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Modelling the pH-dependent Properties of Kv1 Potassium Channels

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It is known that the pH dependence of conductance for the rat potassium channel Kv1.4 is susbstantially reduced upon mutation of either H508 or K532. These residues lie in the extracellular mouth of the channel pore. We have used continuum electrostatics to investigate their interactions with K^+ sites in the pore. The predicted scale of interactions between H508/K532 and potassium sites is sufficient to significantly alter potassium occupancy and thus channel function. We interpret the effect of K532 mutation as indicating that the pH-dependent effect requires not only an ionisable group with a suitable pK_a value (i.e. histidine), but also that other charged groups set the potential profile at a threshold level. This hypothesis is examined in the context of pH dependence for other members of the Kv1 family, and may represent a general tool with which to study potassium channels.

Keywords: potassium channels; pH dependence; molecular modelling; protein electrostatics; Kv1.4

Our understanding of potassium channel function (selectivity, conductance, gating) has been greatly aided by atomic resolution structural analysis,[1–3] and by molecular modelling based on these structures.[4–8] The selectivity filter provides carbonyl groups to interact with potassium ions, substituting for ion–water interactions in bulk solution and providing distinction of ions through solvation geometry.[9] Molecular dynamics and continuum electrostatics calculations model the differential solvation between water and channel, concurring with the multiple ion occupancy models for conductance[10,11] suggested by experimental work that pre-dated structural analysis.[12,13] Superposed on what appears to be a highly conserved filter structure for potassium selective channels,[14] and presumably a common conductance mechanism in the selectivity filter, are a wealth of variations in channel properties, such as gating and pH dependence.[15] Some of these variations map to additional domains, such as the voltage sensing domain,[16] whilst others are mediated by alteration of amino acids within the pore domain. Examples of the latter type are the subject of the current study. It is known that charged residues play key roles in the conduction properties of potassium channels, shown for example by studies of the aspartate in the GYGD sequence at the extracellular mouth of Kv channels,[17] and of negative charges in the intracellular mouth.[18]

Mutational analysis has revealed that histidine residues at least partially mediate pH dependence for several members of Kv1 voltage-gated K^+ channels. These residues are located in the extracellular mouth, and may couple to K^+ concentration dependence of conduction and C-type inactivation, as well as pH dependence.[19,20] Specifically, channels within the Kv1.4 (H508 of rat and ferret Kv1.4)[21,22] and Kv1.5 (H463 of human)[23,24] subfamilies show these properties, where pH dependence can be altered by mutation of the histidine residues. These systems also possess a lysine (K532, Kv1.4) or arginine (R487, Kv1.5) residue at equivalent locations in the extracellular mouth, mutation of which to a non-charged side-chain also affects pH-

Abbreviations used: PD, pore domain; FDPB, finite difference Poisson–Boltzmann.

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dependent conductance properties.[23–25] In Kv1.4, for example, mutation of K532 to a non-charged residue leads to a similar loss of pH dependence as does mutation of H508.[26] On the basis of such measurements, and the coupling of pH and K^+ concentration in regulation of conductance, it has been suggested that positive charges in the extracellular mouth contribute to regulation of potassium ion access to the selectivity filter, and to C-type inactivation.[26,27] The Kv1.3 subfamily also exhibits a histidine-related pH dependence (H399 of human Kv1.3).[28]

Electrostatic calculations are commonly used to study structure and function in potassium channels,[29] often with Poisson–Boltzmann continuum modelling.[30] Static calculation of interactions between protein charges and a single potassium ion moving along the pore axis can be combined with the energetics of multiple ion occupancies,[4] and simulation of conduction with molecular dynamics or a continuum diffusion scheme.[31] This current study looks at the predicted interactions between charge sites implicated in Kv1 family pH dependence, and the channel pore. Our computa-

