Abstract

The High affinity glutathione transporter (Hgt1) of Saccharomyces cerevisiae is a 799 amino acid protein, located on the plasma membrane and predicted to have 12 transmembrane domains (TMD). Here we aim to identify residues involved in glutathione binding and transport. In the present study, alanine scanning mutagenesis of 5 transmembrane domains of Hgt1p was attempted to obtain insight into the residue which are involved in substrate binding and translocation. All alanine mutants were analyzed using a plate-based growth assay. This analysis identified W110A (in TMD1), L282A (in TMD5), L429A (in TMD7), Y449A (in TMD7) and W484A (in TMD8) mutants which exhibited severe loss of functional activity. The detailed biochemical characterization of these mutants includes their effect on protein expression levels. It was found that mutants W110A, L282A, Y449A and W484A had a drastic effect on protein level while mutant L429A had no significant effect on protein level and this was likely to be involved in either trafficking or substrate translocation. Multiple sequence alignment of TMD regions of fungal OPT family revealed that the residues W110, L429, Y449 and W484 are conserved. In addition to this, a study was also initiated to identify if residues which are shown to be important for glutathione transport are also important for Leu- Enkephalin transport by Hgt1p.