Tuning the Voltage-Sensor Motion with a Single Residue

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ABSTRACT The Ciona intestinalis voltage-sensitive phosphatase (Ci-VSP) represents the first discovered member of enzymes regulated by a voltage-sensor domain (VSD) related to the VSD found in voltage-gated ion channels. Although the VSD operation in Ci-VSP exhibits original voltage dependence and kinetics compared to ion channels, it has been poorly investigated. Here, we show that the kinetics and voltage dependence of VSD movement in Ci-VSP can be tuned over 2 orders of magnitude and shifted over 120 mV, respectively, by the size of a conserved isoleucine (I126) in the S1 segment, thus indicating the importance of this residue in Ci-VSP activation. Mutations of the conserved Phe in the S2 segment (F161) do not significantly perturb the voltage dependence of the VSD movement, suggesting a unique voltage sensing mechanism in Ci-VSP.

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Voltage-sensitive phosphatases (VSPs) represent a recently identified family of enzymes that are regulated by the membrane potential (1). VSP regulation by the membrane voltage depends on the conformational changes of a S4-based voltage-sensing domain (VSD), related to the VSD found in voltage-gated ion channels. However, the VSD in VSPs exhibits unique biophysical properties that differ from those in ion channels. For example, in Ci-VSP the speed of the VSD movement (assessed by the gating or sensing currents) is around sixfold slower during activation, and its voltage dependence is shifted by almost +100 mV compared to prototypical Kv channels (2,3). To our knowledge, there is no previous report of the role of this residue in Ci-VSP.

I126 REGULATES KINETICS AND STEADY STATE OF THE VOLTAGE SENSOR IN CI-VSP

We generated every genetically encodable I126 mutant and functionally characterized their sensing currents with the cut-open oocyte voltage-clamp technique (8) by using activation and deactivation pulse protocols. All mutants were functional except I126W and I126R. To avoid relaxation of the VSD during the depolarizing prepulse of the deactivation protocols (3), we employed short depolarizing prepulses, albeit long enough to develop the sensing charge. Typical recordings are shown for mutants I126A and I126F (Fig. 1, C and D).

The charge-versus-voltage (Q-V) curve was determined for each functional mutant using a standard activation pulse protocol. Sensing-current decays produced during activation and deactivation pulse protocols were fitted using a mono- or double-exponential function, and a weighted-average time-constant (τ) was determined as a function of the voltage (τ-V curve).

We then determined the midpoint of the Q-V curves (V\text{1/2}) and the slowest τ values during activation (Ta) and deactivation (Td). Typical sensing-current analyses are depicted for the wild-type Ci-VSP and the I126A and I126F mutants (Fig. 1, E–G). These two mutants exhibit the most extreme phenotypes: I126A produced the slowest kinetics (Ta = 134 ± 4 ms; Td = 121 ± 18 ms) and the most right-shifted Q-V (V\text{1/2} = +79 ± 7 mV), whereas the I126F mutant produced the fastest kinetics (Ta = 1.30 ± 0.1 ms; Td = 1.16 ± 0.2 ms) and the most negatively shifted Q-V (V\text{1/2} = −37 ± 5 mV). The sensing-current parameters for the other mutants are summarized in Table S1 in the Supporting Material.

To study the effects of all I126 mutations, we plotted the V\text{1/2}, Ta, and Td values as a function of the residue present at position I126 (Fig. 2A). The plot shows an unambiguous positive correlation between the three sensing parameters: as the Q-V curve is shifted toward negative voltages by the mutation, the corresponding sensing kinetics accelerate. Interestingly, the effect of the I126 mutations on the V\text{1/2} values appear very well negatively correlated with the size of the side chain at position I126 (Fig. 2B)
rather than its hydrophobicity (see Fig. S1). Thus, larger side chains at position 126 favor the gating-charge transport, suggesting that a direct van der Waals-like interaction between I126 and S4 Arg promotes voltage sensing in Ci-VSP. Using a simple two-state model of the activation gating transition, we show that the changes of both the $V_{1/2}$ and $T_a$ values by the I126 mutations can be well reproduced by modifying mainly the voltage-independent energy barrier for the forward transition (see Supporting Material), in accordance with our hypothesis of interaction between I126 and the S4 Arg. It is interesting to note that the fit to the model shows an exponential dependence between $\alpha_0$, the voltage-independent forward rate, and the surface of the residue in position 126 (Fig. S4). It is expected that the probability of guanidinium translocation depends on an effective collision with the side chain in position 126. Thus, van der Waals interactions between the side chain and the guanidinium group would be reflected in $\alpha_0$, which in turn is exponentially dependent on enthalpy. In addition, $\alpha_0$ depends exponentially on the entropic term that reflects...
the multiple conformations for effective collisions. It will be interesting to investigate whether the VSD of ion channels can be tuned by the side chain of the residue homolog to I126.

F161 HAS A MINOR ROLE IN THE KINETICS AND STEADY STATE OF CI-VSP

In the Shaker Kv channel, mutations of a conserved Phe residue in S2 termed Phe gap (homolog to F233 in Kv1.2; see Fig. 1A) produce nonambiguous bisigmoid Q-V curves that isolate a late gating-charge component presumably carried by the fourth S4 Arg (9), thus dramatically shifting the $V_{1/2}$ of the conductance versus voltage curves (10). Yet, mutations of the homolog residue F161 in Ci-VSP produce WT-like Q-V curves (Fig. 2C), suggesting that F161 has little influence on the charge movement in Ci-VSP.

In summary, our results, to our knowledge, shed new light on the unique sensing mechanism in Ci-VSP and suggest that the fundamental principles of voltage sensing by the VSD may exhibit more variability than predicted from studies mainly focused on ion channels.

SUPPORTING MATERIAL

The methods, four figures, and two tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(12)00719-9.

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REFERENCES and FOOTNOTES