European Union
European Social Fund
OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union

CLONING AND CHARACTERIZATION OF HERBICIDE-DEGRADING GLUTATHIONE TRANSFERASES FROM *CICER ARIETINUM*

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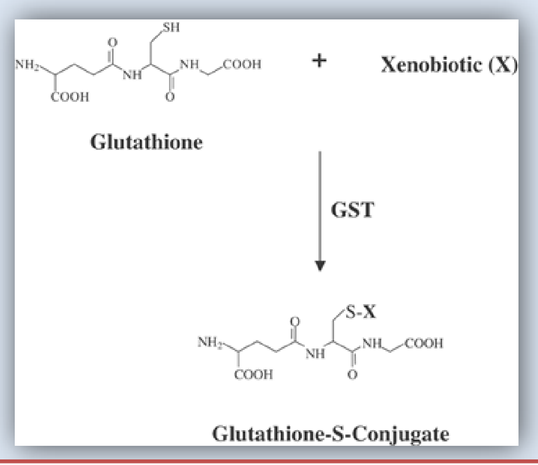
Introduction

GSTs are multifunctional enzymes that catalyze the conjugation of glutathione (GSH) to reactive electrophiles. These electrophiles are diverse and include important endogenous compounds, as well as xenobiotic chemicals, therefore GSTs play an important role in stress tolerance and herbicide detoxification. GSTs are usually active as a dimer of 24–29 kDa subunits. Each monomer of dimeric GSTs contains a G-site, at the N-terminal, capable of binding the GSH substrate and an H-site, at the C-terminal, that has xenobiotic compound-binding capabilities. Different classes of herbicides such as triazines, thiocarbamates, chloroacetanilides, diphenylethers, and aryloxyphenoxypropionates can be metabolized by GSTs. Herbicide tolerance in plants is based primarily on the differential ability of plant species to detoxify a herbicide, with the formation of a herbicide-GSH conjugate in the resistant but not in the susceptible species. The plant-specific phi and tau GSTs are primarily responsible for herbicide detoxification, showing class specificity in substrate preference. In present work, we report the cloning, kinetic and structural characterization of three members of the GST family from *Cicer arietinum* leaves (*Ca*GSTs).

Methods and materials

GSTs of *Cicer arietinum* were identified by in silico transcriptome analysis and their full length cDNAs with complete open reading frame were isolated using RT-PCR. Analysis of the cDNA clones showed that the deduced amino acid sequences share high homology with GSTs that belong to tau classes. The GST isoenzymes were expressed in *E. coli* and purified by affinity chromatography and their substrate specificity was determined towards nineteen different electrophilic substrates including herbicides. Comparative molecular modelling was used to identify key structural characteristics and to provide insights into the substrate specificity and the catalytic mechanism of these enzymes.



Results

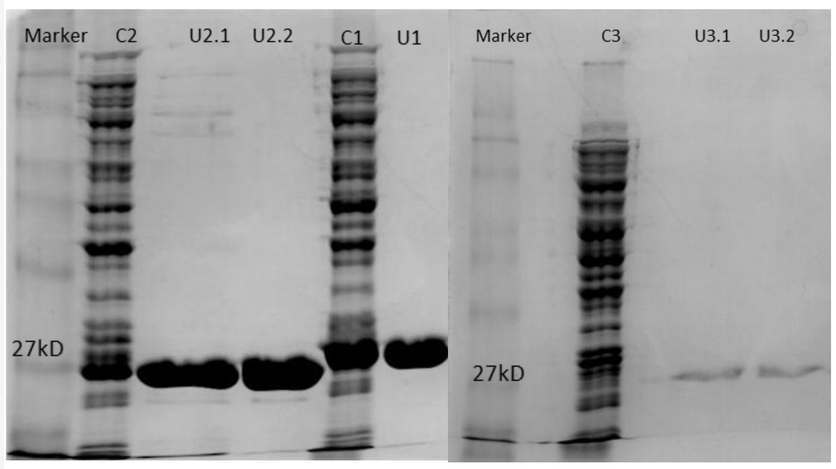


Fig 1: SDS-PAGE analysis of purified *Ca*GST-U1, *Ca*GST-U2, *Ca*GST-U3. Elution was carried out using GSH (10 mM).M protein markers, lane C1,C2,C3 crude extract, lanes U1-U3 eluted fractions from the affinity column.

- Purified recombinant *Ca*GST-U1, *Ca*GST-U2 and *Ca*GST-U3 (**Fig 1)** were assayed as glutathione transferase, glutathione peroxidase, dehydroascorbate reductase and as thiolotransferase and specific activities were determined using different substrates (**Fig 2)**.

