Identification of amino acid residues critical for distinguishing mono- and di-carboxylate permeases in the lactate/pyruvate:H+symporter subfamily

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Lactic, acetic and propionic acids have been used for many years in industrial and pharmaceutical companies and, more recently, lactate as been used for production of biodegradable polymers and as substitute for petroleum-derived chemicals. Understanding in detail the mechanisms underlying the transport of carboxylic acids is crucial towards an efficient biological production of these compounds. In *Saccharomyces cerevisiae*, Jen1p is major monocarboxylate H^+ symporter specific primarily for lactate, pyruvate and for acetate, encoded by the JEN1 gene (TC 2.A.1.12.2) (Casal et al, 1999). A phylogenetic tree of ScJen1p homologues (Casal et al, 2008) showed the existence of two main clusters: a Jen1 group of proteins (monocarboxylate transporters) and a Jen2-like proteins (dicarboxylate transporters). By homology threading of the Jen1p with the LacY permease we were able to obtain a 3D dimensional model, that together with site directed mutagenesis strategies, pointed to the existence of common structure between these two permeases. Furthermore, we have also shown that a highly conserved motif in 7th transmembrane segment (TMS7) is part of the substrate translocation pathway (Soares-Silva et al, 2007). Conserved mutations in this motif affect the kinetics of Jen1p, as well as, its specificity towards physiological substrates. In this work, we rationally design, combine and analyse novel mutations in two other conserved regions located in TMS5 and TMS11 of Jen1p, which we predicted to affect more dramatically Jen1p specificity. The domain in TMS5 was identified by structure/function studies based on phylogenetic molecular comparisons among Jen1p homologues with different specificities and is critical for distinguishing mono- and di-carboxylate permeases. The conserved aminoacids in TMS11 domain pointed to the importance of this domain that was demonstrated to be involved in substrate binding. We thus identify several residues critical for Jen1p activity, among which some also function as critical specificity determinants for the distinction of mono- from di-carboxylates. Overall, our results constitute a first step towards the elucidation and genetic manipulation of substrate specificity in the lactate/pyruvate:H^+ symporter subfamily (TC#2.A.1.12.2) and a tool for the *in silico* prediction of the function of Jen1p homologues in other fungi of industrial importance.

Casal, M., et al. (1999) J. Bacteriol. 181, 2620-2623.

Soares-Silva, I., et al. (2007) Mol. Membr. Biol. 24, 464-474.

Casal, M., et al. (2008) FEMS Microbiol Rev. 32, 974-994.

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(1) Casal M *et al.* (1999) *JOURNAL OF BACTERIOLOGY* **181**:2620-2623.

(2) Casal M *et al.* (2008) *FEMS Microbiol Rev* **32**:974-994.

(3) Soares-Silva I *et al.* (2007) *Molecular Membrane Biology* **24**:464-474.