The Mechano-Activated Potassium Channel from Human Erythrocyte (HEMKCA): Effect of Oxidative Stress and its Implication on the Senescence Process

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Human erythrocyte (hRBC) shows a tightly controlled programmed cell death normally at 17.43 ± 17.15 events/burst, gets diminished only by approximately 25%, similarly, the open dwell time presents only a final of 40% under the same conditions. Although there is an effect on the intraburst variables, the dramatic effect on Po seems more likely to be related to the opening mechanism. Here we present a partial kinetic model for this channel, and the oxidative stress is presented as a down-regulation mechanism for the senescence process of the Human Red Blood Cells.

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Exploring the Co-Multimerization of bCNG Channels

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The bacterial cyclic nucleotide gated (bCNG) channel family is a subfamily of the mechanosensitive channel of large conductance (MscL) superfamily. Members of the bCNG channel family have been shown to gate in response to cyclic adenosine monophosphate alone, but are incapable of rescuing hRBC membranes at microcirculation level are exposed to mechanical shear, and somehow these periodical changes lead to the senescence process of these cells. Using Patch Clamp we had characterized a mechano-activated K+ channel (HEMKCA), which shows a sigmoidal dependence of Po on applied pressure, a mean conductance of 17pS, and is Ca2+ modulated (140mM KCl, 10mM NaCl, 1mM CaCl2, pH 7.0). We had proposed a new hypothesis for the process of senescence, with this channel as the molecular entity behind this biological clock. How is this process controlled? A widely held hypothesis proposes that hRBC membranes at microcirculation level are exposed to oxidative stress. Here, we present the effects of the oxidizing agent tert-Butyl hydroperoxide on the HEMKCA activity. The Po of the channel is dramatically reduced by approximately one order of magnitude, this effect being dependent on the concentration of the oxidizing agent (0μM to 25μM). However the burst mode activity, normally at 17.43 ± 17.15 events/burst, gets diminished only by approximately 25%, similarly, the open dwell time presents only a final of 40% under the same conditions. Although there is an effect on the intraburst variables, the dramatic effect on Po seems more likely to be related to the opening mechanism. Here we present a partial kinetic model for this channel, and the oxidative stress is presented as a down-regulation mechanism for the senescence process of the Human Red Blood Cells.

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Structural and Mechanistic Insights into Gating of K2P Channels


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Two-pore domain (K2P) potassium channels are important regulators of cellular electrical excitation and are capable of integrating a wide range of signalling pathways. However, the structure of these channels and their gating mechanism, in particular the role of the helix-bundle-crossing gate, are not well understood. We recently reported that quaternary ammonium (QAM) ions bind with high-affinity to several different K2P channels and can be used as a tool to probe their inner pore structure and to explore their gating mechanisms itself. Our results identify a QAM ion-binding site deep within the pore of TREK-1 and suggest that the pore structure of this channel most closely resembles the open-state structure of KvAP. We also used the slow binding kinetics of large QA ions (e.g. tetraethylammonium) to investigate the pH and pressure-gating mechanisms of TREK-1. Interestingly, these studies suggested that the helix-bundle-crossing gate remains open even when the channel is closed. We also found that mutations close to the selectivity filter and the nature of the permeant ion (K+, Rb+ or T1+) both had a profound influence upon intracellular pH-gating suggesting that the primary gating mechanism in K2P channels resides close to or within the selectivity filter. Here, we further investigate the state of the helix-bundle gate and structural changes in the pore for several gating principles in K2P channels. To this end we introduced cysteins in TM2 and TM4 of TREK-1 and probed the state dependence of their chemical modification. Furthermore we will report about the intimate coupling of gating and permeation in K2P channels by exploring the effect of different ions and voltage protocols.

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The Hollow Domain of the Mechanosensitive Channel MscS is a Sensor of Cytoplasmic Crowding

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Mechanosensitive channel MscS is a ubiquitous osmolyte release valve found in walled cells the resting state of single pores from bacteria to higher plants. In excised patches, applied tension of 5-8 mN/m activates E. coli MscS, followed by adaptive closure and complete inactivation, both of which are strongly promoted by crowding agents in the cytoplasm and by depolarizing voltages. Previous genetic screens1 and models derived from the crystal structure suggest allosteric coupling between the cytoplasmic ‘cage’ domain and the gate through association of the beta domain and the cytoplasmic end of the pore-lining TM3 helix. This association imposes kinks on TM3 that lead to closing or inactivation. Our analysis shows that mutations disrupting the TM3-beta association dramatically decrease closing rates, impede inactivation and make the channel insensitive to crowding agents and voltage. Conversely, mutations predicted to strengthen the TM3-beta interactions make the channel hard to open and inactivate silently under ramp stimulation. Models with strengthened TM3s and separated beta domains removed the kink in the resting state of MscS. In TM3 beta, the cage is prolate in shape, protruding more into the cytoplasm, whereas conversion to an oblate shape and approaching the membrane favors TM3-beta association. We conclude that the shape changes make the cage an intracellular crowding sensor which functions through labile kink-stabilizing TM3-beta interactions. The cage is the first example of a hollow channel domain that can provide feedback on the degree of cytoplasmic condensation (crowding) that disengages the gate and prevents efflux of osmolytes. 1. Koprnowski P, Gajkowski W, Isaccoff EY and Kubalski A. 2011. J. Biol. Chem. 286:877-88.

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A Model of the Open Pole MscL Based on Experimental Data and Restrained Coarse Grained Simulations

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Mechanosensitive channels are ubiquitous membrane proteins where gating is induced by tension in the lipid bilayer. A high resolution structure of the closed pore of the mechanosensitive channel of large conductance (MscL) is known and the protein has been characterized in different functional states using EPR [1] and FRET [2] spectroscopy. However, details of the open-channel structure and the gating mechanism remain unknown. We present an open pore model obtained from restrained simulations in which inter-subunit distances [1,2] and solvent accessibility data [2] were incorporated into a coarse grained model of MscL. 2 ns simulations combining restraints and tension were carried out. The open-channel structures show a pore diameter between 26A and 32A and TM1 and TM2 tilts of ~60° and ~46°. These measurements are in good agreement with data from previously reported open pore models. In contrast, there is little evidence of rotation of the TM helices. In all simulations the N-terminal lies along the membrane surface making it unlikely to serve as a second gate. The C-terminal does not dissociate during gating but shows indication of upward motion that may stabilize the open state. Results further suggest the open pore the opening is associated with an outward motion of the periplasmic loop in combination with the formation of a kink in the periplasmic end of TM1 and tension-induced thinning of the membrane is necessary for these structural changes to occur.