists dramatically enhances proton currents.^{9,18,22-31} The full enhanced gating mode includes increased maximum proton conductance, $g_{H, \text{max}}$, faster activation, slower deactivation, and a 40 mV negative shift of the g_H - V relationship. A qualitatively similar, but smaller response occurs in cells that lack NADPH oxidase activity,³¹ such as basophils.³⁰ Figure 3 illustrates the enhancement of proton

currents in a human basophil stimulated first with anti-IgE and then with the phorbol ester, PMA. The response to anti-IgE was variable among basophils and often weaker than the PMA response. Both agonists produced negative shifts of the proton current, I_H - V (Fig. 3D) and g_H - V relationships (Fig. 3E). Both also accelerated the turn-on of current during depolarizing pulses (Fig. 3F). The time course of the effects of anti-IgE and PMA on proton currents during test pulses can be seen in Figure 3G, as well as partial reversal of these effects by the PKC antagonist, GF109203X (GFX).

It has been speculated that interconversion between monomeric and dimeric oligomerization states (perhaps the ultimate level of dimer interface lability!) might be responsible for the "enhanced gating mode" in phagocytes.¹ Although attractive, this hypothesis leads to three predictions that now appear to have been contradicted: (1) Because the monomer activates faster than the dimer^{1,6} (Figs. 2B and D), in this hypothesis, enhanced gating mode must reflect dimer to monomer conversion. However, when we compared the g_H - V relationships of monomers and dimers (Fig. 2C), we find that monomeric $H_V1\Delta C$ expressed in HEK-293 cells activated somewhat more positively than the dimer. The shift between dimer and monomer is not only in the wrong direction but also much less than the 40 mV shift seen in activated phagocytes. Most likely, enhanced gating is the result of phosphorylation of Thr²⁹ in the N-terminus.^{18,29} The mechanism by which phosphorylation influences gating remains to be determined. (2) Because monomeric proton channels display significantly reduced Zn²⁺ sensitivity,⁶ the Zn²⁺ sensitivity of proton channels in activated phagocytes should decrease. But comparison of Zn²⁺ effects on resting and PMA-activated proton currents in human eosinophils indicated indistinguishable sensitivity.²⁴ Figure 4 shows the effects of Zn²⁺ in human basophils, before and after stimulation by PMA, again revealing similar Zn²⁺ affinity. (3) If enhanced gating reflects dimer to monomer conversion, activation kinetics should be exponential in activated cells. Instead, activation kinetics is sigmoid both in resting and

activated states (e.g., Fig. 3A vs. B or C). Change in oligomerization status does not appear to be responsible for the enhanced gating mode.

Materials and Methods

The methods used were described previously.^{6,30}

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References

- Koch HP, Kurokawa T, Okochi Y, Sasaki M, Okamura Y, Larsson HP. Multimeric nature of voltage-gated proton channels. *Proc Natl Acad Sci USA* 2008; 105:9111-6.
- Lee S-Y, Letts JA, MacKinnon R. Dimeric subunit stoichiometry of the human voltage-dependent proton channel H_V1 . *Proc Natl Acad Sci USA* 2008; 105:7692-5.
- Tombola F, Ulbrich MH, Isacoff EY. The voltage-gated proton channel H_V1 has two pores, each controlled by one voltage sensor. *Neuron* 2008; 58:546-56.
- Gonzalez C, Koch HP, Drum BM, Larsson HP. Strong cooperativity between subunits in voltage-gated proton channels. *Nat Struct Mol Biol* 2010; 17:51-6.
- Tombola F, Ulbrich MH, Kohout SC, Isacoff EY. The opening of the two pores of the H_V1 voltage-gated proton channel is tuned by cooperativity. *Nat Struct Mol Biol* 2010; 17:44-50.
- Musset B, Smith SME, Rajan S, Cherny VV, Sujai S, Morgan D, et al. Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating. *J Physiol* 2010; 588:1435-49.
- Byerly L, Suen Y. Characterization of proton currents in neurones of the snail, *Lymnaea stagnalis*. *J Physiol* 1989; 413:75-89.
- Okochi Y, Sasaki M, Iwasaki H, Okamura Y. Voltage-gated proton channel is expressed on phagosomes. *Biochem Biophys Res Commun* 2009; 382:274-9.
- Capasso M, Bhamrah MK, Henley T, Boyd RS, Langlais C, Cain K, et al. HVCN1 modulates BCR signal strength via regulation of BCR-dependent generation of reactive oxygen species. *Nature Immunol* 2010; 11:265-72.
- Moutevelis E, Woolfson DN. A periodic table of coiled-coil protein structures. *J Mol Biol* 2009; 385:726-32.
- Li SJ, Zhao Q, Zhou Q, Unno H, Zhai Y, Sun F. The role and structure of the carboxyl-terminal domain of the human voltage-gated proton channel H_V1 . *J Biol Chem* 2010; 285:12047-54.
- Byerly L, Meech R, Moody W Jr. Rapidly activating hydrogen ion currents in perfused neurones of the snail, *Lymnaea stagnalis*. *J Physiol* 1984; 351:199-216.
- Mahaut-Smith M. Separation of hydrogen ion currents in intact molluscan neurones. *J Exp Biol* 1989; 145:439-54.

