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A Shaker K^+ Channel with a Miniature Engineered Voltage Sensor

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SUMMARY

Voltage-gated ion channels sense transmembrane voltage changes via a paddle-shaped motif that includes the C-terminal part of the third transmembrane segment (S3b) and the N-terminal part of the fourth segment (^{NT}S4) that harbors voltage-sensing arginines. Here, we find that residue triplets in S3b and ^{NT}S4 can be deleted individually, or even in some combinations, without compromising the channels' basic voltage-gating capability. Thus, a high degree of complementarity between these S3b and ^{NT}S4 regions is not required for basic voltage gating per se. Remarkably, the voltage-gated Shaker K^+ channel remains voltage gated after a 43 residue paddle sequence is replaced by a glycine triplet. Therefore, the paddle motif comprises a minimal core that suffices to confer voltage gating in the physiological voltage range, and a larger, modulatory part. Our study also shows that the hydrophobic residues between the voltage-sensing arginines help set the sensor's characteristic chemical equilibrium between activated and deactivated states.

INTRODUCTION

Coordinated activity of voltage-gated ion channels generates action potentials, the electric signals used by nerve, muscle, and endocrine cells. A fundamental question in the field has been how these electric signals are detected by the channel protein and how the resulting conformational changes are coupled to channel opening and closing. In the case of voltage-gated K^+ (Kv) channels, they are composed of an ion conduction module surrounded by four voltage-sensing modules (Kubo et al., 1993; Lu et al., 2001; Jiang et al., 2003). Positively charged residues of the channel protein's fourth transmembrane segment (S4) function as the main voltage-sensing residues, e.g., the four arginines (R1–R4) in the N-terminal part of S4 (^{NT}S4) of the Shaker Kv channel (Noda et al., 1984; Catterall, 1988; Stühmer et al., 1989; Liman et al., 1991; Lopez et al., 1991; Papazian et al., 1991; Yang and Horn, 1995; Aggarwal and MacKinnon, 1996; Larsson et al., 1996; Mannuzzu et al., 1996; Seoh et al., 1996; Yang et al., 1996). Movement of these voltage-sensing residues results in transfer of more than 12 elementary charges (or equivalent) across the transmembrane electric field (Schoppa et al., 1992; Aggarwal and MacKinnon, 1996; Seoh et al., 1996; Islas and Sigworth, 1999). Positively charged residues usually occupy every third position within S4 and are stabilized in the membrane plane by negatively charged protein residues or the phospho-head group of membrane phospholipids (Armstrong, 1981; Papazian et al., 1995; Seoh et al., 1996; Cuello et al., 2004; Freites et al., 2005; Ramu et al., 2006; Schmidt et al., 2006; Long et al., 2007; Xu et al., 2008; Milescu et al., 2009). The C-terminal part of S3 (S3b), ^{NT}S4, and their linker together form a helix-turn-helix motif termed the voltage-sensing paddle (Jiang et al., 2003; Long et al., 2007) (Figure 1). Remarkably, the paddle from a given voltage-gated ion (or proton) channel or enzyme (Murata et al., 2005; Sasaki et al., 2006; Ramsey et al., 2006) can be transferred to another voltage-gated channel without loss of voltage-sensing function (Alabi et al., 2007; Bosmans et al., 2008).

Membrane hyperpolarization drives ^{NT}S4 from the extracellular phase, via a short low-dielectric (hydrophobic) region, toward the intracellular phase (Yang and Horn, 1995; Aggarwal and MacKinnon, 1996; Larsson et al., 1996; Mannuzzu et al., 1996; Seoh et al., 1996; Yang et al., 1996; Starace et al., 1997; Glauner et al., 1999; Silverman et al., 2003; Ahern and Horn, 2004; Phillips et al., 2005; Ruta et al., 2005; Campos et al., 2007; Grabe et al., 2007; Pathak et al., 2007; Broomand and Elinder, 2008; Posson and Selvin, 2008; Tao et al., 2010). The accessibility pattern of different-length biotin-reagents tethered to substituted cysteines in the bacterial KvAP voltage-gated K^+ channel (whose S3-S4 linker is short) led Ruta et al. (2005) to conclude that S3b also undergoes substantial voltage-induced movement, consistent with the notion that S3b and ^{NT}S4 move together as a rigid body. On the other hand, the disulfide bond pattern of cysteine pairs substituted in S3b and ^{NT}S4 in the eukaryotic Shaker channel (whose S3-S4 linker is long) led Broomand and Elinder (2008) to conclude that the two helices exhibit a large motion relative to each other. It is unclear whether these different conclusions reflect a difference in channel type and/or in experimental method. Additionally, it has been suggested that ^{NT}S4 alternates between different secondary structures during voltage gating (Long et al., 2007; Khalili-Araghi et al., 2010). If so, complementarity at the S3b-^{NT}S4 interface should not be of such high degree as to prevent ^{NT}S4 from switching conformation.

