Title:	Substrate specificity and mapping of residues critical for transport in the high-affinity glutathione transporter Hgt1p
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Abstract:	The high-affinity glutathione transporter Hgt1p of Saccharomyces cerevisiae belongs to a relatively new and structurally uncharacterized oligopeptide transporter (OPT) family. To understand the structural features required for interaction with Hgt1p, a quantitative investigation of substrate specificity of Hgt1p was carried out. Hgt1p showed a higher affinity for reduced glutathione (GSH), whereas it transported oxidized glutathione (GSSG) and other glutathione conjugates with lower affinity. To identify the residues of Hgt1p critical for substrate binding and translocation, all amino acid residues of the 13 predicted transmembrane domains (TMDs) have been subjected to mutagenesis. Functional evaluation of these 269 mutants by growth and biochemical assay followed by kinetic analysis of the severely defective mutants including previous mutagenic studies on this transporter have led to the identification of N124 (TMD1), V185 (TMD3), Q222, G225 and Y226 (TMD4), P292 (TMD5), Y374 (TMD6), L429 (TMD7) and F523 and Q526 (TMD9) as critical for substrate binding with at least 3-fold increase in Km upon mutagenesis to alanine. In addition residues Y226 and Y374 appeared to be important for differential substrate specificity. An ab initio model of Hgt1p was built and refined using these mutagenic data that yielded a helical arrangement that includes TMD3, TMD4, TMD5, TMD6, TMD7, TMD9 and TMD13 as pore-lining helices with the functionally important residues in a channel-facing orientation. Taken together the results of this study provides the first mechanistic insights into glutathione transport by a eukaryotic high-affinity glutathione transporter.
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