- DeCoursey TE. Hydrogen ion currents in rat alveolar epithelial cells. *Biophys J* 1991; 60:1243-53.
- Cherny VV, Markin VS, DeCoursey TE. The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient. *J Gen Physiol* 1995; 105:861896.
- Woolfson DN. The design of coiled-coil structures and assemblies. *Adv Prot Chem* 2005; 70:79-112.
- Sakata S, Kurokawa T, Nørholm MHH, Takagi M, Okochi Y, von Heijne G, et al. Functionality of the voltage-gated proton channel truncated in S4. *Proc Natl Acad Sci USA* 2010; 107:2313-8.
- Musset B, Capasso M, Cherny VV, Morgan D, Bhamrah M, Dyer MJS, et al. Identification of Thr²⁹ as a critical phosphorylation site that activates the human proton channel Hvcn1 in leukocytes. *J Biol Chem* 2010; 285:5117-21.
- Iovannisci D, Illek B, Fischer H. Function of the HVCN1 proton channel in airway epithelia and a naturally occurring mutation, M91T. *J Gen Physiol* 2010; 136:35-46.
- Ruta V, Chen J, MacKinnon R. Calibrated measurement of gating-charge arginine displacement in the K_vAP voltage-dependent K^+ channel. *Cell* 2005; 123:463-75.
- Swartz KJ. Sensing voltage across lipid membranes. *Nature* 2008; 456:891-7.
- Bánfi B, Schrenzel J, Nüsse O, Lew DP, Ligeti E, Krause K-H, et al. A novel H^+ conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease. *J Exp Med* 1999; 190:183-94.
- DeCoursey TE, Cherny VV, Zhou W, Thomas LL. Simultaneous activation of NADPH oxidase-related proton and electron currents in human neutrophils. *Proc Natl Acad Sci USA* 2000; 97:6885-9.
- DeCoursey TE, Cherny VV, DeCoursey AG, Xu W, Thomas LL. Interactions between NADPH oxidase-related proton and electron currents in human eosinophils. *J Physiol* 2001; 535:767-81.
- DeCoursey TE, Cherny VV, Morgan D, Katz BZ, Dinauer MC. The gp91^{phox} component of NADPH oxidase is not the voltage-gated proton channel in phagocytes, but it helps. *J Biol Chem* 2001; 276:36063-6.
- Bankers-Fulbright JL, Kita H, Gleich GJ, O'Grady SM. Regulation of human eosinophil NADPH oxidase activity: a central role for PKC δ . *J Cell Physiol* 2001; 189:306-15.
- Cherny VV, Henderson LM, Xu W, Thomas LL, DeCoursey TE. Activation of NADPH oxidase-related proton and electron currents in human eosinophils by arachidonic acid. *J Physiol* 2001; 535:783-94.
- Mori H, Sakai H, Morihata H, Kawawaki J, Amano H, Yamano T, et al. Regulatory mechanisms and physiological relevance of a voltage-gated H^+ channel in murine osteoclasts: phorbol myristate acetate induces cell acidosis and the channel activation. *J Bone Miner Res* 2003; 18:2069-76.
- Morgan D, Cherny VV, Finnegan A, Bollinger J, Gelb MH, DeCoursey TE. Sustained activation of proton channels and NADPH oxidase in human eosinophils and murine granulocytes requires PKC but not cPLA α activity. *J Physiol* 2007; 579:327-44.
- Musset B, Morgan D, Cherny VV, MacGlashan DW Jr, Thomas LL, Ríos E, et al. A pH-stabilizing role of voltage gated proton channels in IgE-mediated activation of human basophils. *Proc Natl Acad Sci USA* 2008; 105:11020-5.
- Musset B, Cherny VV, Morgan D, DeCoursey TE. The intimate and mysterious relationship between proton channels and NADPH oxidase. *FEBS Lett* 2009; 583:7-12.
- Ramsey IS, Moran MM, Chong JA, Clapham DE. A voltage-gated proton-selective channel lacking the pore domain. *Nature* 2006; 440:1213-6.
- Cherny VV, DeCoursey TE. pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn²⁺ and other divalent cations. *J Gen Physiol* 1999; 114:819-38.