Membrane Pumps, Transporters, and Exchangers III

2314-Pos Board B451
Vibrational Studies of Channelrhodopsin-1 from Chlamydomonas Augustae: Protonation Changes during the Early Photocycle
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Channelrhodopsins (ChRs) from green algae function as light-gated ion channels when expressed heterologously in mammalian cells. Considerable interest has focused on understanding the molecular mechanisms of ChRs in order to bioengineer their properties for specific optogenetic applications. Most studies have focused so far on channelrhodopsin-2 from Chlamydomonas reinhardtii (CvChR2). Here, low-temperature and time-resolved rapid scan FTIR-difference spectroscopy, along with kinetic UV-Visible transient absorbance spectroscopy, are applied to study protein conformational changes occurring during the photocycle of the red-shifted channelrhodopsin-1 from Chlamydomonas augustae (CvChR1). Substitutions with isotope-labeled retinols or the retinal analog A2, site-directed mutagenesis, hydrogen-deuterium exchange and H\textsubscript{18}O substitution are used to assign bands to the retinal chromophore, protein, and internal water molecules. The primary phototransition of CvChR1 at 80 kV in involves, in contrast to CvChR2, almost exclusively all-trans to 13-cis isomerization of the retinal chromophore, similar to the primary phototransition of bacteriorhodopsin (BR). A negative amide II band is identified in the retinal ethylenic stretch region of CvChR1 which, along with amide I bands, reflects alterations in protein backbone structure early in the photocycle. A decrease in the hydrogen bond strength of a weakly hydrogen bonded internal water is detected in both CvChR1 and CvChR2, but the bands are much broader in CvChR2 indicating a more heterogeneous environment. Based on the data from mutations involving the residues Glu169 and Asp299 (homologs of the Asp85 and Asp212 Schiff base counterions in BR), as well as Phe139 (Lys132 in the C1C2 chimera located within hydrogen bonding distance of the homologs of Asp85 and Asp212), we propose a model for protonation changes occurring near the Schiff base during the P1 and P2 steps of the photocycle.

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Reconstitution of Potassium-Coupled Substrate Transport in an Archaeal Homologue of Glutamate Transporters
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Excitatory neurotransmitter glutamate is packaged into vesicles in presynaptic neurons and is released into the synaptic cleft via exocytosis upon arrival of the action potential. Following activation of the receptors on postsynaptic neurons, glutamate is rapidly removed from the cleft to prevent excitotoxicity. Excitatory amino acid transporters (EAATs) uptake glutamate into the cytoplasm of glial cells and neurons via a process coupled to symport of three Na\textsuperscript{+} ions and a proton and antiport of one K\textsuperscript{+} ion. Studies on an archaeal homologue GltPh elucidated key mechanistic aspects of Na\textsuperscript{+} symport, including the location of Na\textsuperscript{+} binding sites and the mechanisms of Na\textsuperscript{+}-mediated transporter gating and coupled binding of the substrate. In contrast, the mechanism of coupling to K\textsuperscript{+} antiport remains largely unknown. Previous studies on K\textsuperscript{+} coupling were largely based on ‘loss-of-function’ mutations of EAATs and appeared inconclusive because a surprising number of mutations disrupted coupling. To elucidate K\textsuperscript{+} coupling mechanism, we pursued a ‘gain-of-function’ approach where we aimed to reconstitute coupling in K\textsuperscript{+}-independent GltPh. Toward this end, we engineered ‘humanized’ GltPh variants bearing several modifications to closer resemble EAATs. One of the variants showed substrate uptake in proteoliposomes that was strongly dependent on the presence of internal K\textsuperscript{+}. Using isothermal titration calorimetry, we further show that K\textsuperscript{+} ions inhibit substrate binding to this variant but not to the wild type GltPh. Taken together with our previous structural studies, these data allow us to propose a mechanism of K\textsuperscript{+} coupling in mammalian glutamate transporters.

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A Mutation in TM7 of Excitatory Amino Acid Transporters Disrupts the Substrate-Dependent Gating of the Intrinsic Anion Conductance and Drives the Channel into a Constitutively Open State
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In the mammalian central nervous system, excitatory amino acid transporters (EAATs) are responsible for the clearance of glutamate after synaptic release. This energetically-demanding activity is crucial for precise neuronal communication and for maintaining extracellular glutamate concentrations below neurotoxic levels. In addition to their ability to re-capture glutamate from the extracellular space, EAATs exhibit an uncoupled sodium- and glutamate-gated anion conductance. Here we show that substitution of a conserved positively charged residue (R388, hEAAT1) in transmembrane domain 7 (TM7) with a negatively charged amino acid, dramatically disrupts the gating of the substrate activated anion conductance. When expressed in oocytes, R388D or R388A show no increase in anion currents upon application of saturating concentrations of sodium and glutamate, but exhibit permeation properties similar to wild type carriers and retain the ability to transport substrate. These findings suggest that in the mutant transporters, the associated ion channel preferentially exist in a sodium- and glutamate-independent constitutive open state. In addition, the accessibility of cytoplasmic residues to membrane-permeant modifying reagents supports the idea that this substrate-independent open state correlates with a more inwardly-oriented conformation of the transport domain. Our results are in agreement with recent structural and electrophysiological data that suggest that the permeation pathway opens as the protein transitions through intermediate outward-facing conformations. Our data provide additional insights into the mechanism by which substrates gate the anion conductance in EAATs and suggest that arginine-388 is a critical component of the gating mechanism of the EAAT-associated anion channel.

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Studying Electrogenic Transporters of the Solute Carrier Family (SLC) Utilizing Solid Supported Membrane Technology
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The solute carrier family of membrane transport proteins includes over 300 members. Many of them are related to conditions and diseases, or are involved in the transport of pharmacological agents. Therefore, there is a strong need for efficient and flexible assays to study those uptake transporters.
Here we utilize solid supported membrane based electrophysiology to measure transporter activity in a real-time and high throughput manner. We investigated properties and interactions of PepT1, SGLT1, OCT1, ANT, and NCX. Data and method are presented. We were able to study activation and inhibition properties in a 96-well based format, enabling a method suitable also for drug screenings.