Structural basis for the functional differences between ASCT1 and EAATs

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The alanine, serine and cysteine transporters (ASCT1 and 2) are electroneutral exchangers. They belong to the Solute Carrier Family 1, along with human glutamate transporters (Excitatory Amino Acid Transporters - EAATs). Neutral amino acid exchange *via* ASCT1 is thought to be coupled to only one Na^+ ion. This is in contrast to the EAATs where glutamate transport is coupled to three Na^+, one H^+ ion and the counter transport of K^+ ion. Although amino acid exchange by ASCT is not H^+-coupled, its substrate specificity is pH-dependent. Cysteate is a substrate at pH 5.5, but not at neutral pH. We initiated a study of ASCT1 to provide more detailed comparisons with the well characterized EAATs so as to gain a better understanding of the molecular basis for similarities and differences between ASCT and the EAATs. Residues within the transport domain that differ between the EAATs and ASCT, and are in close proximity to bound substrate, were targeted for mutagenesis. Two mutations (A382T and T459R) were identified that altered the functional properties of ASCT1 towards a more EAAT-like phenotype. A382T, in transmembrane domain 7, was found to relax the substrate specificity towards acidic amino acids. This mutation allows the transport of L-aspartate at pH 7.5, although with a much lower affinity than neutral amino acids (124 ± 20 μM for L-serine, 429 ± 20 μM for L-aspartate). However at pH 5.5, the affinities of L-aspartate and L-cysteate increase dramatically to 30.0 ± 4 μM and 36.2 ± 7 μM respectively, which is 10 fold larger than that of neutral amino acids. Strikingly, T459R in transmembrane domain 8 switches the substrate specificity of ASCT1 from neutral to acidic amino acids. Neutral amino acid transport is impaired with the EC_{50} of L-serine >1mM. On the other hand, L-aspartate, L-glutamate and L-cysteate are transported with remarkably high affinity, considering they are not transported by wild type ASCT1 at neutral pH (156.9 ± 33 μM, 420.8 ± 114 μM and 1.8 ± 0.2 μM respectively). This illustrates the importance of an arginine at this position in determining acidic amino acid binding ability of the transporter. It is astonishing that a single point mutation is able to cause such a drastic change in substrate specificity. Interestingly, the combination of both of these point mutations in ASCT1 generates a transporter that transports both neutral and acidic amino acids. For example, L-cysteine transport is unaffected by the A382T mutation, generating large outward currents similar to that seen in wild type ASCT1. However, L-cysteine transport is abolished by the T459R mutation. By combining both A382T and T459R mutations in ASCT1, L-cysteine transport once again reflects that of wild type ASCT1. This indicates that threonine is an important ligand within the substrate binding site for recognition of neutral amino acids. Further investigation into the transport characteristics of both ASCT1 and the EAATs may aid our understanding of the varying transport mechanisms of the SLC1 family.