Molecular and catalytic characterization of the herbicide-inducible glutathione transferases from Phaseolus vulgaris

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ABSTRACT

Plant glutathione transferases (GSTs) comprise a large family of inducible enzymes that play important roles in stress tolerance and herbicide detoxification. Treatment of Phaseolus vulgaris leaves with the aryloxyphenoxypropionic herbicide fluazifop-p-butyl resulted in induction of GST activities. Three inducible GST isoenzymes were identified and separated by affinity chromatography. Their full-length cDNAs with complete open reading frame were isolated using RACE-RT and information from N-terminal amino acid sequences. Analysis of the cDNA clones showed that the deduced amino acid sequences share high homology with GSTs that belong to phi and tau classes. The three isoenzymes were expressed in *E. coli* and their substrate specificity was determined towards 20 different substrates. The results showed that the fluazifop-inducible glutathione transferases from *P. vulgaris* (*Pv*GSTs) catalyze a broad range of reactions and exhibit quite varied substrate specificity. Molecular modeling and structural analysis was used to identify key structural characteristics and to provide insights into the substrate specificity and the catalytic mechanism of these enzymes. These results provide new insights into catalytic and structural diversity of GSTs and the detoxifying mechanism used by *P. vulgaris*.

RESULTS

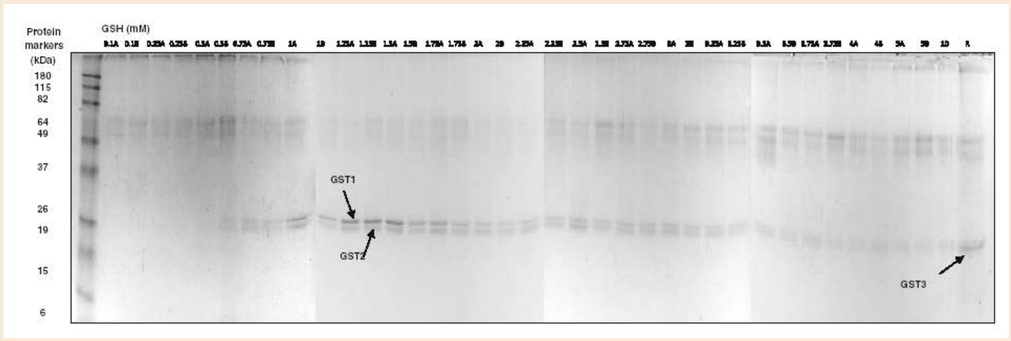


Fig 1. SDS-PAGE analysis of GST elution profiles from the GSH-Sepharose affinity adsorbent. Separation of fluazifop-butyl-induced *P. vulgaris* GSTs by affinity chromatography on GSH-Sepharose adsorbent. Stepwise elution was carried out using GSH (0.1–10 mM). R the fraction eluted using 50 mM NaH2PO4, 1M NaCl pH 7.5

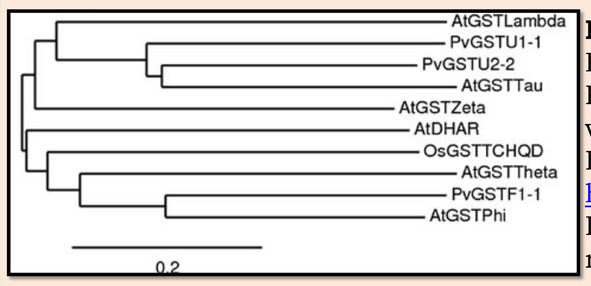


Fig.2. Phylogenetic analysis of PvGSTF1-1, PvGSTU1-1 and PvGSTU2-2. Phylogenetic tree was constructed by the DrawGram program (run at http://www.phylogeny.fr/, Dereeper et al. 2008) and representative members from all known plant GST classes.

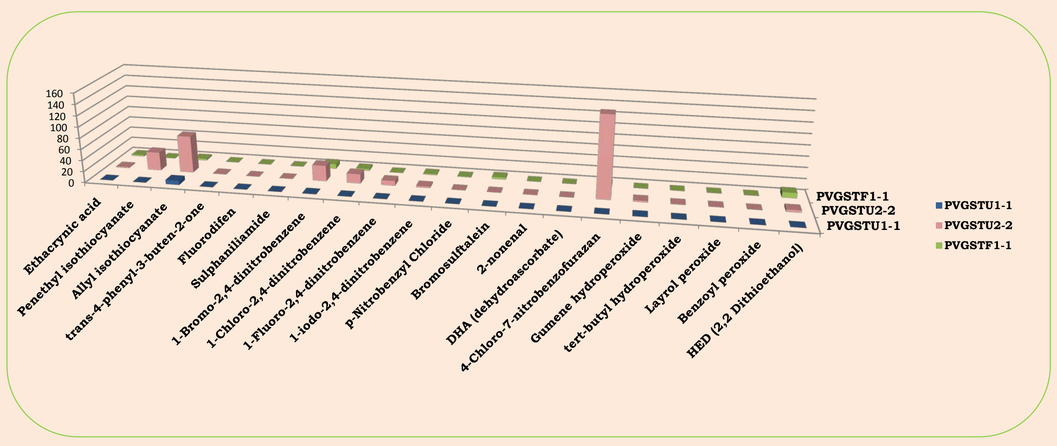
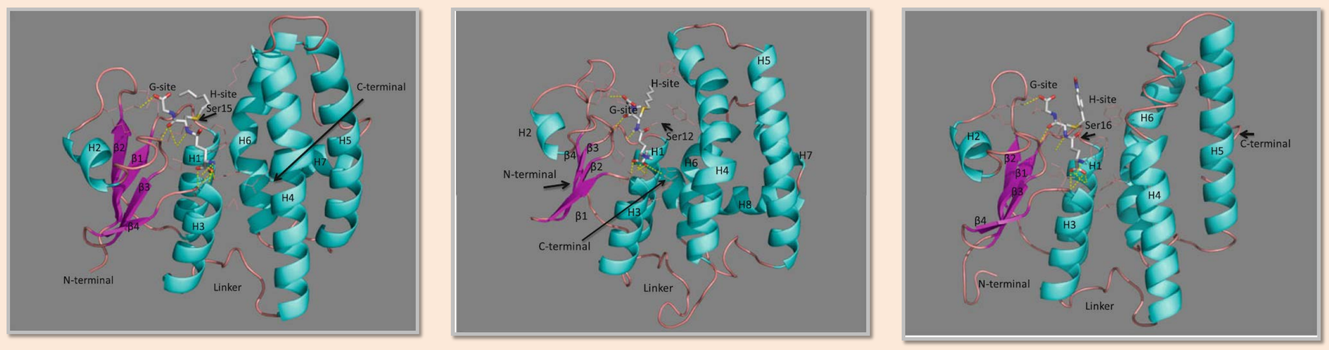