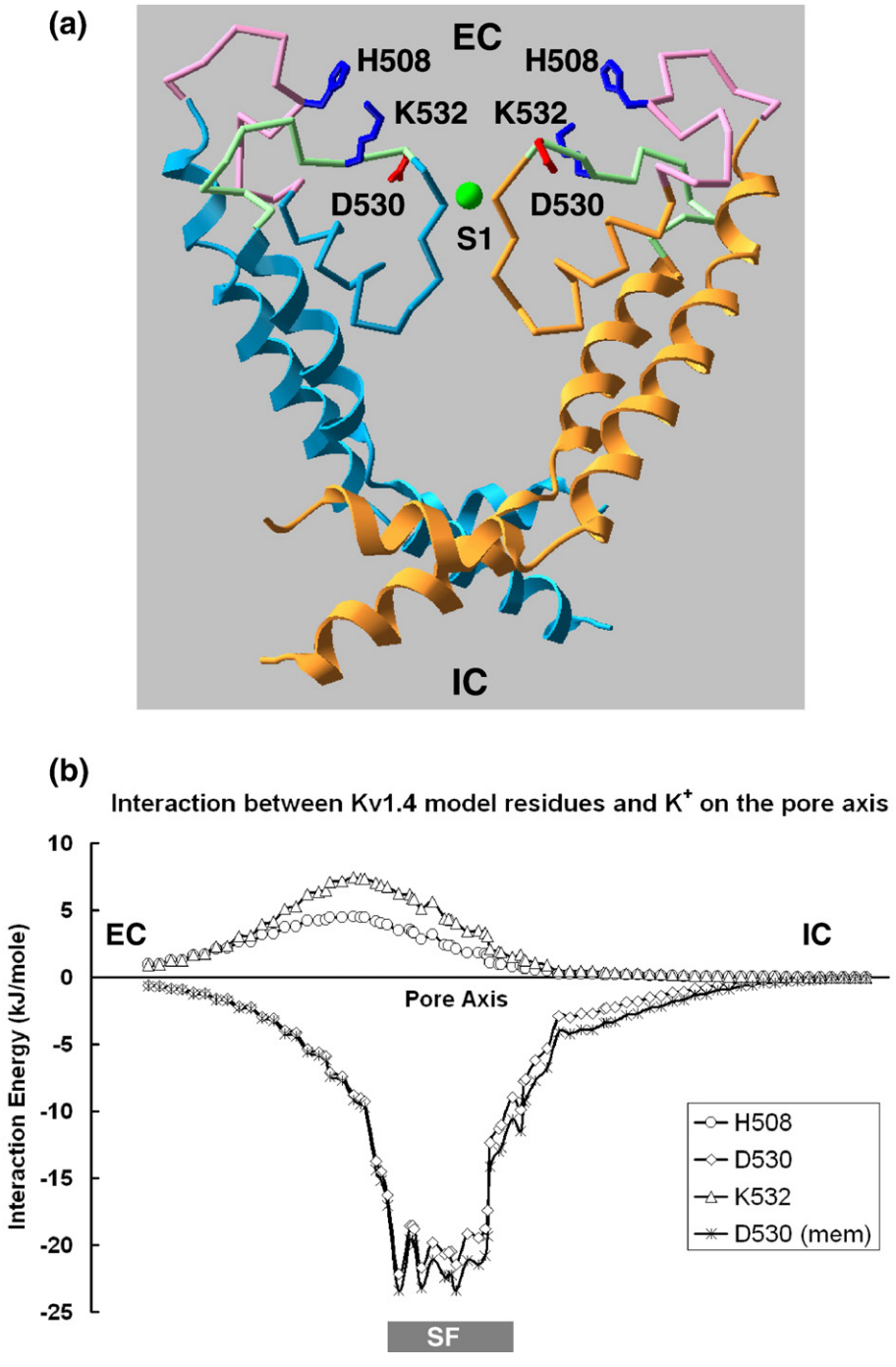

Figure 1 (*legend on next page*)