Mapping the ion translocation pathway in the glutamine transporter SNAT3

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The glutamine transporter SNAT3 plays a pivotal role in the release of glutamine from brain astrocytes, the uptake and release of glutamine from hepatocytes and the uptake of glutamine in epithelial cells of the kidney. SNAT3 has a complex mechanism involving the cotransport of Na+ and the antiport of protons. In addition substrate-dependent and independent ion-conductances are observed. In order to understand the mechanism of the transporter in more detail, we explored the ion translocation pathway by experimental and theoretical approaches.

Until recently, database searches have not revealed homology to any known transporter structure. Comparison of hydropathy plots of the hydantoin permease Mhp1 with the hydropathy plot of SNAT3, however, revealed a significant similarity allowing us to generate a homology model of the transporter. The SNAT3 model suggests an overall topology that is similar to the Mhp1 structure. In this model helix 1 and 6 are lining the translocation pore and a putative Na+-binding site was identified involving residues asparagine 76, methionine 79 in helix 1 and valine 377 and threonine 380 in helix 8.

In line with this model we previously found that mutation of threonine 380 and of asparagine 76 changed the permeability properties of the transporter. Mutation of valine 377 to larger hydrophobic residues resulted in transporters with little activity. Mutation of valine 377 to leucine, however resulted in an active transporter molecule. This conservative exchange abolished the substrate-dependent conductance at pH 8.4, but not at pH 7.4. This is opposite to the change observed in a threonine 380 to alanine substitution, where the conductance at pH 7.4 is abolished. This confirms previous observations that the transporter has different conductance properties at different pH-values. As shown previously mutation of asparagine 76 to aspartate introduces a chloride-conductance that is tightly controlled by pH. We combined this mutation with a truncation of the C-terminal histidine in order to explore whether this residue changes the pH-dependence of the conductance. In this double mutant the anion conductance was still pH-gated, but showed little rectification compared to the N76D mutant.

The results suggest that asparagine 76, valine 377 and threonine 380 line the translocation pore of the glutamine transporter SNAT3. All three residues significantly alter the conductance of the transporter which is consistent with their suggested position close to the substrate and ion binding site of the transporter.