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ORIGINAL PAPER

Multi-ion versus single-ion conduction mechanisms can yield current rectification in biological ion channels

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Abstract There is clear evidence that the net magnitude of negative charge at the intracellular end of inwardly rectifying potassium channels helps to generate an asymmetry in the magnitude of the current that will pass in each direction. However, a complete understanding of the physical mechanism that links these charges to current rectification has yet to be obtained. Using Brownian dynamics, we compare the conduction mechanism and binding sites in rectifying and non-rectifying channel models. We find that in our models, rectification is a consequence of asymmetry in the hydrophobicity and charge of the pore lining. As a consequence, inward conduction can occur by a multi-ion conduction mechanism. However, outward conduction is restricted, since there are fewer ions at the intracellular entrance and outwardly moving ions must cross the pore on their own. We pose the question as to whether the same mechanism could be at play in inwardly rectifying potassium channels.

Keywords Kir channels · Transmembrane domain · Binding sites · Brownian dynamics · Rectification mechanism

1 Introduction

A number of ion channels are able to conduct ions more rapidly in one direction than another, a property known as current rectification. The most prominent of these are the inwardly rectifying potassium (Kir) channels, which selectively allow potassium ions to move more freely into rather than out of the cell. Their main role is to maintain membrane resting potential, and regulate the action potential in electrically excitable cells [1]. Rectification in these channels is related to either physical or electrostatic blockage of

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outward currents by divalent cations or polyamines [1–9] and is closely linked to the presence of specific charges at the intracellular end of the pore [10–14]. A number of studies have shown that pronounced rectification arises in the presence of divalent cations or polyamines [15–19] and it has been demonstrated that these can be bound by the negatively charged intracellular pore [11, 12]. Although some rectification has been reported in the absence of these compounds [15], Guo and Lu [20] suggest that this is likely to be due to impurities in the recording solutions. Rectification is also seen if the negative charges in the intracellular pore are removed by mutation [10, 12–14]. However, the physical reason why these situations yield current rectification in these channels, and the conditions that can create rectification in a more general sense, remain to be clearly elucidated.

Brownian dynamics (BD) simulations of models of the transmembrane domain of Kir channels have demonstrated that inward rectification can occur in the models in the absence of divalent cations [21, 22]. Despite the absence of an energy barrier, ions were shown to move more easily inwardly rather than outwardly through the truncated Kir channels. Mutation of the hydrophobic residues that guard the intracellular gate of all Kir channels to acidic residues conferred a linear current–voltage relationship, as shown in Fig. 1b. This suggested that the lack of acidic residues at the intracellular gate is important in contributing to the attenuation of outward currents [21, 22]. Unlike Kir channels, KcsA channels have a glutamate residue (E118) at the intracellular gate. It has been demonstrated in BD simulations that KcsA can be made to become inwardly rectifying by removing the charge on the glutamate residue at the intracellular gate [23], as illustrated in Fig. 1a. Therefore, a plausible explanation for inward rectification is that an ion attempting to enter the intracellular entrance of the pore encounters an insurmountable energy barrier, whereas no barrier is presented to an ion leaving the pore to the intracellular space. However, it has been shown previously that no such energy barrier is encountered by a potassium ion moving across the intracellular gate of a rectifying channel [8, 21, 22].

Although our BD models represent simplifications of real Kir channels, especially given the lack of a cytoplasmic domain, they do present a simple situation in which current rectification is seen. Furthermore, as we can follow the trajectories of individual ions in these studies, it is possible to determine the exact reason why currents are larger in one direction than another. In this paper, we utilize data from previous BD simulations [21–23] to more thoroughly examine the regions in the conducting pathways of Kir3.2, Kir2.1 and Kir1.1b where ions dwell preferentially, and the steps involved in conduction so as to elucidate the mechanisms of rectification within these models. We compare the wild-type channel permeation characteristics to those where the intracellular gates are mutated to acidic residues. We compare these Kir channels to a previously published simplified model of KcsA where KcsA was made to become inwardly rectifying by removing the charge on the glutamate residues at the intracellular gate [23]. Although we use these simplified models, lacking the cytoplasmic domain, we hope that explaining how rectification occurs in these cases may provide insight into mechanisms that could play a role in complete rectifying channels.

