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The mechanism of inward rectification in Kir channels: A novel kinetic model with non-equilibrium thermodynamics approach

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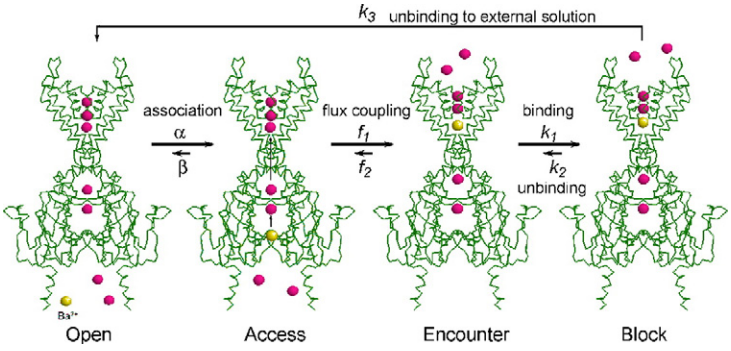
HIGHLIGHTS

- "Driving force"-dependent block in Kir channels is simulated with a kinetic model.
- The "steep voltage dependence" near E_K is due to flux-dependent block.
- The single-file multi-ion cytoplasmic pore is essential for flux coupling.
- The flux-dependent block can be demonstrated by concentration gradient alone.
- Fluctuation theorem in small systems is applied to explain the flux ratio.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanisms of the strong inward rectification in inward rectifier K^+ (Kir) channels are controversial because the drop in electrical potential due to the movement of the blocker and coupling ions is insufficient to explain the steep voltage-dependent block near the equilibrium potential. Here, we study the "driving force"-dependent block in Kir channels with a novel approach incorporating concepts from the non-equilibrium thermodynamics of small systems, and computer kinetic simulations based on the experimental data of internal Ba^{2+} block on Kir2.1 channels. The steep exponential increase in the apparent binding rate near the equilibrium potential is explained, when the encounter frequency is construed as the likelihood of transfer events down or against the electrochemical potential gradient. The exponent of flux ratio, $n_f = 2.62$, implies that the blockage of the internal blocker may be coupled with the outward transport of 2 to 3 K^+ ions. The flux-coupled block in the single-file multi-ion pore can be demonstrated by the concentration gradient alone, as well as when the driving force is the electrochemical potential difference across the membrane.

1. Introduction

The steep inward rectification of the inward rectifier K^+ channels (Kir channels) is intriguing because the block-controlled gating in Kir channels depends on the driving force for conducting K^+ ions. Intracellular cations, such as Mg^{2+} and polyamines, are known to act as physiological blockers that occlude the channel from the cytoplasmic solution

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[1–4] and produce a steep voltage-dependent block at membrane potentials (V_m) near the equilibrium potential (E_K). The rectified current–voltage (I–V_m) curve of the Kir channel shifts along the voltage axis in parallel with shifts in E_K as extracellular or intracellular K^+ concentration changes (as in Fig. 3B). The extent of channel block correlates with the driving force, ($V_m - E_K$), for K^+ conduction. The steep voltage dependence of the block near E_K could not be explained by the Woodhull view [5], which assumed independent movements between the conducting ions and the blocker and attributed the voltage dependence to the fractional electrical field traversed by the positively charged blocker. Previous blockage studies using various internal cation blockers on Kir channels revealed that the measured voltage dependence could be larger than unity, and that blockers with different valences could have similar apparent voltage dependence [6,7].

The steep voltage dependence has been attributed to the multi-ionic nature in early theoretical models [8,9]. A single-file multi-ion long pore is a prerequisite for flux coupling. Movement and occupancy during the binding process of the blockers are coupled with the conducting K^+ ions in the long pore [10–13]. X-ray crystal structural studies of the Kir channels have identified narrow long multi-ion cytoplasmic pores, which extend the single-file region to a 60 Å-long K^+ conduction pathway [14–17] (Fig. 1). The cytoplasmic pore provides electronegative lining and weak binding ion sites for partial dehydrated K^+ ions and can thereby accommodate several K^+ ions and facilitate flux-coupling effects. However, even when the movement of multiple K^+ ions within the cytoplasmic pore is taken into consideration, it seems to be difficult to reconcile the strong voltage-dependent block with the fact that the transmembrane potential drops only a small fraction along the cytoplasmic pore [18,19], because the transmembrane potential gradient is mostly concentrated at the selectivity filter.

