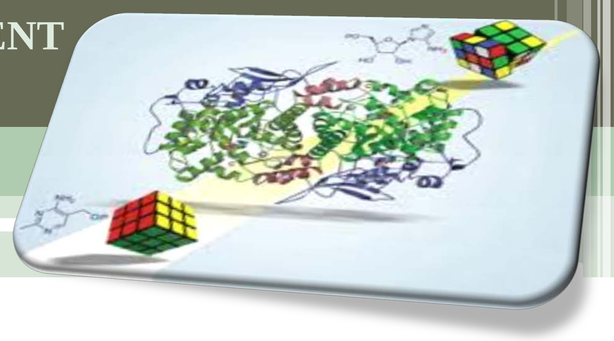
PROTEIN ENGINEERING OF GLUTATHIONE TRANSFERASE FOR THE DEVELOPMENT OF OPTICAL BIOSENSOR TO DETECT XENOBIOTICS



E. Chronopoulou, N.E. Labrou
Agricultural University of Athens, Enzyme Technology Laboratory
Iera Odos 75, 118 55 Athens

Abstract

Glutathione transferases (GSTs, EC. 2.5.1.18) are inducible enzymes that play essential role in detoxification and degradation of toxic compounds, including pesticides. The purpose of the present study is the development of an optical enzyme biosensor based on GSTs, for the detection and determination of pesticides in environmental samples. Protein engineering was used for the creation of a GST variant with higher selectivity towards pesticides. cDNA libraries were created from *Phaseolus vulgaris* and *Glycine max* stressed plants using degenerated primers and reverse transcription-PCR. Large diversity in GST genes was accomplished employing directed evolution through DNA shuffling of a mixture of GST genes from *P. vulgaris* and *G. max* stressed plants. The shuffled library of chimaeric GST genes was cloned in *E. coli* expression plasmid. Screening of the library led to the isolation of a novel GST enzyme that displays both glutathione transferase and glutathione peroxidase activities. The enzyme was purified by affinity chromatography and characterized by kinetic analysis towards 20 different substrates and 66 different pesticides. The results showed that the organoclorine insecticides and strobilurins (fungicides) are strong inhibitors of the enzyme. The specificity of the enzyme towards pesticides was further improved using site-saturation mutagenesis at position Phe117. The mutant Phe117Ile displays 5-fold higher catalytic efficiency and selectivity towards organochlorine insecticides. Therefore, the mutant GSTPhe117Ile was used for the development of an optical biosensor. The enzyme was immobilized in alkoxysilane (TEOS/PTMOS) sol-gel system in the presence of the pH indicators bromocresol purple (acidic) and phenol red (basic). The bioactive material exhibits linearity in the range of 0.625-30 μM α-endosulfan (pH=4-7) at 562 nm and was used for the development of an analytical method for the determination of α-endosulfan in environmental samples.

Results

Stressed *Phaseolus vulgaris* and *Glycine max* plants were used for the creation of cDNA libraries using degenerated primers and reverse transcription-PCR. Large diversity in GST genes was accomplished employing directed evolution through DNA shuffling of a mixture of GST genes from *P. vulgaris* and *G. max* stressed plants. The shuffled library of chimaeric GST genes was cloned in *E. coli* expression plasmid. Screening of the library led to the isolation of a novel GST enzyme *PvGm*GSTUG that displays both glutathione transferase and glutathione peroxidase activity. This novel GST enzyme belongs to *tau* class and is strongly inhibited by organochlorines (insecticides) and strobilurins (fungicides).

