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- Protein Engineering and Bioactive Molecules Laboratory, LIP-MB INSAT, **Pr M. Nejib Marzouki**
- Bioorganic and Bioinorganic Chemistry Team, UMR, CNRS 8182, Molecular and Materials Chemistry Institute, Orsay, France, **Dr Hafsa Youssoufi Korri**
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New peroxidases enzymes study and analysis of their applications in pollutants detection by innovative biosensors

Determination of hydrogen peroxide is of practical importance in chemical, industrial, environmental and other fields. Peroxidases (EC: 1.11.1.7) have been used in chemical, biological and clinical trials but novel applications in biosensors design are arousing more and more interest. Horseradish peroxidase (HRP) is the most widely used enzyme for biosensors design but new sources of peroxidases are now of great interest. Chitosan possesses many properties such as good biofilm-forming ability, chemical inertness, high mechanical strength, high hydrophilicity and biocompatibility which let it be an interesting immobilization method for enhancing stability of the peroxidase-based biosensor.

Peroxidase X1 (POX1) iso-enzyme was purified from garlic bulb (Allium sativum L.). Native-PAGE profile showed two isoforms partially purified (designated POX1A and POX1B). A POX1B-based electrode showed great potential for monitoring hydrogen peroxide in biological samples.

Chitosan was used as a matrix for enzyme immobilization. The enzyme activities were studied by photometry. Immobilization was accomplished by either inclusion in a thin film or adsorption to cross-linked microspheres. Two linkage agents were used: glutaraldehyde and glyoxal.

The effect of temperature on the immobilized enzyme was tested. Anchored POX1B inside chitosan was used for biosensor design.

Cyclic voltammetry and impedance spectroscopy were employed to analyze electrochemical properties of the modified gold electrode and to monitor hydrogen peroxide. The biosensor was very sensitive and attained a detection limit of 100 nM.

Construction of enzyme biosensors for phenolic compounds monitoring has already been investigated. With the aim to extend the practical use of new peroxidase POX_{1B} which has already shown great potential in hydrogen peroxide biosensing, a multifunctional enzyme electrode was designed for the monitoring of phenolic compounds in waste waters. The immobilized enzyme into chitosan microspheres retained full activity and stability. The mediator free POX_{1B}-based biosensor exhibited high sensitivity towards 2,6-dichlorophenol, 4-chlorophenol and pentachlorophenol since detection limits attained the nanomolar range and even the picomolar range for 4-chlorophenol and pentachlorophenol.

No reported biosensor for phenolic compounds detection has attained these limits at present. The kinetic constants of the enzyme reactions for the chlorophenols tested were calculated. Rapidity of the biosensor electrochemical response was evaluated by chrono-amperometry.