2 Theoretical calculations

The data is generated from previously conducted BD simulations through Kir3.2, Kir1.1b, Kir2.1, and KcsA channels [21–23]. Detailed descriptions of the BD simulation methodology are given in earlier papers [21–24]. Although it is theoretically possible to perform molecular dynamics simulations to obtain conduction data, such simulations would require extensive computational time. BD simulations reduce the complexity of the system, and

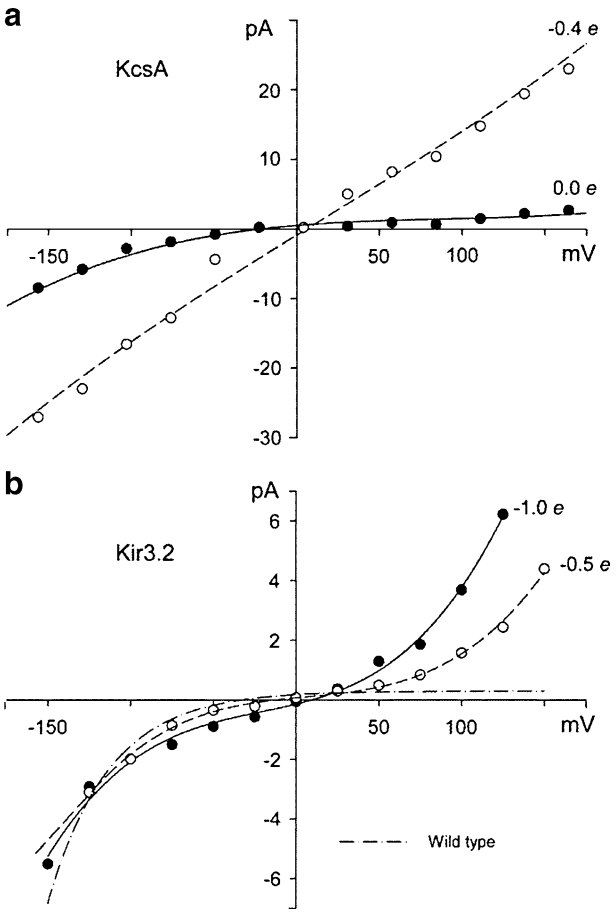

Fig. 1 Current–voltage profiles of the simplified model of (**a**) KcsA and (**b**) Kir3.2 channels [20, 22]. In each case, the charges on the intracellular gate residues (E118 and F192, respectively) are modified as indicated in the figure to demonstrate the effect on rectification. Part (A) reprinted from [27], Copyright 2002, with permission from Elsevier. Part (B) reprinted from [21], Copyright 2013, with permission from Elsevier

therefore allow for much longer timescales to be conducted with reasonable computational time. We note here that the cytoplasmic domain was excluded in all channels investigated in those studies. Kir3.2 coordinates were obtained from the protein database (PDB ID: 3SYA) [25]. Homology models were generated for Kir2.1 and Kir1.1b using the crystal structure coordinates of Kir2.2 (PDB ID: 3JYC) and Kir2.2 (R186A) (PDB ID: 3SPG), respectively [26, 27]. For details on the homology models we refer the reader to [22].