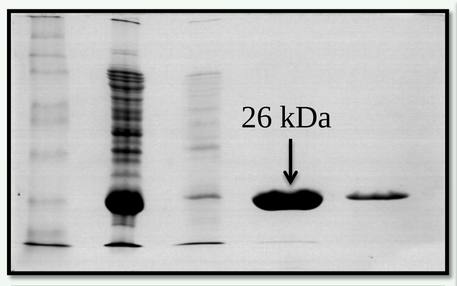


Fig.1. **SDS-PAGE electrophoresis of *PvGm*GSTUG purification by affinity chromatography.**

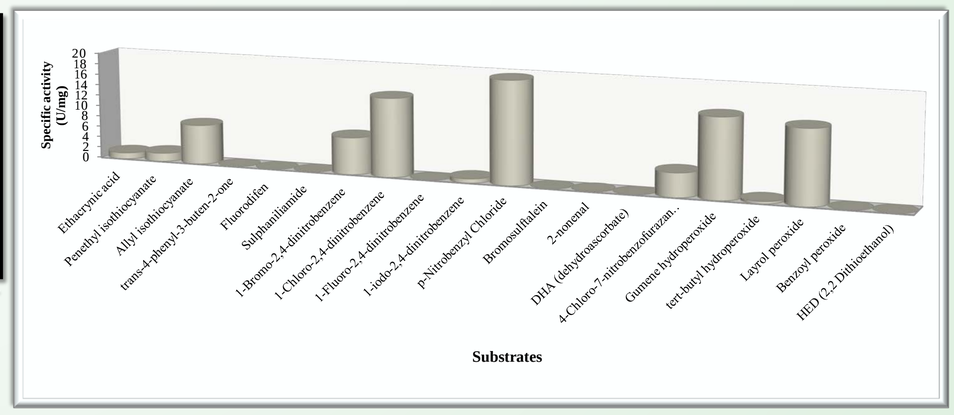


Fig.2. **Specific activity of P*vGm*GSTUG towards 20 different substrates**

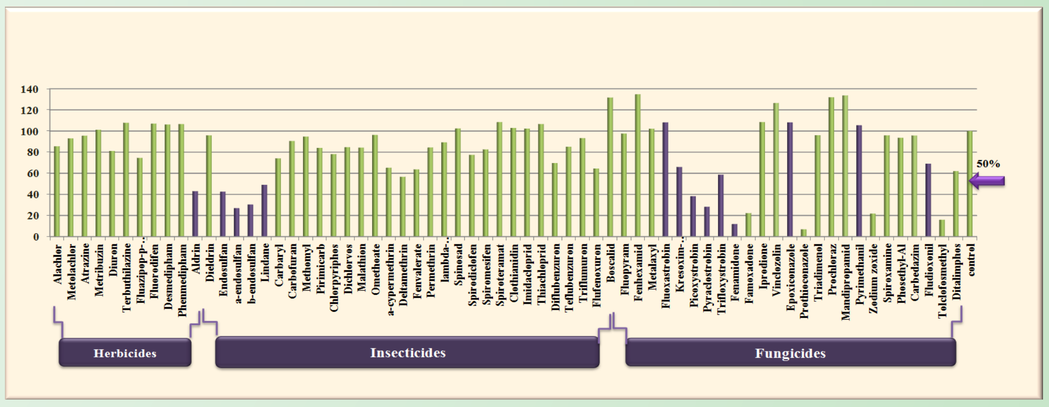
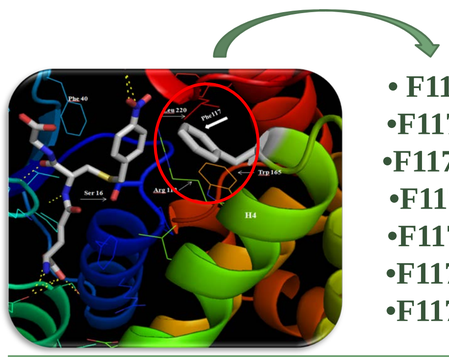


Fig.3. **Inhibition (% residual activity) of P*vGm*GSTUG by 66 different pesticides**

For the enhancement of *PvGm*GSTUG pesticide selectivity, site-saturation mutagenesis was carried out at position Phe117. Phe117 is located at the entrance of the substrate binding-site. The enhanced selectivity of each mutant is shown in Fig.5. The mutant Phe117Ile exhibits strong inhibition by organochlorine insecticides. Therefore, the mutant F117I was selected for the development of the biosensor. This mutant also shows 5-fold higher k_{cat}/K_m than wild type enzyme. The mutant F117I was immobilized in sol-gel composed by alkoxysilanes (TEOS and PTMOS), with aging in TEOS. Fig 6. depicts kinetic analysis of the immobilized mutant enzyme.



