ANALYSIS OF THE STRUCTURAL DETERMINANTS OF CHLORIDE INTERACTION IN THE NSS AMINO ACID TRANSPORTER KAAT1.

Giovanola M, Castagna M, Santacroce M, Sacchi V.F.

Department of Molecular Sciences Applied to Biosystems, Università degli Studi di Milano, Via Trentacoste 2, 20134 Milano, Italy

KAAT1 is an amino acid cotransporter belonging to the NSS family of solute transporters cloned from the intestine of the tobacco hornworm *Manduca sexta* (Castagna *et al*., 1998) where it realizes the cotransport of neutral amino acids with a peculiar cation selectivity (Bossi *et al*., 1999; Castagna *et al*., 2009). Despite the fact that many eukaryotic members of the family present a strict chloride dependence, KAAT1 activity is only weakly dependent on this anion (Bettè *et al*., 2008). Aim of this work has been investigating the structural determinants of KAAT1 chloride dependence. Comparison of KAAT1 sequence with chloride-dependent and chloride-independent transporters of the family has revealed some differences in residues forming the putative anion binding site and only T339 seems to be relevant for chloride dependence of KAAT1. T339C mutant showed the same chloride dependence as wt but when the same residue was substituted with serine or with glutamate, the transport activity became almost completely chloride dependent. By sequence comparison we also identified the ATS sequence (66-68, KAAT1 numbering) that is conserved only in KAAT1 and in the other weakly chloride dependent transporter of the family CAATCH1. According to the structure of the model protein of the family, the bacterial transporter LeuT (Yamashita *et al*., 2005), A66 corresponds to G20, that is a member of the sodium binding site Na2, while S68 takes part of the Na1 site corresponding to A22 in LeuT sequence, therefore these residues could have a functional relevance also in KAAT1. A66G and S68A mutants presented a chloride dependence comparable to that of wt but the mutation of T67 had different results: T67Y mutant was fully chloride independent whereas T67S and T67A showed an enhancement in chloride dependence. In order to confirm the role of position 67, we built the reciprocal mutant of KAAT1 T67Y in the chloride dependent GABA transporter rGAT1: Y60T, interestingly, showed a reduced chloride dependence compared to wt. Our results suggest that T339, located in the putative chloride binding site, is implicated in KAAT1 interaction with the anion but we also obtained evidences that the dimensions and polarity of the lateral chains of amino acids located in position 67 can modulate KAAT1 chloride dependence.