The molecular mechanisms of Na^+-coupling of Na^+/amino acid co-transporters

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Summary

For the understanding of the mechanisms of coupling of Na^+ transport to the organic solute transport, we have performed structure-function analyses of Na^+-dependent neutral amino acid transporters ASCT1 and ASCT2.

ASCT1 and ASCT2 belong to the Na^+-dependent neutral and acidic amino acid transporter family which includes glutamate transporters and ASC transporters. ASCT1 and ASCT2 exhibit 57% amino acid sequence identity with each other. Both transported alanine, serine, threonine and cysteine as common substrates; however they still exhibited different functional properties. ASCT2 transported glutamine as a high-affinity substrate, whereas ASCT1 did not transport glutamine. This suggests that substrate binding sites of ASCT1 and ASCT2 are different in their interaction with substrate amino acid side chains. In order to identify the exact site to recognize substrate amino acid side chains we have performed chimera analyses based on the differential substrate selectivity in ASCT1 and ASCT2. ASCT2/ASCT1 chimeras (N-terminus ASCT2/C-terminus ASCT1) were constructed by homologous recombination of their cDNAs in $recA^+$ *E.coli*. cRNAs were *in vitro* synthesized from the chimera cDNAs and expressed in *Xenopus laevis* oocytes. Among 26 functional chimeras analyzed, 9 chimeras transported both threonine and glutamine, whereas others transported only threonine. All the chimeras which transported both threonine and glutamine possessed chimera points in the highly conserved long hydrophobic stretch close to C-terminus, suggesting that the site of recognition of substrate amino acid side chains lies in this region.

The Hill analyses of Na^+-dependent amino acid transports revealed that ASCT2 couples to the co-transport of single Na^+, whereas ASCT1 couples to at least two Na^+. ASCT2 was high affinity to Na^+ with a Km value of 2.5 mM for Na^+. ASCT2 accepted Li^+ instead of Na^+ whereas ASCT1 did not work in LiCl solution without Na^+. Thus, two Na^+-binding sites were postulated: "Na^+-binding site A" which is common to both ASCT2 and ASCT1 and "Na^+-binding site B" which exists only in ASCT1. "Na^+-binding site A" is high-affinity to Na^+ and accepts Li^+. On the other hand, "Na^+-binding site B" is low-affinity and dose not accept Li^+. Thus, the analyzing ASCT2/ASCT1 chimeras would enable us to identify the structure responsible for the differential Na^+-coupling between ASCT2 and ASCT1, that is, the "Na^+ binding site B". The ASCT2/ASCT1 chimeras were first analyzed in the view of Li^+ acceptance. Among 26 functional chimeras analyzed, 10 chimeras transported threonine in Li^+. All the chimeras which accepted Li^+ possessed chimera points in the long hydrophobic stretch, indicating that the "Na^+-binding site B" is mapped close to the proposed amino acid side chain recognition site. Na^+-dependence of four selected chimeras were analyzed. One exhibited sigmoidal dependence on Na^+ and the rest of three showed hyperbolic dependence on Na^+. We found an interesting chimera (HTJ 186) which accepted Li^+ and showed hyperbolic dependence on Na^+ ($ASCT2$ type Na^+-coupling), whereas did not transport glutamine ($ASCT1$ type substrate selectivity). This indicate that the chimera point of HTJ 186 lies between the "Na^+-binding site B" and the substrate amino acid side chain recognition site. In the extension of this chimera study would it be possible to identify the sites responsible for the substrate recognition and for the interaction with Na^+.