Our recent study on the mechanism of the "driving force"-dependent block by intracellular Ba^{2+} on the cloned Kir2.1 channel presented novel findings which may provide proper experimental and theoretical explanations of inward rectification [11]. First, the increase in the apparent affinity of Ba^{2+} blockage near E_K results from the steep increase in the flux-coupled encounter frequency between Ba^{2+} and the high-affinity binding site, located near T141 at the internal entrance of the selectivity filter (TIGYG, residues 142 to 146). Because the direction and the magnitude of the unidirectional K^+ flux changes dramatically at voltages increase above E_K, the encounter frequency and the apparent binding rate experience a steep increase at a voltage range of ($V_m - E_K$) ~+10 to +40 mV. When the driving force ($V_m - E_K$) is greater than +40 mV, the apparent binding rates are limited by the intrinsic activation barrier of the Ba^{2+} binding reaction to the high-affinity binding site because the flux-dependent encounter frequency exceeds the activation rate.

Second, the slow unbinding rates of the internal Ba^{2+} block in the Kir2.1 channel increase monotonically with voltage. These rates depend mildly on voltage across the positive tested voltages, indicating that the unbinding rates of Ba^{2+} are determined primarily by the intrinsic chemical affinity of the high-affinity binding site. The positive voltage dependence implies that Ba^{2+} ions may traverse through the selectivity filter and dissociate outward to the extracellular solution [11]. The unbinding process is little affected by changes in the direction of net K^+ flux.

More interestingly, the driving force-dependence block has been demonstrated even in the presence of a concentration gradient alone by altering the extracellular K^+ concentration and fixing the intracellular K^+ concentration at a membrane voltage of 0 mV (see Fig. 8 in ref. [11]), when a voltage difference across the membrane is absent. Under this condition, the thermodynamic driving force is the chemical potential difference across the membrane. The relationship between the unblocked current and the driving force is also demonstrated by the strong rectification feature. These results suggest that the electrical potential gradient across the flux-coupling region in the channel pore is not essential for the steep change in the apparent blocking affinity near the equilibrium point. Flux-coupled block can occur when K^+ flux is driven either by concentration differences or by voltage differences across the membrane in the single-file multi-ion cytoplasmic pore in Kir channels.

In this paper, we present a novel model, which combines computer kinetic simulation and concepts from non-equilibrium thermodynamics, to elucidate the mechanisms of inward rectification. The simulations are based on experimental data from our recent study of the internal Ba^{2+} block in the Kir2.1 channel [11]. The discrete binding kinetics of the Ba^{2+} block have made it possible to dissect the blocking events. The processes of the internal Ba^{2+} blockage on Kir channels are described by sequential steps including association, "driving force"- or flux-dependent encounter, binding, and dissociation. We apply the fluctuation theorem in a non-equilibrium small system [20] to explain the flux-coupled encounter frequency by interpreting the flux ratio of the unidirectional efflux to the influx as the likelihood for a transfer event to occur down or against the electrochemical potential gradient [21,22]. The theoretical and simulation studies here enforces that the "steep voltage-dependence" in the block on Kir channels actually results from the driving force-dependent block. Under this view, the inward rectification curve, the molecular structural model, and the physiological experimental data are consistent with one another.

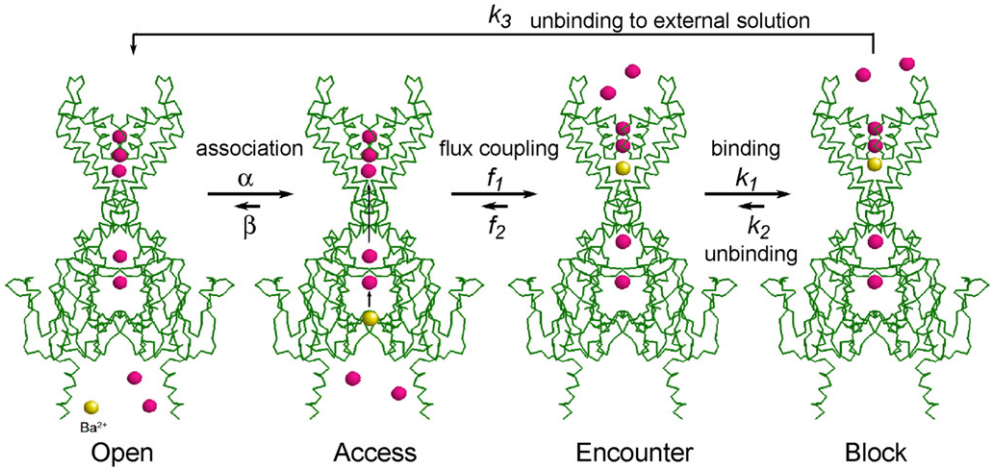

Fig. 1. The schematic model of the intracellular Ba^{2+} block in the Kir channel. The blocking process involves association, flux-coupled encounter, and chemical binding. The Ba^{2+} ion then unbinds and dissociates to the external or internal solution. The backbone channel structure is a Kir2.1 channel model shown with Rasmol from the SWISS-MODEL Repository based on the crystallography template structure of a Kir2.2 channel (PDB: 3sph) [14].