In BD, we make two simplifying assumptions. First, each atom in the protein that forms an ion channel is placed at a fixed position. In reality, proteins are malleable and undergo fluctuations at high frequencies. Second, instead of simulating water molecules explicitly, as in molecular dynamics, we lump together the net effects of incessant collisions between ions and water molecules and represent them as the frictional and random forces. With these simplifications, we are able to calculate the currents flowing through the channel under various conditions, something that is computationally prohibitive with all-atom simulations. To test the validity of the rigidity assumption, Chung and Corry [28] introduced a method

of incorporating the motion of charged atoms lining the selectivity filter of KcsA into BD simulations of ion conduction. As the root mean-square fluctuations of the pore lining carbonyl groups in KcsA were increased, the channel conductance and number and location of resident ions in the channel remained relatively unaffected. This demonstrates that restraining the motion of the polar residues in the selectivity filter has little discernible effects on the channel conductance. The diffusion coefficients of an ion in different segments of a narrow conduit can be estimated from the momentum autocorrelation function measured using molecular dynamics (see [29]). In the gramicidin channel, for example, the diffusion coefficient of an ion sodium ion is about a half that observed in the bulk. In the wider segment of the potassium channel, including the inner cavity, the diffusion coefficient of potassium ion is nearly the same as that in bulk solution [30]. The value in the selectivity filter of KcsA is on average $1/2$ of the bulk value. In practice, however, the precise value of the diffusion coefficient used in BD simulations has only a small effect on the measured currents [31].

To construct dwell histograms, each channel is divided into 1 Å thin sections along the z-coordinate (pore axis), and the number of ions in each section is obtained from five 1 μs BD simulations. Kir channels share common structural features and the pores are made up of three distinct zones: the selectivity filter, located close to the extracellular side of the membrane, is connected to a water cavity of 8–10 Å in diameter, which then narrows towards the intracellular side of the membrane forming an intracellular gate. The region between the intracellular gate and the water cavity is surrounded by hydrophobic residues. Figure 2 illustrates the pore radius and main channel regions for the Kir3.2 channel: the intracellular vestibule, the hydrophobic region, the water cavity, the selectivity filter, and the extracellular vestibule. The pore radius and channel regions for Kir2.1 and Kir1.1b are shown in Figure S1. We also use the ion conduction data to determine the number of ions present within critical regions, such as in the region surrounded by hydrophobic residues.

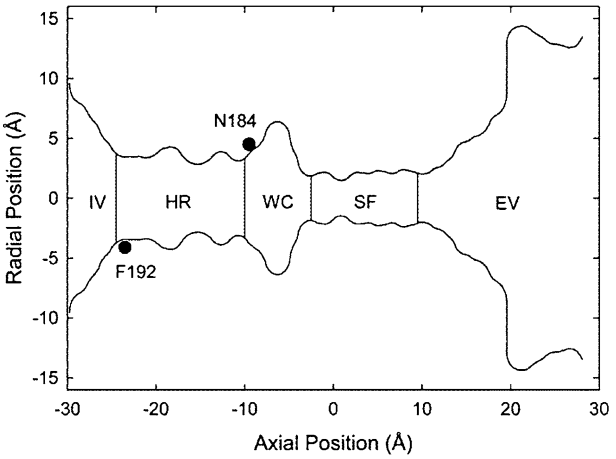

Fig. 2 Pore radius profiles of the open Kir3.2 channel with the pore regions highlighted: intracellular vestibule (IV), hydrophobic region (HR), water cavity (WC), selectivity filter (SF), and extracellular vestibule (EV). *Filled circles* indicate the positions of the hydrophobic gate residues and important residues near the water cavity. The pore profile is generated from the crystal structure of Kir3.2 [29]. Reprinted from [21], Copyright 2013, with permission from Elsevier

3 Results

In this paper, we more thoroughly analyze previous simulations [21–23] in the hope of elucidating the rectification mechanism. A clear pattern emerges when we examine the dwell histograms at both positive and negative voltages for potassium ions through rectifying channels. Figure 3 illustrates the dwell histograms generated from the wild-type Kir3.2 channel at −100 mV and +100 mV. No ions are present at the intracellular gate or in the hydrophobic region extending to the water cavity regardless of the applied potential. Moreover, ion occupancy in this region does not fluctuate during the simulations. The same is observed for the Kir2.1 and Kir1.1b models, as shown in Figure S2. A similar effect is also observed for KscA (Figure S4): when there is no charge on the intracellular gate residues the total number of ions in this hydrophobic-lined region is 0 and rectification occurs. The

