Engineering of Tau class GSTs for the development of biosensor

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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 are involved in the detoxification mechanism of endogenous and xenobiotic electrophile compounds by catalyzing the nucleophilic attack of reduced glutathione (GSH) on the electrophilic center of xenobiotic compounds including pesticides. This catalytic activity is the basis for the development of enzyme biosensor for herbicide determination in environmental samples. 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 for the development of enzyme biosensor. The inhibition potency of 47 different pesticides towards the chimeric enzyme was evaluated using activity assays. Five compounds, one insecticide and four fungicides, showed high inhibition potency (IC50) towards the chimeric GST. Kinetic inhibition studies revealed that pesticides appeared to bind at the substrate-binding region in a competitive manner with respect to the substrate. The chimeric enzyme will be immobilized and will be explored for the construction of an optical biosensor. This biosensor will be portable, easy to use, allowing the direct determination of pesticides in environmental samples.

Biography

Foteini M Pouliou is a PhD candidate at the Agricultural University of Athens since 2014. She majored in Biotechnology from the Agricultural University of Athens in 2012. She has done her Master of Science studies in 2013 focusing on the Bioactive Products and Protein Technology. Her research interests include protein engineering, enzyme and environmental biotechnology.

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