Fig.4. Substrate specificity of *Pv*GSTU1-1, *Pv*GSTU2-2 and *Pv*GSTF1-1



GST1/GSTU1-1 **GST2/GSTF1-1** **GST3/GSTU2-2**

Fig. 3 Ribbon diagrams of PvGSTU1-1, PvGSTF1-1, and PvGSTU2-2 protein models. Helices (H) are in turquoise and b-strands (b) in magenta. The GSH analogues (S-hexyl-GSH for PvGSTF1-1 and PvGSTU1-1 and p-nitrobenzyl-GSH for PvGSTU2-2) are represented in a stick and colored according to atom type. The location of active site Ser residue, the G- and H-site as well as the C-, and N-terminal and the linker are labeled. The molecular figures were created using PyMOL (DeLano 2002).

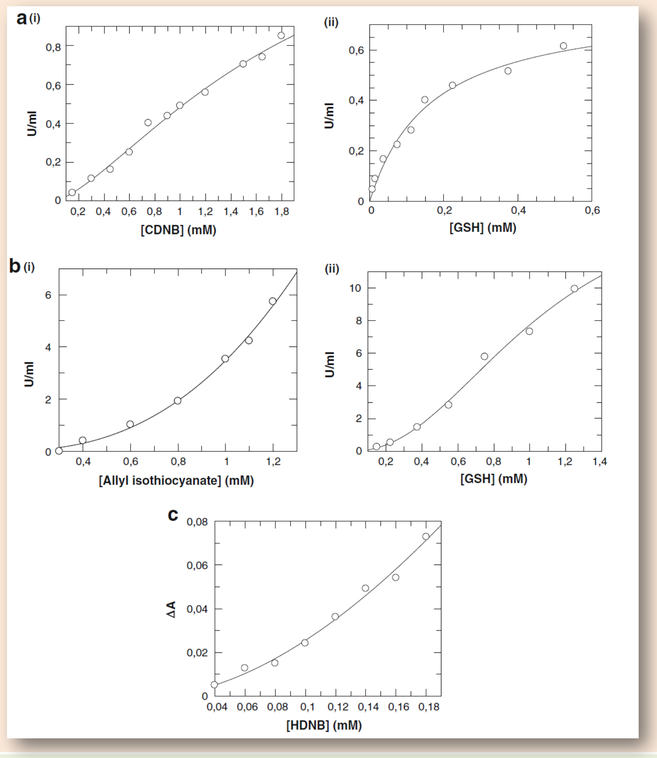


Fig. 5. a Kinetic analysis of *Pv*GSTU1-1 using the CDNB as a variable substrate (i) and GSH at a fixed concentration. Kinetic analysis of *Pv*GSTU1-1 using the GSH as variable substrate (ii) and CDNB at a fixed concentration. **b** Kinetic analysis of *Pv*GSTU1-1 using the allyl-isothiocyanate as a variable substrate (i) and GSH at a fixed concentration. Kinetic analysis of *Pv*GSTU1-1 using the GSH as variable substrate (ii) and allyl-isothiocyanate at a fixed concentration. Experiments were performed in triplicate and lines were that calculated by least-squares regression analysis. **c** Spectroscopic analysis of HDNB binding to PvGSTU1-1. The graph shows the difference absorbance at 310 nm as a function of the total HDNB concentration.

CONCLUSION

In the present work, we describe the characterization of three fluazifop-inducible GSTs from P. vulgaris. The results showed that PvGSTs are capable of catalyzing several different reactions and substrates, including herbicides, and exhibit wide substrate specificity. Structural analysis showed that PvGSTs share the same overall fold and domain organization of other plant cytosolic GSTs, with major differences at their active site and some differences at the level of C-terminal domain and the linker between the C- and N-terminal domains. The structural heterogeneity within the C-terminal domain seems to be responsible for the substrate variability and specificity across PvGSTs. Taking into account the inducible expression of PvGSTs by fluazifop-p-butyl and their high catalytic activity towards fluazifop-p-butyl, it is conceivable to assume that PvGSTs may contribute to P. vulgaris stress response mechanism towards fluazifop-p-butyl. The methodology reported in the present study for the discovery of novel enzymes involved in herbicide stress response may find applications in other plant/stress systems.

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