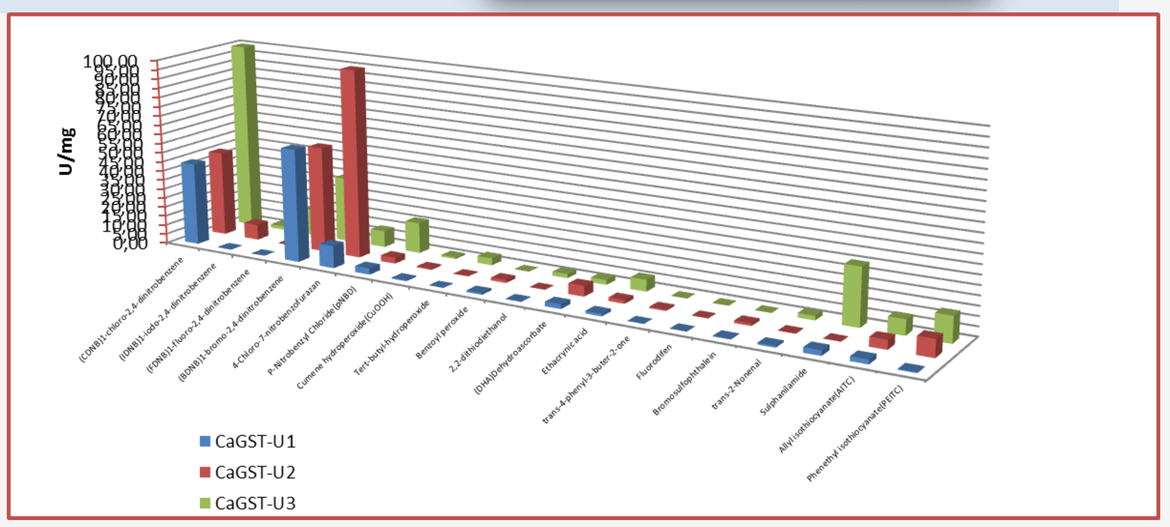


Fig 2: Substrate specificity for purified recombinant *Ca*GSH-U1,*Ca*GSH-U2,*Ca*GSH-U3, towards 19 different electrophilic substrates. Enzyme assays were carried out under standard conditions. Results represent the means of triplicate determinations.

