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MolTag 1st Funding Period.

Defensio: February 2016

Title: Biophysical characterization of substrate transport in the aspartate transporter - GltPh

Abstract in English:

Uptake of glutamate by the glutamate transporter plays an important role in maintaining the glutamate homeostasis in brain. Accumulation of extracellular glutamate at the neuronal synapse can lead to excitotoxicity. Defective glutamate transporter function has been proposed to be involved in various neurological disorders including amyotropic lateral sclerosis and schizoprenia. Studies on structure function relationship in human glutamate transporters have largely advanced after the publication of the crystal structure of an aspartate transporter (GltPh) from an archaea named Pyrococcus horikoshii in 2004.

By now, crystal structures of GltPh are available in several states and they provide important insights into the mechanism of substrate transport across the membrane. In order to understand the structure function relationship better in this class of transporters, it essential to study the transport process at atomic resolution. In this study, an accelerated molecular dynamics approach called as steered molecular dynamics was used in combination with conventional molecular dynamics simulation to model the substrate translocation pathway. The modeled translocation pathway showed two important local conformational changes as key switches in the translocation pathway. The first involved the maturation of the second sodium binding site and simulations showed T308 to play an important role in second sodium binding. The second conformational change involved the disruption of an ionic interaction network involving E192, K290, and Y195 at the cytoplasmic side of the transporter. To probe the role of T308 in second sodium binding, site directed mutagenesis was performed and the purified protein was reconstituted into proteoliposomes. [3H]L-aspartate uptake assays performed with proteoliposomes revealed an important role for the side chain hydroxyl group of T308 in sodium binding. Charge reversal of lysine at position 290 to a glutamate resulted in the reduction of substrate uptake in proteoliposomes, in comparison to wild type GltPh.

Taken together, these observation provide information on the sequence of events leading to the internalization of substrate in GltPh, at an atomic resolution.