The Origin of Subconductance Levels in Voltage-gated K^+ Channels

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Typical single channel recordings in voltage-dependent K^+ channels show two simple conductance states: open or shut. Considering the fact that K^+ channels are assembled with four identical subunits, the classical interpretation has been that the conduction pathway is not formed unless all four subunits are in their active positions. In fact, this agrees with the molecular interpretation of the open probability of n^4 as presented originally by Hodgkin and Huxley (1952), whereby they postulated four n particles that needed to be in the correct position to open the channel. The simple two-conductance level of K^+ channels was experimentally challenged by Chapman et al. (1997) when they described intermediate conductance levels in drk1 K^+ channels. In most cases they found that the subconductance levels were extremely short lived, and that could be interpreted as an artifact produced by very fast switching observed under limited bandwidth. However, in a few cases, they observed longer-lived subconductance levels that exceeded the filter risetime, giving them confidence that those intermediate current levels were indeed the result of a channel conformation with a smaller conductance. The subconductance levels were much more frequent at potentials, called the threshold region, where the overall open probability was small. Under these conditions, only one or two of the subunits make a transition to the active state. This led them to propose a reasonable hypothesis that the intermediate levels were the result of channels that had a smaller conductance because the conduction pathway was formed by only one or two of the subunits instead of the four required for full conduction.

In this issue, Chapman and Van Dongen (2005) have studied the subconductance of drk1 with a new, ingenious approach that clears any doubt about their existence. These authors have taken advantage of the properties of two different constructs, based on the wild-type drk1 channel, to record the subconductance levels completely separated from the full open channel. One construct opens at normal voltages and has a large single channel conductance (the low threshold channel, LT), while the other construct opens at very large positive potentials and has the normal drk1 single channel conductance (high threshold channel, HT). These two constructs were first fully characterized in their macroscopic and single channel properties and then they built a tandem dimer construct that combined the two subunits into a single polypeptide. The resulting channel, which is made up of stoichiometrically equal number of low threshold and high threshold subunits, was functional when expressed in *Xenopus* oocytes but had a peculiar conductance versus voltage characteristics. For small depolarizations, they found that the channels opened mostly with two subconductance levels, as expected from the conductance of the low threshold channel. On the other hand, for larger depolarizations, they found that single channel openings showed mostly the conductance of the high threshold channel. In a specific example, they showed that a depolarization to 0 mV exhibited openings with three subconductance levels with clear predominance of the two smaller levels and occasional openings to a higher level. This is exactly what is expected from a channel assembled by two LT and two HT subunits that will conduct ions even when the high threshold subunits are not activated. Thus, the lowest level represents the conduction through the pore formed by only one LT subunit, the second level by two, and the highest level by two LT and the HT subunits. In another example, the authors showed that for a pulse to +40 mV, most of the openings had the typical binary open–closed characteristics as expected at potentials where both the LT and the HT subunits would be fully activated. The fact that they obtained consistent channel openings at small depolarizations that did have the conductance of half of the dimer discards the possibility that intermediate subconductance levels observed in their original work (Chapman et al., 1997) may have been an artifact originated from flicker of full conductance levels.

The predictions of how this dimer would operate came from the previous work of Chapman et al. (1997)

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Abbreviations used in this paper: HT, high threshold; LT, low threshold.

where a kinetic model was presented in which each subunit evolved from a closed state to an intermediate state to an active state, generating a total of 15 distinct kinetic states. This model is very similar to the activation model of *Shaker* presented by Zagotta et al. (1994), except that the active state in Chapman et al. (1997) could conduct ions with partial single channel conductance, generating four conducting states, all with different single channel conductance, while in the Zagotta et al. (1994) model only one state was conducting and corresponded to the case of all four subunits in the active state.

For a given depolarization, the first transition (presumably the movement of the voltage sensor) of the LT subunit will be fast while it will be slow for the HT subunit. It is then expected that at intermediate depolarizations, many channels will have one or two of the LT subunits in the active state, thus producing two subconductance states, while at higher depolarizations, channels will have all four subunits in the active state and they will exhibit mostly a single open state. The model predicts four sublevels but only three were observed: the two small ones and the fully conducting level. The authors pose three possible explanations: the sublevel three is visited rarely, its lifetime is too brief, or its amplitude is not too different from the fully open state. The other important prediction from this model is on the first latency of openings. In particular, the latency to first opening of subconductance level one should be fast, while for subconductance level two should be slower and sigmoidal, and for the fully open level should be even slower and significantly more sigmoidal. The authors showed that these predictions were fulfilled by the measurements of first latencies in the dimer construct. Furthermore, the time course of the open probability (P_o) of the subconductance states rose to a maximum and then declined while the P_o of the fully conducting channel was delayed and reached a plateau. These results nicely confirm that the subconductance states were transient states that get populated while the sensors are recruited to the active state during the depolarizing pulse.

The dimer data of Chapman and VanDongen (2005) were quantitatively analyzed by fitting models to the time courses of the open probability, the conditional open probabilities, and the first latencies for all the sublevels and the fully open state for 0 and +40 mV. The 15 state model was simplified to a reduced model of a total of five closed states, three subconductance states, and one open state, and the rate constants were fitted to different kinetic relations between the states. The fits revealed that the best model requires that the sublevels are partially activated channels where one or two sensors have activated and that the opening and closing rate constants are dependent on the number of activated subunits. A further improvement in the fit occurred when the activation and opening are allosterically coupled. A strictly sequential model did not fit the data. Since suppression of intermediates is a feature of allosteric models, this would also explain why only three and not four conductance states were seen.

