

4th European Chemistry Congress

May 11-13, 2017 Barcelona, Spain

Investigation of the interaction of the tau class glutathione transferase with pesticides by differential scanning fluorimetry

Foteini M Pouliou and Nikolaos E Labrou
Agricultural University of Athens, Greece

Glutathione transferases (GSTs, EC 2.5.1.18) constitute one of the most important families of detoxifying enzymes in nature with multiple biotechnological applications. GSTs contribute to the phase II biotransformation of xenobiotics in a variety of organisms, with members of the family being involved in both the metabolism and transportation of potentially toxic ligands (e.g. pesticides). The goal of this study was to apply GST as a model in order to investigate whether ligands' mode of inhibition could be discerned through thermal denaturation profiles. A library of Tau class GSTs was constructed by DNA shuffling using the DNA encoding the *Glycine max* GSTs GmGSTU2-2, GmGSTU4-4 and GmGSTU10-10. The DNA library contained chimeric structures of alternated segments of the parental sequences and point mutations. Chimeric GST sequences were expressed in *Escherichia coli*, purified by affinity chromatography and their enzymatic activities towards CDNB (1-chloro-2,4-dinitrobenzene) were determined. A selected chimeric enzyme which exhibited high catalytic activity and stability was used as a model for the assessment of the GST-pesticide interaction with a differential scanning fluorimetry (DSF). DSF signatures were obtained and analysed in the absence and in the presence of different concentrations of the substrate GSH, the inhibitor S-hexyl GSH and a range of different pesticides. The results of the study are explored for the development of a DSF-based assay for the direct determination of pesticides in environmental samples.

Biography

Foteini Pouliou is a PhD candidate at the Agricultural University of Athens since 2014. She majored in biotechnology at the department of biotechnology of the same university in 2012 and enrolled in her Master of Science (MSc) studies in 2013 focusing on bioactive products and protein technology. Her research interests include protein engineering, enzyme and environmental biotechnology. She has been working on Glutathione Transferases (GSTs), a group of detoxification enzymes. She has been awarded a grant by the State Scholarships Foundation under the scheme research projects for excellence iky/siemens.

poulioufot@gmail.com

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