View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

As the endosomal NHE display significant similarity to NHE1, we used the NHE1 model as a template and used a unique modeling approach to model these other human transporters. Three NHE9 mutations (S438P, L236S, V176I) could be readily extrapolated to yeast Nhx1 from sequence conservation. We used site-directed mutagenesis to replace the yeast residues with the human equivalent, as well as the disease-associated mutation. Mutants were expressed in *nhx1* deletion background and evaluated for pH, salt and trafficking phenotypes. While S43P and L236S mutations led to loss of function phenotypes in yeast, unexpectedly, V167I had no discernable effect. The latter may reveal isoform specific functions that remain to be identified or may call to question the functional contribution to the disease phenotype. In a parallel approach, these NHE9 mutations were expressed in glial cells for functional evaluation in a mammalian cell model. We observed altered trafficking and surface expression of glutamate transporters, consistent with elevated glutamate levels reported in patient brains.

1358-Pos Board B268

Substrate and Inhibitor Binding to a Glutamate Transporter Homologue

Nicolas Reyes, Olga Boudker.
Weill Cornell Medical College, New York, NY, USA.

Glutamate transporters tightly control extracellular glutamate concentration by pumping the transmitter into neurons and glia. Active transport is achieved by coupling the energy of the transmembrane ionic gradients to the conformational changes of the transporter, which alternates between outward- and inward-facing states with the substrate-binding site accessible from the extra- and intracellular solutions, respectively. In order to concentrate the substrate into the cytoplasm, the transporter's binding site must exhibit a high affinity from the outside and a low affinity from the inside. To better understand this change, we have characterized the binding properties of a prokaryotic homologue of the glutamate transporters, GltPh, by means of isothermal titration calorimetry. We designed double cysteine mutants that, upon cross-linking, lock the transporter in either the outward- or the inward-facing state and measured the thermodynamic binding parameters of the substrate aspartate and a competitive inhibitor TBOA. Surprisingly, the free energies of aspartate binding to the outward- and inward-facing states of the transporter are very similar at 25 °C, although the enthalpic and entropic contributions differ significantly. GltPh affinity for aspartate depends steeply on the concentration of sodium ions (Na), consistent with binding of ~3 Na being thermodynamically coupled to binding of each substrate molecule. In contrast to the substrate, TBOA shows significantly lower affinity for the inward-facing state compared to the outward-facing state. In both states, TBOA exhibits a weaker sodium-dependence with an apparent number of coupled Na near 1. Our results show that in the absence of a sodium gradient, GltPh binds aspartate and Na with the same affinity in the outward- and inward-facing states. Therefore, the higher substrate affinity on the extracellular side of the membrane is determined by the higher Na concentration in the extracellular space compared to the cytoplasm.

1359-Pos Board B269

Formation of a Leaky State and Molecular Mechanism of Water Co-Transport in Secondary Transporters

Jing Li, Emad Tajkhorshid.

Accumulating experimental evidence suggesting water co-transport and even ion leak currents in specific secondary transporters continues to challenge the principles of the prevailing alternating-access mechanism in this class of transporters. In the alternating-access model, transporter could only expose the central binding site to one side of the membrane, and the ions and the substrate are coupled across the membrane through the carrier-mediated style, which is difficult to explain the high water to solute transport stoichiometry in water co-transport and transport-independent leak current in recent experimental observations. However, the precise mechanisms underlying these observations are still in debate and direct structural evidence of how water and ions might be co-transported along with the substrate is still lacking. We have used extended molecular dynamics simulations of a secondary transporter (vSGLT) for which water co-transport has been well documented in order to investigate the molecular mechanism of the phenomenon. The protein has been simulated in the membrane and initiated from the inward-facing crystal structure in various bound states. The simulations capture a leaky state in which a continuous water channel is formed within the lumen of the transporter, resulting in the accessibility of the central substrate-binding site to solutions at both sides of the membrane simultaneously. During the performed 200-ns simulations, hundreds of water molecules completed full permeation events respectively in either direction. Further structural analysis and comparative investigation identify a gating network as the main mechanism controlling the formation of the leak state, which probably decides the distinct water-cotransport permeability in specific secondary transporters. The leak state captured in our simulations provides novel and deeper insight to the mechanism of transport cycle, and reshape the definition of transporter.

1360-Pos Board B270

Water Permeation Through the Sodium-Dependent Galactose Cotransporter VSGLT

Seungho Choe, John M. Rosenberg, Jeff Abramson, Ernest M. Wright, Michael Grabe.

