616-Pos

Quantum Calculations: A Test of Accuracy on a Solvated Crown Ether with an Ion, a System Large Enough to Model a Useful Section of A Protein

Alisher M. Kariev, **Michael E. Green**.
City College of the City Univ of NY, New York, NY, USA.

A quantum calculation (DFT) has been carried out on the crown ether (CE) 14-C-4, together with up to 14 methanol molecules, or 27 water molecules, plus one ion. The free energy of complex formation is known experimentally for both Na^+ and K^+ ions, allowing comparison for methanol; neither ion can be complexed from bulk water. We calculate that the ions could be complexed from a more limited water solvent shell. In order to avoid leaving an unrealistic CE-vapor interface, 8 water molecules or 4 methanol molecules were placed on the side of the CE opposite the ion. Thus ''bulk solvation'' was 10 methanol or 19 water molecules. In addition to optimizing the geometry at B3LYP/6-311++G** level, we did frequency calculations to obtain the thermodynamic quantities. The errors average approximately 2 kcal/mole. The methanol complex formation values are, for K^+, −2.40kcal/mole (calculated), −1.80 kcal/mole (experimental); for Na^+, −5.4 kcal/mole(calculated), −2.2 or-3.0 kcal/mole (experimental, from 2 laboratories). For water, there is only the qualitative observation that complexes do not form; however, in the calculation, removing 7 water molecules results in only one shell of solvation. The free energy of complex formation is then negative, so that a complex could form. Since the system has over 100 atoms, it is large enough to model the interaction of a protein with an ion at the surface of the protein (see abstract of Kariev and Green: ''Quantum calculations on the KcsA channel 1/4rdquo;), suggesting that quantum calculation is useful in a case where polarizability and charge transfer, neither present in standard molecular dynamics, are important. (Acknowledgement for calculations: W.R. Wiley supercomputer facility of the EMSL at PNNL).

617-Pos

Quantum Calculations on the KcsA Channel Cavity: N_A^+ and K^+ Solvation, and the Energetics of the Transfer of the Ion to the Selectivity Filter

Alisher M. Kariev, **Michael E. Green**.
City College of the City Univ of NY, New York, NY, USA.

We have calculated the energy of the Na^+ and K^+ ions as solvated in the cavity of the KcsA channel, using the DFT method B3LYP, with basis set 6-31+G**. This method in a comparable calculation on a crown ether system for which the free energy of complex formation is known experimentally turned out in calculations (using frequency calculation for room temperature values) to be accurate to ±2 kcal/mole (but with a slightly larger basis set: see Kariev and Green abstract ''Quantum Calculations: A test of accuracy 1/4''). The transfer free energy of the two ions to the S4 (lowest) position of the selectivity filter can be calculated, and should therefore be approximately this accurate. The geometry of the hydrated ion in the center of the cavity agrees with the experimental X-ray structure (Zhou and MacKinnon, 2004). We are also calculating the barrier to the transfer from the center to the S4 position. We observe that the Na^+ ion can move slightly to the side of the S4 position, which helps in understanding the observation that Na^+ ion can block the channel. The solvation in the S4 position comes from the hydroxyl and carbonyl groups on the threonines that constitute the site, and we have determined the geometry of this structure as well. We will also show a first step toward a calculation of the salt bridges that we postulate are responsible for the open structure of the voltage sensing domain of a voltage sensitive K^+ channel. (Acknowledgement for calculations: W.R. Wiley supercomputer facility of the EMSL at PNNL).

618-Pos

Immunomodulation of Voltage-Dependent K^+ Channels in Macrophages: Molecular and Biophysical Consequences

Miren David[1], Nuria Villalonga[2], Joanna Bielanska[2], Ruben Vicente[2], Nuria Comes[2], Antonio Felipe[2], Carmen Valenzuela[1].
[1]Institute of Biomedical Research CSIC-UAM, Madrid, Spain, [2]Departament de Bioquímica i Biología Molecular. Universidad de Barcelona, Barcelona, Spain.

