

Extracellular Blockade of Potassium Channels by TEA⁺: The Tip of the Iceberg?

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Extracellular blockade of potassium channels by TEA⁺ has long been an important tool in electrophysiology (Armstrong, 1969). It is known that the binding affinity of TEA⁺ for the external side is directly affected by the amino acid side chain at position 449 (Shaker) (Heginbotham and MacKinnon, 1992). If an aromatic side chain is present (Tyr or Phe), TEA⁺ binding is strong, whereas there is a loss of affinity for almost any other side chain (Trp, Val, Thr, etc.). This has led to the suggestion that some amount of cation- π electron interaction was involved in the stabilization of the TEA⁺ at the external site. In classical terms, this interaction corresponds roughly to the favorable attraction between a positive charge and the quadrupolar moment of the aromatic ring. In broad agreement with the experimental data on external TEA⁺ blockade, the crystallographic structure of the KcsA channel (Doyle et al., 1998) subsequently confirmed that the residue corresponding to Shaker position 449 (Tyr82 in KcsA) is near the extracellular entrance to the selectivity filter.

From this point on, however, the situation has become increasingly puzzling. It turned out that the x-ray structure was not really consistent with the concept of cation- π electron interactions because the side chains of Tyr82 are not properly positioned. The aromatic side chains are, in fact, too far from the pore entrance to contact a TEA⁺ bound at the channel mouth (Crouzy et al., 2001). Moreover, in the KcsA structure, the side chain is “edge on” while an “en face” orientation would be needed to give rise to a favorable cation- π electron (charge-quadrupole) interaction. Electrophysiological studies on KcsA in planar membrane do show that TEA⁺ is an external channel blocker and that blocker affinity is higher for the wild-type channel with Tyr82 than for the mutant Y82T (LeMasurier et al., 2001), but the wild-type channel’s TEA⁺ affinity is not as high as in *Shaker* channels. Computational studies considered the situation and found that, indeed, TEA⁺ does bind reasonably well to the KcsA structure despite the edge-on orientation of Tyr82 (Crouzy et al., 2001; Luzhkov and Åqvist, 2001; Guidoni and Carloni, 2002), and that it

binds less well to the Y82T mutant. Finally, the binding of an arsonium analogue of TEA⁺ to the KcsA channel, with the Tyr82 side chain in the edge-on orientation, was confirmed directly using x-ray crystallography (Lenaeus et al., 2005). At this point, one might have concluded that the concept of cation- π interaction did not apply to extracellular blockade of K⁺ channels by TEA⁺, but new results have given a new twist to this ongoing saga.

In the study by Ahern et al. in this issue of the Journal (p. 649), the authors use the nonsense suppression methodology for unnatural amino acid incorporation to progressively substitute the H’s in Phe449 by F’s in order to observe the effect on the affinity of TEA⁺ for external blockade. The elegant technique allows one to dissect the microscopic interactions controlling TEA⁺ binding using a much finer scalpel than could be afforded, for example, by traditional site-directed amino acid substitutions. Using this approach it was found that the degree of fluorination of Phe449 is strongly correlated with the observed loss of TEA⁺ affinity. The experimental work is then complemented by a quantitative analysis based on ab initio quantum chemistry computations, which show that this qualitative trend cannot be reproduced with the edge-on orientation of Phe449. The trend can, however, be reproduced assuming an en-face orientation.

This study illustrates how molecular modeling increasingly is becoming an essential aspect of biophysics, blurring the frontier between experiment and theory. Here, the ab initio computations play a key role in the interpretation of the data and in drawing the final conclusions. More computations will undoubtedly be needed to assess the various effects that arise from changes in hydration and protein flexibility on TEA binding, though the present analysis is an important step and the conclusion is compelling.

In final analysis, the results from Ahern et al. present us with the possibility that the functional conformation of *Shaker* must be compatible with an en-face orientation of the Phe449 residues. One might be inclined to posit that the structure of the bacterial channel KcsA differs slightly from that of *Shaker*, but the x-ray structure of the close homologue Kv1.2 (Long et al., 2005)

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seems to argue against this: although Kv1.2 has a valine at the corresponding position (381), the conformation of the backbone is essentially identical to that of KcsA and would be consistent only with the edge-on orientation. In the KcsA structure (Doyle et al., 1998), the side chain of Tyr82 is tightly constrained by the neighboring residues (e.g., the C β of Asp80 from adjacent subunits) and a simple reorientation of the aromatic ring without some rearrangement of the backbone seems to be unlikely. The results from Ahern et al., therefore, raise the possibility that the conducting state (states?) of K⁺ channels might display sufficient conformational flexibility to allow the en-face orientation of an aromatic residue at the external mouth of the selectivity filter. Interestingly, the en-face orientation of Tyr82 is observed in one of the crystal structures of the E71A mutant of KcsA, the so-called “flipped” conformation, which also has Asp80 displaced significantly (Cordero-Morales et al., 2006). While proton activation is rapidly followed by inactivation in wild-type KcsA, this process is suppressed in the E71A mutant. This suggests that the functional selectivity filter can undergo much larger excursions than previously thought and that significantly different conformational states may be accessible when the channel conducts.

Resolving the intriguing TEA⁺ puzzle is important because these results could very well be the tip of a large iceberg of surprises and misconceptions about the structure of the conducting pore, the range of its conformational flexibility, and its accessible conformational states. More efforts will be needed to address this fundamental issue.

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