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Analysis of Ion-Binding Sites in the Potassium-Chloride Cotransporter KCC2 Using Molecular Dynamics Simulations

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ABSTRACT

The potassium-chloride cotransporter KCC2 (SLC12A5) is the primary chloride extrusion transporter in most adult inhibitory neurons of the central nervous system. KCC2 mediates the electroneutral secondary active symport of potassium cation (K^+) coupled with chloride anion (Cl^-) across the plasma membrane of cells. Dysfunction of KCC2 is associated with a number of neurological disorders, especially epilepsy. The transport stoichiometry of KCC2 is 1K+:1Cl-, however, recent cryo-EM structures of KCCs revealed three non-protein electron densities, identified as two Cl^- and one K^+. Some studies suggest the presence of both the main Cl^- transport site and an additional allosteric site, but their exact functional roles remain unexplored. Molecular dynamics (MD) simulations were performed with GROMACS program and CHARMM36 force field. The investigation focused on coordination interactions and conformational changes resulting from site-specific mutagenesis of amino acid residues forming the ion-binding sites. The transmembrane domain monomer of the human KCC2 dimer (PDB ID: 6M23) was embedded in a phosphatidylcholine bilayer using CHARMM-GUI. The system was solvated with TIP3P water molecules and neutralized with 150 mM KCl. Two conserved disulfide bonds (C287–C302, C322–C331) were added to the protein structure. The positions of transported Cl^- and K^+ were taken from the nearest KCC4 homolog (PDB ID: 7D99). The coordinates of the allosteric Cl^- were derived from the KCC2 structure. Based on MD trajectories, we identified localization of ions and coordinating residues, (defined as being within $\leq$ 0.4 nm of the ions). Ions were considered stably associated when their displacement remained within 0.3 nm of the binding site center during the simulation. The analysis revealed local conformational changes in the structure of binding sites associated with amino acid substitutions, reflecting the functional sensitivity of these regions to point mutations. These results clarify the molecular details of KCC2 function at the atomic level and may further contribute to the development of antiepileptic drugs.

Keywords: membrane proteins, ion transporters, mutations, molecular dynamics

References:

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