Voltage-dependent potassium channels play a pivotal role in the modulation of macrophage physiology. Macrophages are professional antigen-presenting cells and produce inflammatory and immunoactive substances that modulate the immune response. Blockage of K_v channels by specific antagonists decreases macrophage cytokine production and inhibits proliferation. Numerous pharmacological agents exert their effects on specific target cells by modifying the activity of their plasma membrane ion channels. Investigation of the mechanisms involved in the regulation of potassium ion conduction is, therefore, essential to the understanding of potassium channel functions in the immune response to infection and inflammation. Here we demonstrate that the biophysical properties of voltage-dependent K^+ currents are modified upon activation or immunosuppression in macrophages. This regulation is in accordance with changes in the molecular characteristics of the heterotetrameric $K_v1.3/K_v1.5$ channels, which generate the main K_v in macrophages. An increase in K^+ current amplitude in LPS-activated macrophages is characterized by a faster C-type inactivation, a greater percentage of cumulative inactivation and a more effective Margatoxin inhibition than control cells. These biophysical parameters are related to an increase in $K_v1.3$ subunits in the $K_v1.3/K_v1.5$ hybrid channel. In contrast, DEX decreased the C-type, the cumulative inactivation and the sensitivity to Margatoxin concomitantly with a decrease in $K_v1.3$ expression. Neither of these treatments apparently altered the expression of $K_v1.5$. Our results demonstrate that the immunomodulation of macrophages triggers molecular and biophysical consequences in $K_v1.3/K_v1.5$ hybrid channels by altering the subunit stoichiometry.

619-Pos

Functional KCNH1 Potassium Channels in Danio Rerio are Essential for Early Development

Rayk Stengel[1], Nirakar Sahoo[1], Christina Ebert[2], Frank Bollig[2], Christoph Englert[2], Stefan H. Heinemann[1], Roland Schönherr[1].
[1]Center for Molecular Biomedicine, University Jena, Jena, Germany, [2]Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena, Germany.

The physiological role of KCNH1 channels has not been clarified in detail yet, but the predominant neuronal expression in mammals indicates a role in electrical signaling. The only described physiological function of KCNH1 so far is the promotion of myoblast fusion. Moreover, KCNH1 channels apparently enhance proliferation of cancer cells, thus exhibiting oncogenic potential. Many genes involved in cancerogenesis play a physiological role during embryonic development. To study KCNH1 channel function in this respect, we surveyed genomic databases of *Danio rerio*, a widely used vertebrate-development model organism, and found two putative *kcnh1* paralogs located on chromosomes 17 (*kcnh1a*) and 22 (*kcnh1b*). The corresponding amino acid sequences show >80% identity to that of human KCNH1. We observed both *kcnh1* genes to be endogenously expressed in different organs of adult fish and at early developmental stages, most prominently in neuronal tissues. We electrophysiologically analyzed the encoded channels by two-electrode voltage-clamp and patch-clamp techniques on *Xenopus* oocytes, showing the gene products to form functional potassium channels. The *Danio rerio* channels exhibited typical characteristics known from other species, such as dependence of activation kinetics on prepulse potential and extracellular Mg^{2+} concentration, as well as current inhibition by intracellular Ca^{2+}/calmodulin. After morpholino-mediated knockdown of the individual *kcnh1* paralogs in zebrafish embryos we observed severe effects on development. The morphants were retarded in growth, showed abnormal body curvature, exhibited complete body malformation or even died within less than 24 hrs after fertilization, depending on the degree of knockdown. The most remarkable phenotypes were alterations of brain structures like head edemas, incomplete brain growth and necrotic degeneration. Given the electrophysiological similarity of *Kcnh1* channels to the human homolog, we suggest that human KCNH1 potassium channels play a similar role during early embryonic development in humans.

Voltage-gated K Channels-Gating I

620-Pos

Microtubule Dependent Mechanisms Regulate the Trafficking Deficient Phenotype of hERG Mutations Linked to Long QT Syndrome

Jennifer L. Smith[1], Christie M. McBride[1], Daniel C. Bartos[1], Craig T. January[2], Brian Delisle[1].
[1]University of Kentucky, Lexington, KY, USA, [2]University of Wisconsin, Madison, WI, USA.

Type 2 Long QT syndrome (LQT2) is caused by mutations in the *human Ether-a-go-go Related Gene (hERG)*, which encodes the voltage-gated K^+ channel α-subunit that underlies the rapidly activating delayed rectifier K^+ current in the heart. Most LQT2 missense mutations decrease hERG trafficking to the plasmalemma. The trafficking-deficient LQT2 (tdLQT2) phenotype is characterized by a loss of hERG glycosylation and current. The purpose of this study was to test the hypothesis that multiple cellular mechanisms underlie the tdLQT2 phenotype. We studied the tdLQT2 mutations G601S- and R752W-hERG in stably expressed HEK293 cells, and found that G601S-hERG selectively colocalized with the Endoplasmic Reticulum (ER) and Golgi apparatus proteins calnexin and 58K. Nocodazole is an antimicrotubule agent that is used to study microtubule dependent trafficking of proteins between the ER and the Golgi apparatus. Nocodazole treatment (20 ∈ 1/4M, 18-24 hours) did not alter the functional expression of G601S-hERG or R752W-hERG, but it did alter the immunostaining and glycolytic processing