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Characterization of the pesticide detoxifying enzyme C*d*GSTM1-1 by kinetic analysis and differential scanning fluorimetry

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Glutathione transferases (GSTs, EC. 2.5.1.18) form a large group of multifunctional enzymes, best known for their involvement in the metabolism and inactivation of a wide range of xenobiotic compounds. GSTs catalyze the nucleophilic attack of the reduced form of glutathione (γ-L-Glu-L-Cys-Gly, GSH) on the electrophilic center of a variety of compounds such as pesticides, herbicides etc. The conjugation of GSH to such molecules results in the increase of their solubility and the reduction of their toxicity. GSTs are useful tools with a variety of biotechnological applications in many fields. The natural ability of the GSTs to interact with xenobiotic compounds gives the opportunity to develop enzyme biosensors for the simple and direct monitoring of environmental pollutants. In the present work we report the cloning, kinetic and structural characterization of the GSTM1-1 from camel (*Camelus dromedarius*) as well as a screening method to identify ligands that bind the protein. The C*d*GSTM1-1 enzyme was expressed in *E. coli* and purified by affinity chromatography. The ligandin function of the enzyme was evaluated by measuring the ability of forty seven xenobiotic compounds to bind and inhibit enzyme activity. The inhibition potency was measured with the CDNB/GSH assay system. The IC_{50} value and the kinetic analysis of the compound that showed the highest inhibition were determined. The results showed that the enzyme exhibits high selectivity towards the fungicide zoxium zoxamide. The thermodynamic stability of C*d*GSTM1-1 and the influence of zoxium zoxamide were investigated using the differential scanning fluorimetry (DSF). The results are explored for the development of an optical biosensor for the determination of zoxium zoxamide in environmental samples.

Biography

Fereniki Perperopoulou studied Agricultural Biotechnology at the Agricultural University of Athens. She remained at the Agricultural Biotechnology Department of the Agricultural University of Athens, where she earned a master's degree on "Bioactive Protein Products & Technology". From 2014 to date she is a PhD candidate at the Department of Biotechnology (Agricultural University of Athens), working on the subject "Protein engineering and molecular study of transferase glutathione", for which she has been awarded a scholarship under the scheme research projects for excellence iky/siemens.

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