Title:	Defining the cytosolic pathway of glutathione degradation in Arabidopsis thaliana: Role of the ChaC/GCG family of γ-glutamyl cyclotransferases as glutathione-degrading enzymes and AtLAP1 as the Cys-Gly peptidase
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Abstract:	Glutathione homoeostasis is critical to plant life and its adaptation to stress. The γ-glutamyl cycle of glutathione biosynthesis and degradation plays a pre-eminent role in glutathione homoeostasis. The genes encoding two enzymatic steps of glutathione degradation, the γ-glutamyl cyclotransferase (GGCT; acting on γ-glutamyl amino acids) and the Cys-Gly dipeptidase, have, however, lacked identification. We have investigated the family of GGCTs in Arabidopsis thaliana. We show through in vivo functional assays in yeast that all three members of the ChaC/GCG subfamily show significant activity towards glutathione but no detectable activity towards γ-glutamyl methionine. Biochemical characterization of the purified recombinant enzymes GGCT2;2 and GGCT2;3 further confirmed that they act specifically to degrade glutathione to yield 5-oxoproline and Cys-Gly peptide and show no significant activity towards γ-glutamyl cysteine. The Km for glutathione was 1.7 and 4.96 mM for GGCT2;2 and GGCT2;3 respectively and was physiologically relevant. Evaluation of representative members of other subfamilies indicates the absence of GGCTs from plants showing significant activity towards γ-glutamyl-amino acids as envisaged in the classical γ-glutamyl cycle. To identify the Cys-Gly peptidase, we evaluated leucine aminopeptidases (LAPs) as candidate enzymes. The cytosolic AtLAP1 (A. thaliana leucine aminopeptidase 1) and the putative chloroplastic AtLAP3 displayed activity towards Cys-Gly peptide through in vivo functional assays in yeast. Biochemical characterization of the in vitro purified hexameric AtLAP1 enzyme revealed a Km for Cys-Gly of 1.3 mM that was physiologically relevant and indicated that AtLAP1 represents a cytosolic Cys-Gly peptidase activity of A. thaliana. The studies provide new insights into the functioning of the γ-glutamyl cycle in plants. © The Authors Journal compilation
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