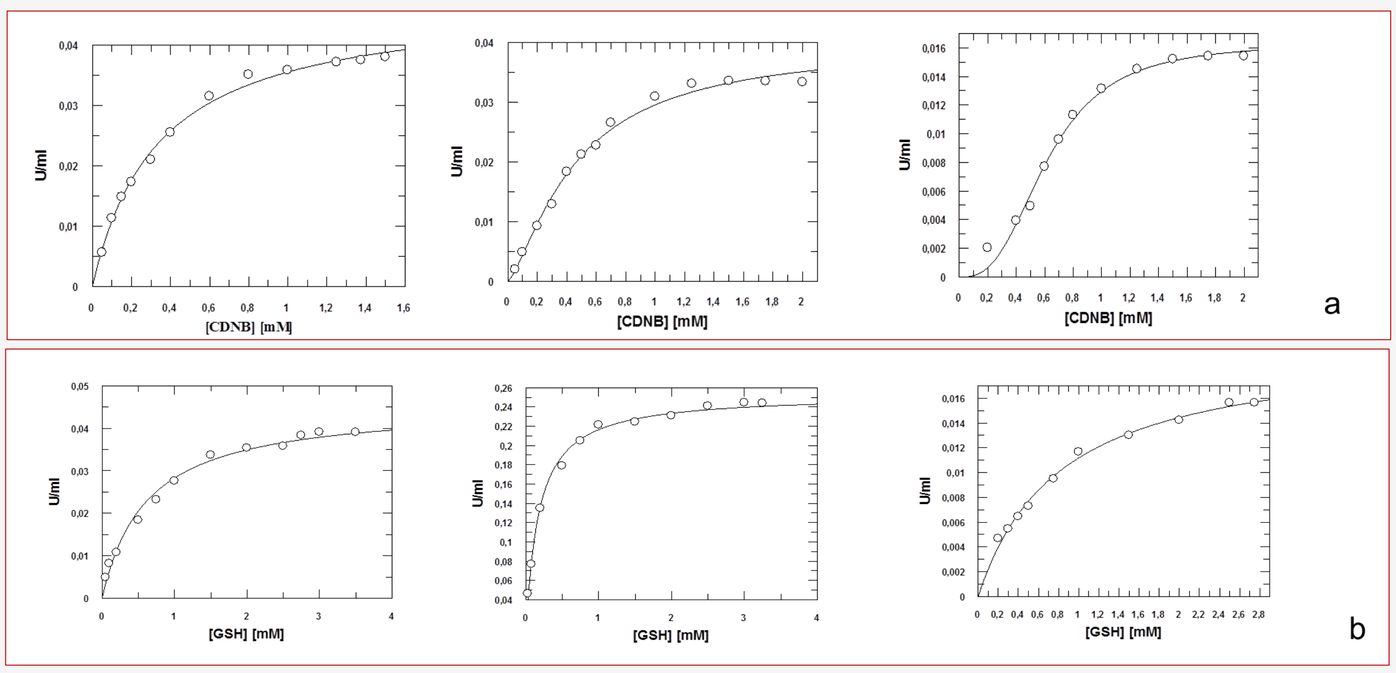


Fig 3: Kinetic analysis of *Ca*GST-U1, *Ca*GST-U2, *Ca*GSH-U3 using the CDNB as a variable substrate (a) and GSH at a fixed concentration. Kinetic analysis of *Ca*GST-U1, *Ca*GST-U2, *Ca*GSH-U3 using the GSH as variable substrate (b) and CDNB at a fixed concentration. Experiments were performed in triplicate, and lines were those calculated by least-squares regression analysis.

- The kinetic parameters k_{cat}, K_m,$S_{0,5}$ and n_H were determined by steady-state kinetic analysis (**Fig 3)** and the results are listed in **Table 1.**

Table 1. Kinetic analysis of *Ca*GSTs.

	K_m (mM) (CDNB)	K_{cat} (min^{-1}) (CDNB)	K_{cat}/K_m (mM^{-1} min^{1})(CDNB)
CaGST-U1	0,3443 ± 0,0184	694	2015,7
	$S_{0,5}$ (mM)(CDNB)	K_{cat} (min^{-1}) (CDNB)	n_H (CDNB)
CaGST-U2	0,3508 ± 0,0611	347	1,535
CaGST-U3	0,4644 ± 0,1687	206	2,0904

	K_m (mM) (GSH)	K_{cat} (min^{-1}) (GSH)	K_{cat}/K_m (mM^{-1} min^{-1})(GSH)
CaGST-U1	0,6128 ± 0,0731	664	1083
CaGST-U2	0,1707 ± 0,0122	154	902
CaGST-U3	0,8221 ± 0,0597	206	250

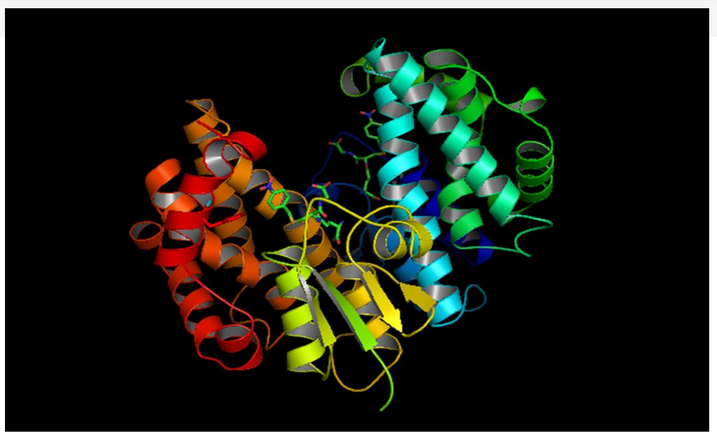


Fig. 4 :A Ribbon diagram of the *Ca*GST-U1 protein model. The figure was created using PyMOL.

Discussion

- The results showed that the glutathione transferases from *C. arietinum* (*Ca*GSTs) catalyze a broad range of reactions and exhibit quite varied substrate specificity.
- CaGST-U1 obeys Michaelis - Menten hyperbolic equation using CDNB and GSH as a variable substrate Steady-state kinetic analysis of *Ca*GSTU2 and *Ca*GST-U3 showed that when CDNB was used as a variable substrate with GSH at fixed concentration, a sigmoid substrate dependence was observed.
- Molecular modelling showed that the isoenzymes shares the same structural organization with other plant cytosolic GSTs and some differences at the level of C-terminal domain (**Fig 4)**. In particular, major variations were identified in helices H4 and H9 that affect xenobiotic substrate recognition and catalytic mechanism.

References
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ACKNOWLEDGEMENTS. The present work was supported by the action "THALES: Glutathione transferases, multifunctional molecular tools in red and green biotechnology". This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) .