Molecular determinants for functional differences between the neutral amino acid transporter ASCT1 and acidic amino acid transporters of the EAAT family

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The Alanine, Serine, Cysteine Transporters (ASCTs) belong to the Solute Carrier Family 1, which also includes the human glutamate transporters (known as Excitatory Amino Acid Transporters, EAATs) and the prokaryotic aspartate transporter Glt_{Ph}. Despite a high degree of amino acid sequence identity between these family members, ASCTs function quite differently from the EAATs and Glt_{Ph}. ASCTs exchange small, neutral amino acids in an electroneutral fashion at pH 7.5. At low pH, L-cysteate and L-glutamate are also substrates of ASCT1 and ASCT2 respectively. The EAATs and Glt_{Ph} transport acidic amino acids such as L-glutamate and L-aspartate, generating a net flux of substrate and charge. We initiated a study of ASCT1 to investigate the molecular basis for the functional differences between members of the Solute Carrier Family 1. Our aim was to generate a transporter with functional properties of the EAATs and Glt_{Ph} by mutating residues in ASCT1. Residues within the transport domain that differ between the EAATs and ASCT1, and are in close proximity to bound substrate, were targeted for mutagenesis. Three residues were identified that play major roles in determining differences between ASCT1, the EAATs and Glt_{Ph}: A382, T459 and Q386. ASCT1 transporters containing the mutations A382T, T459R and Q386E were expressed in *Xenopus laevis* oocytes and analysed using electrophysiology and radiolabelled uptake experiments.

A382T and T459R altered the ligand selectivity of ASCT1 to accommodate acidic amino acid binding. The double mutant transporter A382T/T459R resembles Glt_{Ph} in that D- and L-aspartate share high affinities (0.8 ± 0.1 μM and 2 ± 1 μM respectively), while L-glutamate is much less potent (230 ± 30 μM). Interestingly, activation of the anion conductance by the acidic amino acids did not correlate with substrate transport in the mutant ASCT1 transporters. No significant levels of ^{3}H-L-aspartate uptake were observed for the mutant transporters, nor were acidic amino acids able to stimulate efflux of ^{3}H-L-serine from oocytes. This highlights the distinction between the transport process and activation of the anion conductance.

Q386E impairs the ability of ASCT1 to bind acidic amino acids at low pH. L-cysteate does not stimulate the anion conductance of Q386E, nor is it able to inhibit the uptake of ^{3}H-L-serine into oocytes, suggesting that acidic amino acids are no longer able to bind to this transporter. This is likely due to the positive charge of the introduced glutamate repelling acidic amino acids from entering the substrate binding site. Interestingly, the equivalent glutamate residue in the EAATs is a key factor in the transport process (Pines *et al.*, 1995, Grewer *et al.*, 2003). The contrasting role of this glutamate residue in the EAATs versus ASCT1 suggests that neighbouring residues, such as A382, are a critical influence on the transport process.

Grewer C, Watzke N, Rauen T, Bicho A. (2003) Is the glutamate residue Glu-373 the proton acceptor of the excitatory amino acid carrier 1? *Journal of Biological Chemistry* **278,** 2585-2592.

Pines G, Zhang Y, Kanner BI. (1995) Glutamate 404 is involved in the substrate discrimination of GLT-1, a (Na^+ + K^+)-coupled glutamate transporter from rat brain. *Journal of Biological Chemistry* **270,** 17093-17097.