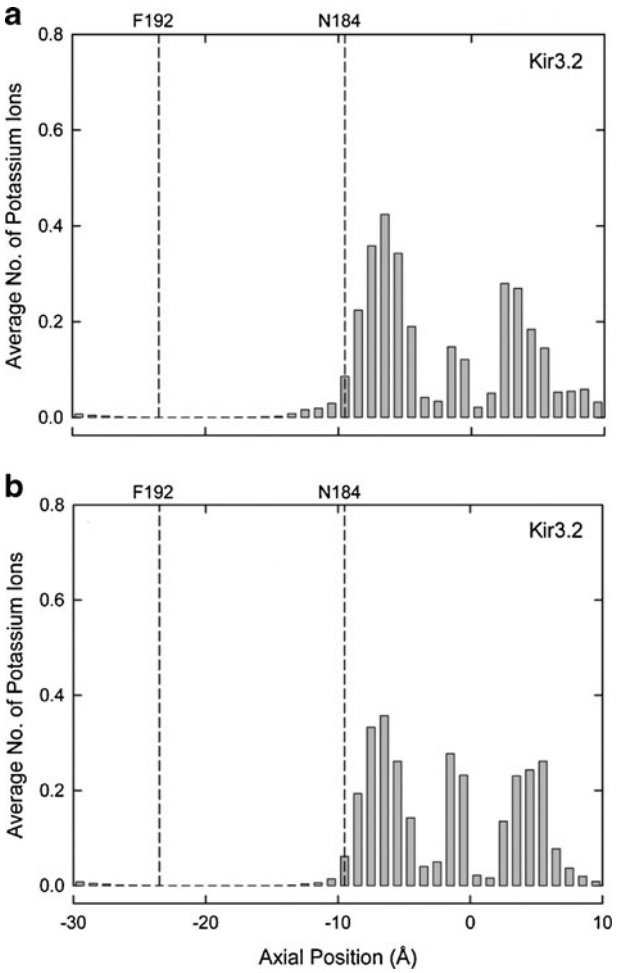

Fig. 3 Dwell histogram for the rectifying Kir3.2 wild-type model channel at −100 mV (**a**) and +100 mV (**b**) plotted between the entrance to the selectivity filter to the intracellular vestibule. The number of ions present in each slice is averaged over five simulations each lasting 1 μs

Kir1.1b channel has the largest region of ion vacancy (Figure S2) as a result of having the narrowest hydrophobic region of the three channels; and the Kir2.1 channel has the smallest region of ion vacancy (Figure S2) due to the presence of the D172 residue. The strong rectification observed in experiments of Kir2.1 is thought to be primary a result of the D172 residue in the water cavity [8, 18]. Moreover, a similar observation is made in experiments of BK channels in which the ring of negatively charged amino acids at the entrance of the intracellular vestibule is shown to increase outward conduction but have little effect on inward conduction, suggesting an electrostatic mechanism [32]. In addition, in the absence of charge, the BK channels were converted into inwardly rectifying channels.

As shown previously [21, 22], inward rectification is eliminated as a result of replacing the hydrophobic residues at the intracellular gate with acidic residues. Figure 4 illustrates the dwell histogram for the mutated Kir3.2 F192E channel at −100 mV and 100 mV. It

