Exploring the role of TM8 as a key domain in influencing the functional properties of human glutamate transporters

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Human Excitatory Amino Acid Transporters (EAATs 1-5) are responsible for the synaptic clearance of extracellular glutamate and play a key role in preventing excitotoxic cell injury. The transport process is coupled to the co-transport of 3 Na^+, $1H^+$, and the counter-transport of $1K^+$. In addition to transport, EAATs also possess a thermodynamically uncoupled chloride conductance which is activated upon binding of the substrate and sodium ions. In 2004, the crystal structure of the bacterial aspartate transporter, *Pyroccoccus horikoshii* (GltPh) was solved and serves as a basis for understanding the structure and function of the EAATs. GltPh shares ~36% amino acid identity with the human EAATs and there is high conservation of regions thought to be important to the transport process. This project seeks to develop a structural model of the EAATs and explore the structural basis for the pharmacology of these transporters. The highly conserved c-terminal half of the transporters (HP1, HP2, TM7, TM8) contain residues that have been implicated in substrate and ion binding/translocation. In TM8, there is a six-amino acid residue motif found in GltPh that is not present in the EAATs. To determine the significance of this motif, EAAT1/GltPh and EAAT2/GltPh chimeras were constructed and expressed in Xenopus laevis oocytes. It was observed that the two chimeras exhibited similar substrate selectivity and affinity as their respective wild-types. Interestingly, the degree of chloride conductance was enhanced in the EAAT2 chimera. Transport of the poor substrate, 4-methylglutamate was supported by the EAAT2 chimera. These results suggest that the TM8 motif that is unique to GltPh does not affect substrate transport, but does impact poor substrate selectivity and chloride conductance in EAAT2.