- **F117I**
- **F117R**
- **F117W**
- **F117S**
- **F117T**
- **F117G**
- **F117H**

Fig.4. **Site-saturation mutagenesis in the position 117**

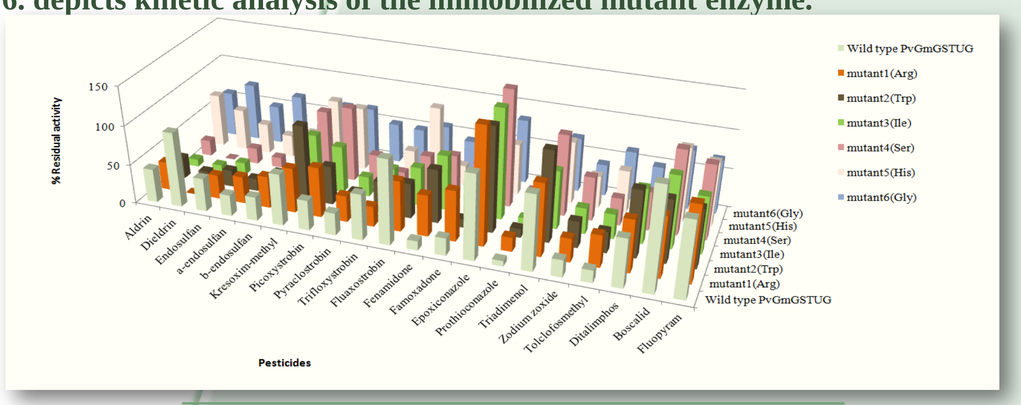


Fig.5. **Screening of the mutants towards pesticides**

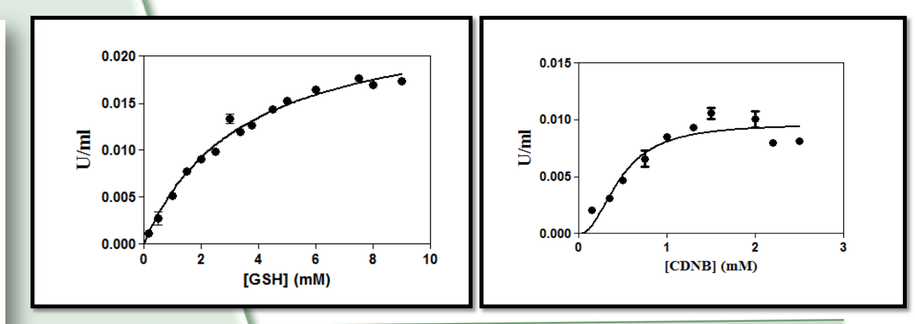


Fig.6. **Kinetic analysis of the immobilized mutant F117I**

The analytical method was based on the enzyme inhibition by α-endosulfan . The bioactive material consists of two different sol-gels that led to the immobilization of F117I mutant and the pH indicators (bromocresol purple and phenol red).