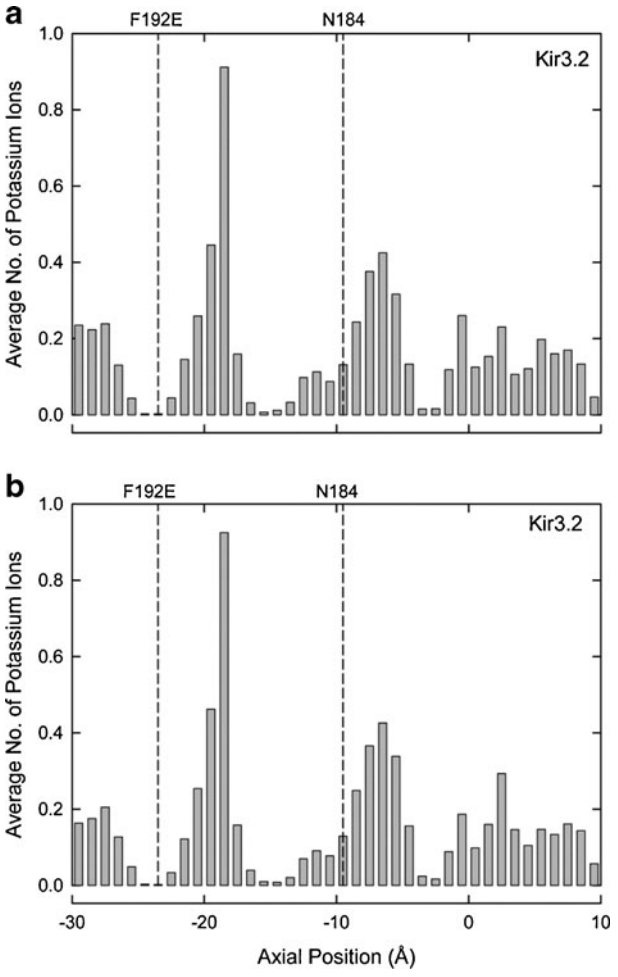

Fig. 4 Dwell histogram for the non-rectifying Kir3.2 F192E model channel (intracellular gate mutated) at −100 mV (**a**) and +100 mV (**b**). The number of ions present in each slice is averaged over five simulations each lasting 1 μs

is clearly evident that ions are now present in the hydrophobic gate region. The same is observed for the Kir2.1 and Kir1.1b mutated models, as shown in Figure S3. Similarly, when the charges on the intracellular glutamate residues of KcsA are -0.4 e, one ion resides in the hydrophobic gate region and the current is non-rectifying.

To understand the reasons why charged residues at the internal end of the pore influence rectification, we plot the number of potassium ions in the intracellular gate as a function of charge and the influence this has on current. The average number of potassium ions in the intracellular and hydrophobic region increases linearly with the charge on the intracellular entrance, as shown in Fig. 5a for Kir3.2. The average number of ions present in the water cavity and selectivity filter remains the same regardless of charge magnitude. However, for