It is well accepted that cotransporters facilitate water movement by two independent mechanisms: osmotic flow through a water channel in the protein and flow driven by ion/substrate cotransport. However, the molecular mechanism of transport-linked water flow is controversial. Some researchers believe that it occurs via cotransport, in which water is pumped along with the transported cargo, while others believe that flow is osmotic in response to an increase in intracellular osmolarity. In this report, we present the results of a 200-ns molecular dynamics simulation of the sodium-dependent galactose cotransporter vSGLT. Our simulation shows that a significant number of water molecules cross the protein through the sugar-binding site in the presence as well as the absence of galactose, and 70-80 water molecules accompany galactose as it moves from the binding site into the intracellular space. During this event, the majority of water molecules in the pathway are unable to diffuse around the galactose, resulting in water in the inner half of the transporter being pushed into the intracellular space and replaced by extracellular water. Thus, our simulation supports the notion that cotransporters act as both passive water channels and active water pumps with the transported substrate acting as a piston to rectify the motion of water.

1361-Pos Board B271

Dynamics of the Outward and Inward Facing States of the Serotonin Transporter

Saher A. Shaikh, Emad Tajkhorshid.

The serotonin transporter (SERT), a member of the neurotransmitter:sodium symporter (NSS) family, is responsible for clearing neuronal synapses of serotonin, and a major drug target in psychopharmacology. Since no structures have been reported for SERT, structural information is deduced from the crystal structure of LeuT, a prokaryotic NSS homologue. SERT is believed to function by alternating between outward-facing (OF) and inward-facing (IF) states, hence a description of these and intermediate states is vital to the understanding of its transport cycle. To date, studies of SERT structure and dynamics have been restricted to models of the OF state, that in which LeuT is crystallized.
We have generated models of substrate/ion-bound as well as free states of SERT, using the OF crystal structure, and our recently published IF models of LeuT. We performed comparative modeling for model generation, docking to position the substrate and extensive optimization of the protein sidechains to generate these models. These SERT models were then simulated in the presence of membrane, water and ions, to characterize the dynamics of the transporter in different states. The simulations reveal differential water permeation behavior among the OF and IF states, and are discussed in view of the known channel-like behavior in SERT. Ion binding patterns hint at a putative binding site for $K+$, which is known to assist in SERT function. We describe major differences in local and global conformational behavior between the OF and IF states, which provide clues to the transition between these states, and the transport cycle. To our knowledge, these studies provide the first description of dynamics of SERT in the IF state, and a comparative view of the dynamics of the OF and IF as well as substrate/ion bound and free states of SERT.

1362-Pos Board B272

Mitochondrial Membrane Potential in Living Neurons Measured by Flim

Elena E. Pohl, Jutta Schueler, Anne Rupprecht.

Uncoupling protein 4 (UCP4) is an inner membrane mitochondrial protein with localisation in neurons and astrocytes (1). In contrast to UCP1 and UCP2 (2-4), no UCP4 - mediated proton transport activity was demonstrated. To gain insight into the protein function we used fluorescence lifetime imaging (FLIM) for the analysis of mitochondrial membrane potential (MMP). The method provides information about the local fluorophore environment. We first evaluated several probes for MMP measurements including rhodamine dyes, JC-1 and TMRM in living cells. MMP changes were then detected by FLIM in neurons after addition of the artificial uncoupler CCCP or respiratory chain inhibitors (rotenone, antimycin A). The putative UCP activator 4-hydroxy-2-nonenal did not significantly alter membrane potential.

Reference List

1. Smorodchenko, A., Rupprecht, A., Sarilova, I., Ninnemann, O., Brauer, A. U., Franke, K., Schumacher, S., Techritz, S., Nitsch, R., Schuelke, M., and Pohl, E. E. (2009) Biochim. Biophys. Acta 1788, 2309-2319.
2. Beck, V., Jaburek, M., Demina, T., Rupprecht, A., Porter, R. K., Jezek, P., and Pohl, E. E. (2007) FASEB J. 21, 1137-1144.
3. Beck, V., Jaburek, M., Breen, E. P., Porter, R. K., Jezek, P., and Pohl, E. E. (2006) Biochim. Biophys. Acta 1757, 474-479.
4. Rupprecht, A., Sokolenko, E. A., Beck, V., Ninnemann, O., Jaburek, M., Trimbuch, T., Klishin, S. S., Jezek, P., Skulachev, V. P., and Pohl, E. E. (2010) Biophys. J. 98, 1503-1511.