Sol-gel immobilization of glutathione transferase: efficient tool for bioremediation

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Introduction

Glutathione transferases (GSTs, EC. 2.5.1.18) are multi-functional enzymes with an important role in xenobiotic detoxification. They catalyse the nucleophilic addition of the sulfur atom of glutathione (γ-L-Glu-L-Cys-Gly, GSH) to the electrophilic groups of a large variety of hydrophobic molecules including organic halides, epoxides, arene oxides, α- and β-unsaturated carbonyls, organic nitrate esters, and organic thiocyanates (Chronopoulou et al., 2014; Labrou et al., 2015). The conjugation of GSH to such molecules increases their solubility and reduces their toxicity.

GSTs represent a versatile tool with a variety of biotechnological applications, in the field of bioremediation to clean up environmentally contaminated sites. Several studies have been carried out exploiting the potential of GSTs, using purified proteins as well as engineered microorganisms and plants expressing GSTs (Rui et al., 2004; Park, 2012; Ryllota et al., 2014). The purpose of this project was the study of GST immobilization for the biodegradation of toxic compounds.

Material and methods

In the present work two different human GSTs and one plant GST were cloned, expressed in *E. coli* BL21(DE3), purified by affinity chromatography and were entrapped in silane derived sol-gel. The sol-gel process, involves hydrolysis of alkoxide precursors, phenyltrimethoxysilane (PTMOS) and tetraethoxysilane (TEOS), under acidic conditions followed by condensation of the hydroxylated units, which lead to the formation of a porous gel (**Fig. 1**). Then, under alkaline conditions a xerogel is constructed. Finally, the xerogel is further treated with TEOS for "aging" and improvement of its stability.

Results

The sol-gel entrapped GSTs exhibited higher storage and operational stability at 4˚C, compared to the free enzymes (**Fig. 2**). Kinetic analysis of the entrapped enzyme using 1-chloro-2,4-dinitrobenzene as model substrate showed that the entrapped GSTs did not show significant changes in their catalytic and kinetic properties compared to the free enzymes (**Fig. 3**). The biocatalysts were evaluated for the biodegradation of 1-chloro-2,4-dinitrobenzene (**Fig. 4**) . Under selected conditions the sol-gel entrapped GSTs were able to degrade >90% of the loaded 1-chloro-2,4-

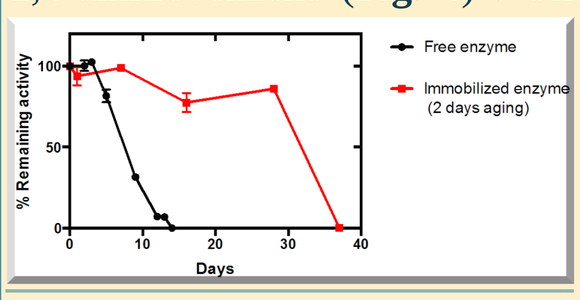


Fig. 2. Storage and operational stability of free and entrapped plant enzyme *PvGm*GST(F117I) at 4^0C.

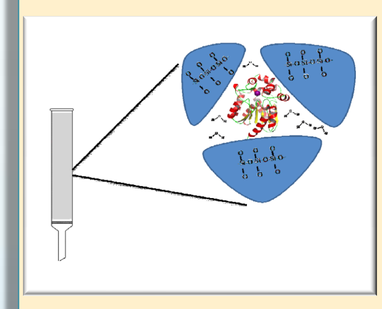
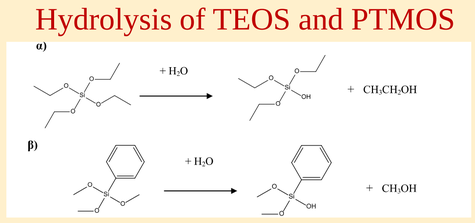


Fig. 4. Schematic diagram of the fixed-bed reactor based on sol-gel immobilization of GST.

The results of the present study suggest that the use of **GST biocatalysis** may provide a 'green chemistry' tool .