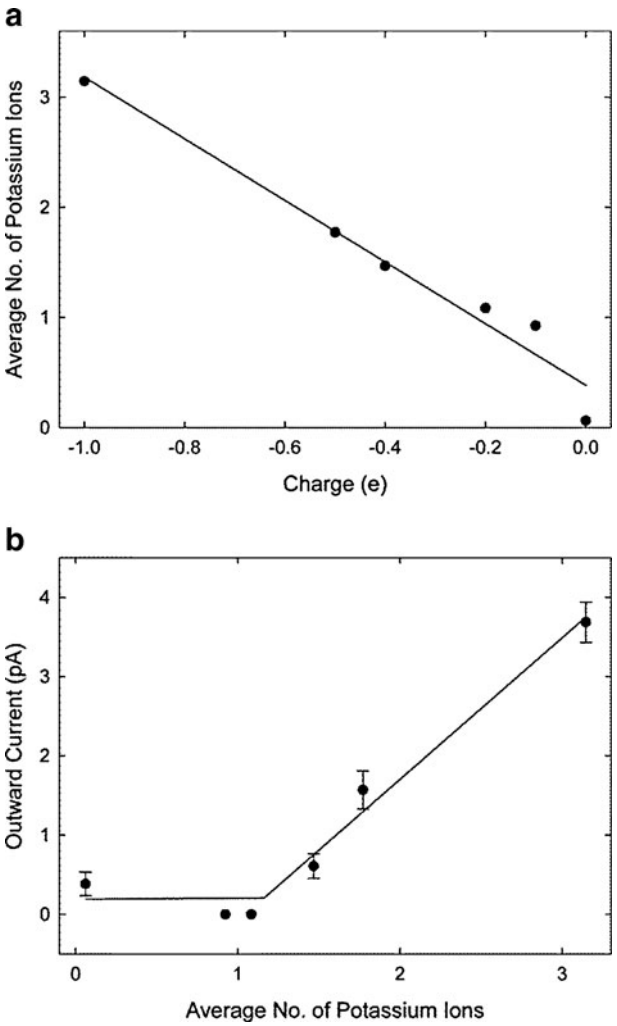

Fig. 5 The effect of the number of potassium ions in the intracellular and hydrophobic regions for the Kir3.2 model. **a** The average number of potassium ions in the intracellular and hydrophobic regions (taken from Fig. 2) as a function of charge at the intracellular entrance. **b** Outward current at an applied potential of 100 mV as a function of the number of potassium ions present in the intracellular and hydrophobic region

significant outward conduction to occur, there must be more than one ion present in the intracellular and hydrophobic region, as shown in Fig. 5b for Kir3.2.

4 Discussion

Since the BD simulation explicitly includes the motion of individual ions, by examining the steps involved in conduction it is possible to determine the physical mechanism that creates rectification in our model channels. In all our models, ions have difficulty crossing the long hydrophobic section of the pore. Our major finding is that inward conduction can involve multiple ions, while outward conduction has to take place in a single ion process, which reduces or eliminates ion current. Since multiple ions are present at the external end of the pore, inward conduction takes place in a multi-ion knock-on process in which coulomb repulsion helps an ion to cross the hydrophobic region (Fig. 6a). Repulsion between the ions effectively couples their thermal motion, increasing the chance of permeation. However, in the rectifying channels, ions rarely enter the intracellular end of the pore, and even if they do they have to cross the hydrophobic region on their own, without the aid of other ions (Fig. 6b). Indeed, for outward conduction to occur, a second ion has to enter the inner end of the pore, something that rarely happens (if at all) in the models displaying rectification.

This explanation of the basis of current rectification in our models also allows us to understand why adding charge at the intracellular end of the pore can remove rectification. Mutating the hydrophobic gate residue in our models to an acidic residue results in multiple ions being present in the intracellular and hydrophobic region (as illustrated in Figs. 4 and 5). Therefore, in non-rectifying channels, both inward and outward conduction

