Charge compensation mechanism of a Na+-coupled, secondary active glutamate transporter.

Abstract:

Forward glutamate transport by the excitatory amino acid carrier EAAC1 is coupled to the inward movement of three Na(+) and one proton and the subsequent outward movement of one K(+) in a separate step. Based on indirect evidence, it was speculated that the cation binding sites bear a negative charge. However, little is known about the electrostatics of the transport process. Valences calculated using the Poisson-Boltzmann equation indicate that negative charge is transferred across the membrane when only one cation is bound. Consistently, transient currents were observed in response to voltage jumps when K(+) was the only cation on both sides of the membrane. Furthermore, rapid extracellular K(+) application to EAAC1 under single turnover conditions (K(+) inside) resulted in outward transient current. We propose a charge compensation mechanism, in which the C-terminal transport domain bears an overall negative charge of -1.23. Charge compensation, together with distribution of charge movement over many steps in the transport cycle, as well as defocusing of the membrane electric field, may be combined strategies used by Na(+)-coupled transporters to avoid prohibitive activation barriers for charge translocation.