The confirmation of subconductance levels produced by partial activation of the channel opens several interesting conjectures on the operation of the gates during voltage activation. Schoppa and Sigworth (1998) made a detailed study of the activation kinetics of the *Shaker* K channel and concluded that a better model would have each subunit undergoing not two but three transitions where the last one would put the subunit in the active state that would only make the channel conductive when all four subunits were in that state. In light of the sublevels discussed above, there would be several sublevels that have not been observed because their lifetimes would be too brief to be detected. It is not clear that the last transition of each subunit would be equivalent to the transition that makes the subunit conductive in the Chapman and VanDongen model (2005), but clearly that last transition is not correlated with the main charge movement that originates the bulk of the gating current. One possibility is that the last transition is related to the extra charge movement that was described with the ILT mutant by Ledwell and Aldrich (1999), although the total charge between 1 and 2 e associated with that step would only correspond to ~0.4 e per subunit. An alternative view of the sublevels was provided by Zheng and Sigworth (1997, 1998), where they proposed a model in which each state associated with one subunit in its active state while the others are in the intermediate nonconducting state but required one more transition to become a sublevel. The results with heteromultimeric channels (Chapman and VanDongen, 2005) cannot be completely explained by this model. On the other hand, the simpler Chapman and VanDongen model, which is similar to the Zagotta et al. model, is not able to explain details of gating currents and activation at extreme potentials (Schoppa and Sigworth, 1998). In the ILT mutant (Ledwell and Aldrich, 1999), the state with voltage sensor in the activated position but with the pore segments in the closed position was stabilized, thus separating the actual open state from the activated voltage sensor. In the results of Chapman and VanDongen, the stabilized transition states are those states that have one or two of the pore segments in the activated position. Therefore, a combination of the ILT mutant and the separation of the threshold region using the ILT mutant in a dimer could be useful to dissect the coupling pathways involved in the last transitions that lead to channel opening. The distinction between alternative models will not be possible until a global fit is done with constructs similar to the one used by Chapman and VanDongen (2005) in a large range of potentials.

Zheng and Sigworth (1997, 1998) described intermediate levels of conductance in *Shaker* mutants and found that these sublevels had different selectivity than the fully open state. This is an extremely important observation because it suggests that the formation of the conductive pore by only one or two subunits creates a conduction path with different selectivity than the pore formed by the four subunits. Some of these observations were done before the structure of KcsA (Doyle et al., 1998) was known, and all were done before the structure of the open pore of MthK (Jiang et al., 2002) and KvAP (Jiang et al., 2003) were published. If we consider the simple view that the K^+ selectivity is completely determined by the pore structure and that conduction occurs when the inner segment (S6) is bent into the open conformation, then the intermediate states would originate when one, two, or three of the S6 segments are bent open. The fully open state would occur when all four are bent into the open conformation. The structure of the conducting pore is not very different between the closed (KcsA) and the open conformation (KvAP). Then, the activation of one, two, or three of the subunits is not expected to affect the selectivity filter and consequently would not explain the change in selectivity observed by Zheng and Sigworth (1997) for the sublevels in *Shaker*. However, we must recognize that the strict separation of the gate in the S6 segment and the selectivity filter is not quite correct because we know that the activation of the channel brings subsequent changes in the pore region that leads to phenomena such as pore- or C-type inactivation. It is, therefore, not unreasonable to think that when the voltage sensor couples its movement to the S6 gate, it brings about other changes in the pore structure that are reflected in the subtle changes in selectivity observed by Zheng and Sigworth (1997). In fact, Perozo et al. (1999), using EPR probes in KcsA, found that such coupling exists.

Regardless of the detailed coupling between gating and selectivity, the simplest assumption is that the channel can conduct ions when only one or two of the voltage sensors become activated. The active conformation of the voltage sensor couples through the S4–S5 linker to the S5 segment, allowing the S6 to bend either in the glycine or the PVP motif (del Camino et al., 2000), thus unblocking the bundle crossing, allowing ion conduction. The results of Chapman and VanDongen (2005) strongly suggest that the opening of only one or more of the S6 gates generate subconductance levels but also suggest new experimental tests. For example, some internal blockers, such as TEA, are known to enter the channel from the internal mouth when the channel opens (Armstrong, 1971). It is then possible to ask whether TEA will enter when only one or two of the subunits are in the open position by observing its blocking effect in the Chapman and VanDongen dimer at potentials where the fully open state is rarely populated. These sizing experiments will be especially instructive if done at the single channel level. By repeating this type of experiments with blockers of different sizes it would be possible to estimate the aperture size as a function of the number of subunits activated. The experiment could be performed with a channel with N-inactivation intact and then test how the inactivating particle interacts with the channel in the intermediate subconductance levels. The inactivating particle is supposed to penetrate into the channel cavity (Zhou et al., 2001) and thus block the channel. By using a dimer construct similar to the channel of Chapman and VanDongen, it would be possible to test whether the inactivating particle can indeed interact with states of the channels that were considered closed because their short lifetimes made them hidden. The subconductance levels may be blocked by the inactivating particle even without penetrating into the channel mouth, indicating that they interact directly with the pore. On the other hand, if the aperture in the subconductance states are too small for the inactivating particle to penetrate, it may not block the conduction of the sublevel but its interaction may decrease the latency to block when the fourth subunit opens.

The dimer construct of Chapman and VanDongen offers an excellent opportunity to carry out a detailed study of the selectivity of the channel while in a subconductance level because there is a relatively large range of potentials where only sublevels are detected with long single channel lifetimes. That study should shed some light on the coupling of the gate and the selectivity of the channel.

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