Hydrolysis of TEOS and PTMOS



Condensation

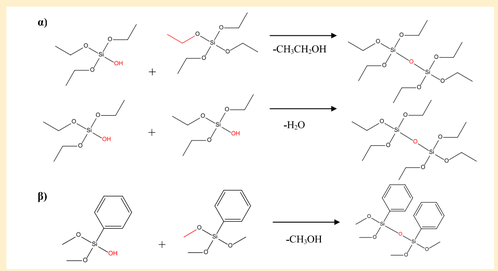


Fig. 1. The procedure of sol-gel is completed in two stages, hydrolysis and condensation.

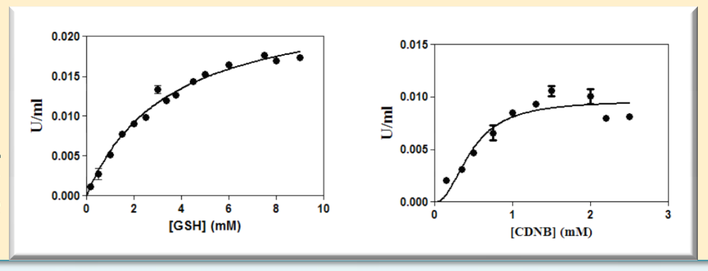


Fig. 3. Kinetic analysis of immobilized enzyme *PvGm*GST(F117I).

GSTs display wide substrate specificity towards halogenated aromatic compounds. The results showed that *PvGm*GST particular high activity towards CDNB (**Table 1**).

Table 1 . Substrate specificity for purified recombinant *PvGm*GST

Substrate	Specific activity (U/mg)
1-Chloro-2,4-dinitrobenzene	14,6
1-Bromo-2,4-dinitrobenzene	6,9
1-Iodo--2,4-dinitrobenzene	0,8

The catalytic efficiency of the immobilized enzymes were also evaluated in the presence of aromatic compounds such as benzophenone. In **Fig. 5** is shown the 1-chloro-2,4-dinitrobenzene degradation reaction by GST in the presence of benzophenone.

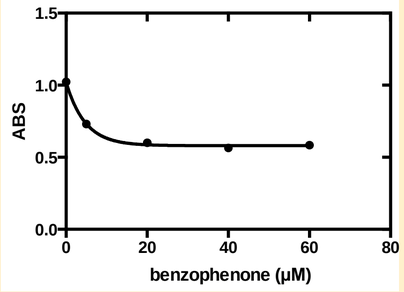


Fig. 5. 1-chloro-2,4-dinitrobenzene degradation reaction in the presence of benzophenone and immobilized enzyme *h*GSTA1-1.

Conclusion

The results of the present study demonstrate a new method of designing a bioreactor that can be operated over a longer period of time with high efficiency. The method has potential for the use of immobilized GSTs in the biodegradation of toxic compounds under process conditions using industrial wastes.

References

Chronopoulou E, Madesis P, Tsaftaris A, Labrou NE (2014). Cloning and characterization of a biotic-stress-inducible glutathione transferase from Phaseolus vulgaris. Appl Biochem Biotechnol. 172(2), 595-609.

Labrou NE, Papageorgiou AC, Pavli O, Flemetakis E (2015). Plant GSTome: structure and functional role in xenome network and plant stress response.Curr Opin Biotechnol. 32, 186-194.

Park H (2012). Reduction of antibiotics using microorganisms containing glutathione S-transferases under immobilized conditions. Environ. Toxicol. Phar. 34, 345-350.

Rui L, Kwon YM, Reardon KF & Wood TK (2004) Environ Microbiol 6, 491–500.

Rylott EL, Gunning V, Tzafestas K, Sparrow H, Johnston EJ, Brentnall AS, Potts JR, Bruce NC (2015). Phytodetoxification of the environmental pollutant and explosive 2,4,6-trinitrotoluene. Plant Signal Behav. 10:e977714.

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