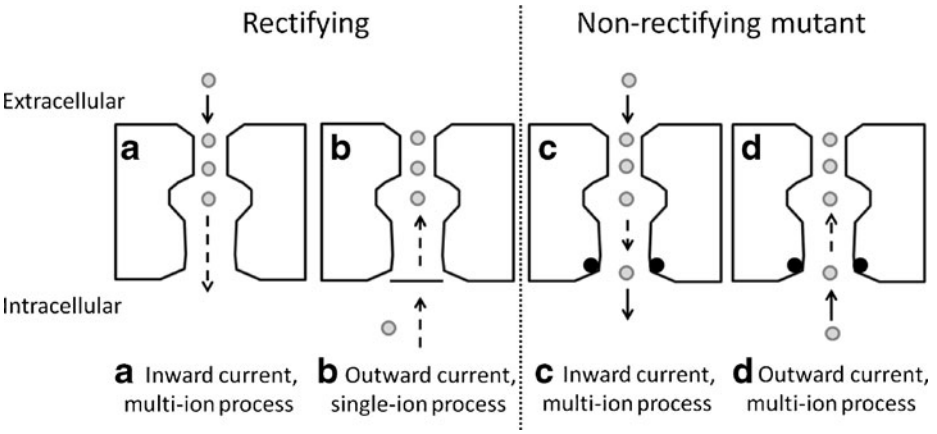

Fig. 6 Schematic illustrating the rate limiting steps in ion conduction through the transmembrane domain of a model channel. **a** Inward conduction through a non-mutant channel: ions (shown as *grey circles*) can cross the hydrophobic region by a multi-ion conduction mechanism. **b** Outward conduction through a non-mutant channel: ions must first find the entrance to the pore, and then cross the hydrophobic region on their own. An imaginary barrier is shown as a *horizontal line*. **c** Inward conduction through a mutant channel, where the intracellular gate residue is replaced with an acidic residue (shown as *black circles*): ions can cross the pore by a multi-ion process. **d** Outward conduction through a mutant channel: ions are attracted to the intracellular entrance and can cross the hydrophobic region by a multi-ion conduction mechanism. Note that the *arrows* indicate the direction of conduction and the *dashed arrows* represent rate limiting steps

are multi-ion processes (Fig. 6c and d), and it is equally probable for an ion to move in either direction. As can be seen in Fig. 5, it is only when multiple ions are present at the intracellular end of the pore that outward conduction becomes possible.

Can these results inform our understanding of complete Kir channels, and in particular could differing numbers of ions contribute to inward and outward conductivity to yield rectification? For methodological reasons, our models completely lack the cytoplasmic domain, but there may be some correspondence to the natural situation if we consider the role of the divalent ions.

In nature, the charged amino acids that line the cytoplasmic domain of Kir channels act to attract potassium ions to the intracellular entrance [8–10, 12, 13, 33, 34] and Kir channels are non-rectifying in the absence of divalent cations or other blockers [20]. We propose that in the absence of divalent cations and other blockers, ions are attracted to the intracellular entrance of the transmembrane domain and outward conduction can occur through full Kir channels via a multi-ion process (similar to that shown in Fig. 6d). Fujiwara and Kubo [12] suggest that the negative charges in the cytoplasmic pore play a role in attracting potassium ions to the cytoplasmic vestibule. Similarly, recent molecular dynamics simulations show that potassium ions accumulate inside the cytoplasmic pore, and that either magnesium ions or spermine can reduce the potassium concentration [9].

When divalent cations or polyamines are present, the acidic residues in the cytoplasmic domain are shielded from the ion-conducting pathway and inward rectification is observed experimentally [4, 5, 8–11]. Experiments also show that outward conduction is restricted when the negative charges on the wall of the cytoplasmic pore are mutated to neutral residues [10, 12–14]. In other words, ions are not attracted to the intracellular gate of the transmembrane domain when divalent cations are present or when the cytoplasmic pore is neutralized. We propose that in the presence of divalent cations, outward conduction through full Kir channels is restricted and becomes a single-ion process, since an ion moving outwardly through the pore must cross the barriers within the transmembrane domain on their own (similar to that shown in Fig. 6b). Of course, it is also possible that divalent cations simply physically block outward conduction through Kir channels.

In summary, we have demonstrated that as a consequence of the hydrophobic-lined intracellular end of the transmembrane domain of our channel models ions are not attracted to the intracellular entrance and, even if they stumble into the pore entrance, they must pass through the intracellular, hydrophobic-lined segment of the pore on their own. In contrast, an ion moving inwardly is able to cross this barrier by utilizing coulomb repulsion between the ions. We propose that there are two factors that contribute to the rectification observed in full Kir channels:

(i) the hydrophobic-lined section of the pore which restricts outward conduction through the transmembrane domain, and
(ii) the presence of divalent cations in the cytoplasmic domain [4, 5, 8–11].

At the moment, we are not able to use BD to simulate the entire channel (i.e., both the transmembrane and cytoplasmic domains), and our proposal remains an untested hypothesis. We hope that this can be tested in the future by either conducting simulations with the intracellular domain present or by gaining experimental data on channels in which the intracellular domain is removed. However, at present, no functional channels are available with the cytoplasmic domain removed.

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