Voltage-induced motion of S4, through a physical coupler, then enables the channel gate, which is formed by the C-terminal

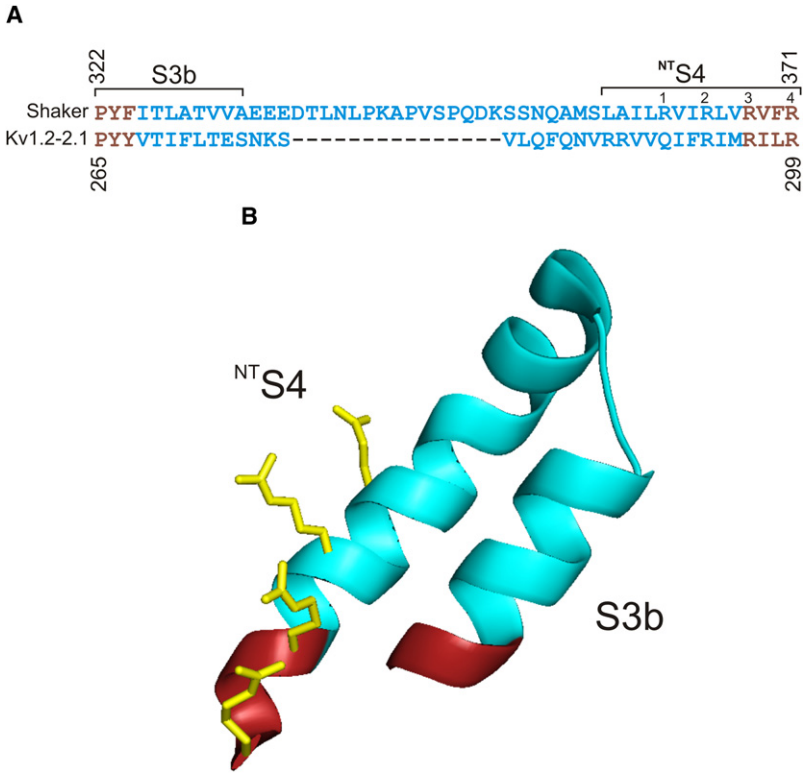

Figure 1. Sequence and Structure of the Voltage-Sensing Paddle

(A) Comparison of the paddle sequences of Shaker (top) and Kv1.2-2.1 (chimeric) channels (bottom) (Long et al., 2007).

(B) Structure of the paddle of the Kv1.2-2.1 channel (PDB: 2R9R). The region of the paddle corresponding to the region of Shaker that we replaced by a glycine triplet in Figure 5C is colored cyan (but recall that Shaker's S3-S4 linker is much longer). The side chains of the residues that correspond to R1–R4 in Shaker are shown as yellow sticks.

end of S6 (^{CT}S6), to move toward and from the central axis of the ion conduction pore and thereby close or open the pore (Liu et al., 1997; Doyle et al., 1998; Jiang et al., 2002; Kitaguchi et al., 2004). Mutagenesis-based functional studies have provided the initial clue as to which regions of the channel protein couple the motion of the voltage sensor to that of the channel gate (Lu et al., 2001, 2002; Tristani-Firouzi et al., 2002). Proper coupling requires two complementary sequences: (1) the S4-S5 linker and (2) ^{CT}S6 plus its immediate extension (^{XT}S6) (Lu et al., 2002). This finding led to the following proposal regarding the crux of the coupling mechanism: as membrane hyperpolarization causes S4 to descend inwardly and rotate, the S4-S5 linker "pushes" onto ^{CT}S6-^{XT}S6 to close the gate. This proposal has received structural support from crystallographic studies on Kv1.2 (Long et al., 2005). If the above coupling concept is valid, the goal of the voltage-sensing process is mainly to move the S4-S5 linker and thereby S6. Effective S4 movement must then both achieve this mechanical goal and transfer enough voltage-sensing residues across the electric field to engender the observed physiological voltage sensitivity.

The aim of the present study is to identify the essential characteristics of the paddle motif that confer voltage-sensing capability upon the Shaker channel.

RESULTS

Deletion Analysis of S3b and ^{NT}S4 Segments

To examine the basic features of the paddle motif, we performed, in the Shaker Kv channel, a systematic deletion analysis of S3b and ^{NT}S4. Remarkably, the channels remain voltage gated even after deletion of any one of the five residue triplets in the sequence encompassing S3b plus four trailing residues (Figures 2A–2G). Deletion of the first triplet causes a marked rightward shift of the conductance-voltage (G-V) curve, whereas deletion of any of the other four causes modest shifts (Figure 2H). The channels also remain voltage gated after deletion of any of the adjacent four triplets in S4 (Figure 3), each of which harbors one of the main voltage-sensing residues R1–R4 (Aggarwal and MacKinnon, 1996; Seoh et al., 1996). All of these results show that none of these triplets (or helical turns) in S3b and ^{NT}S4 is essential for the paddle to be a functional voltage sensor. They also imply that no high degree of complementarity between S3b and ^{NT}S4 is required for basic voltage sensing. We then systematically deleted pairs of adjoining triplets (i.e., six contiguous residues at a time) in ^{NT}S4 (Figure 4A). These mutants, each missing one or two main voltage-sensing residues (R1, R1 and R2, R2 and R3, or R3 and R4, respectively), still remain voltage gated (Figures 4B–4E), albeit with reduced gain, i.e., a shallower G-V relation (Figure 4F). The midpoint ($V_{1/2}$) of the G-V curve of the mutant missing R3 and R4 is rightward shifted, a result consistent with the structural peculiarity that R3 and R4 are hydrogen bonded to certain negatively charged residues, interactions that help to stabilize the activated state (Long et al., 2007).

When we deleted the two triplets containing R1 and R2 in ^{NT}S4 together with (their structural neighbors) the two C-terminal triplets of S3b (i.e., a total of 12 residues; Figure 5A), the construct remained voltage gated (Figure 5B). Given that the S3-S4 linker in Shaker is not essential (Gonzalez et al., 2001), we substituted in the above construct a single glycine triplet for a further deletion comprising the S3-S4 linker proper plus two additional S3b and four additional ^{NT}S4 residues (that is, in the final construct the glycine triplet bridges a 43 residue gap) (Figure 5A). For visual reference, we render in cyan the sequence within the paddle of the Kv1.2-2.1 (chimera) structure (Long et al., 2007) that corresponds to the sequence we replaced in Shaker with a glycine triplet (Figure 1, but recall that Shaker's S3-S4 linker is much longer than the chimera's). This Shaker mutant with minimal voltage sensor is still well gated by voltage