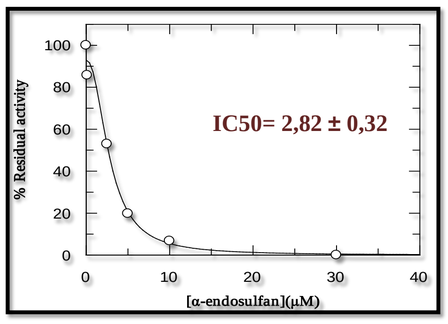


Fig.7. **Dose response curve of α-endosulfan/immobilized F117I mutant enzyme.**

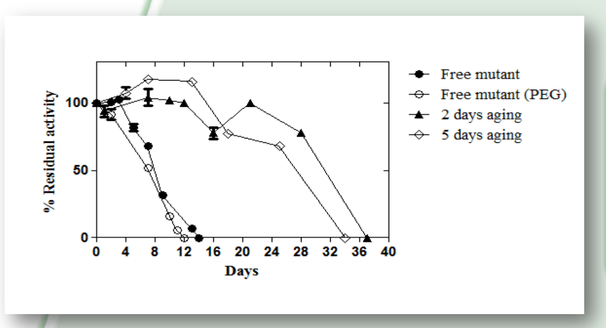


Fig.8. **Stability of the free and immobilized F117I mutant enzyme.**

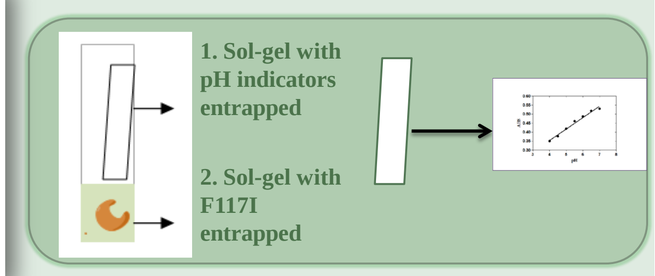
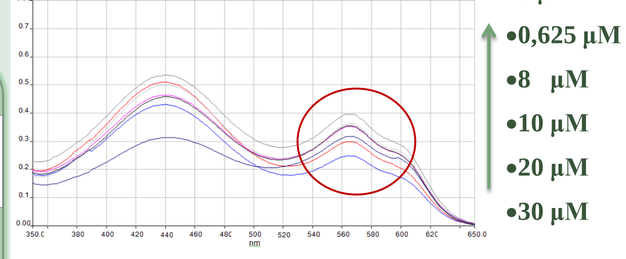
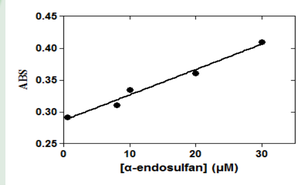


Fig. 9. T**he biosensor used for the development of the analytical method for α-endosulfan determination**





y=0.004 ± 0.287, R^2=0.98

Fig.10. **Spectra of indicators at different concentrations of α-endosulfan and calibration curve at 562 nm**

Conclusion

Sol–gel encapsulated enzymes have been widely employed in the construction of biosensors using different detection methods such as electrochemical and optical methods. In the present work a novel GST was created using DNA shuffling and site-saturation mutagenesis. The mutant F117I displayed higher selectivity towards organochlorine insecticides. The enzyme was immobilized in alkoxysilane (TEOS/PTMOS) sol-gel system in the presence of the pH indicators (bromocresol purple and phenol red). The bioactive material exhibits linearity in the range of 0.625-30 μM α-endosulfan at 562 nm and high operational stability.

REFERENCES

E. Chronopoulou and N.E. Labrou (2009). Glutathione Transferases: Emerging Multidisciplinary Tools in Red and Green Biotechnology. Recent Patents in Biotechnology 3(3):211-23.
Andreou, V.G., Clonis, Y.D. (2002). A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol–gel entrapped bromcresol purple for in-field use. Biosens. Bioelectronics .17, 61–69.
Sassolas, A., Blum, L.J., Leca-Bouvier, B.D. (2012). Immobilization strategies to develop enzymatic biosensors. Biotechn. Adv. 30, 489–511.
Kapoli P, Axarli IA, Platis D, Fragoulaki M, Paine M, Hemingway J, Vontas J, Labrou NE (2008) Engineering sensitive glutathione transferase for the detection of xenobiotics, Biosensors Bioelectronics, 24(3):498-503.

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

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) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.