HRP/AuNPs/Polycyclosiloxane Bioelectrochemical System as a New Peroxide Sensor

Evelyn Ospina[1], M. P. Garcia Armada[1], J. Losada[1], B. Alonso[2], C. M. Casado[2]

[1] Dpto. Ingeniería Química Industrial y M. A., Universidad Politécnica de Madrid, José Gutierrez Abascal, 2,28006 Madrid, Spain
[2] Dpto. Química Inorgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain
pilar.garcia.armada@upm.es

Abstract

Ferrocenyl polymers, containing a cyclotetrasiloxane in the backbone can act as electrocatalyst in the direct oxidation of enzymes, the reduction of hydrogen peroxide generated in enzymatic reactions and in the oxygen spent in the enzyme-catalyzed reaction. Electrodes modified with these polymers have been used as mediators in amperometric enzyme electrodes for the detection of glucose [1].
In the other hand, it is widely know that nanostructures can improve the interface between biomolecules and an electronic transducer. The nanostructured surfaces have the advantages of a reduced distance between the redox center of proteins and the electrode together with the facilitation of electron transfer. Immobilizing metal nanoparticles on an electrode coated with a good electrocatalyst provide a three-dimensional structure similar to the microenvironment of redox proteins in biological systems [2], as well as facilitate the electron transfer. In addition, these surfaces allow a favorable support for immobilizing redox proteins and keep they in their native states.
Gold nanoparticles (AuNPs) have good biocompatibility and can act as a tiny conduction centers to improve electron transfer rates of redox proteins. AuNPs can be self-ensambled by means of the thiol functional groups which are present in the protein structure.
We present here a new peroxide biosensor with HRP covalently bonded at an AuNPs/ polycyclosiloxane electrochemical system with high reproducibility, sensitivity and good linear range. The biosensor was prepared by electrodepositing the cyclotetrasiloxane polymer on a Pt electrode surface that was further used for the electrochemical deposition of gold nanoparticles. AuNPs were obtained in a 4 g/L HAuCl4 solution by the adequate number of potential cycles into the margins from -0.4 V to 0.2 V at scan rate 0.02 V/s [2]. By means of this procedure, AuNPs aggregates between 185-200 nm with nanoparticles size about 20-44 nm were obtained. Figure 1 shows the AuNPs grown in the polymer-electrode surface.
The immobilization of HRP was done by incubating the modified electrode for 2 h 30 min in a 2mg/mL HRP in 0.1 M pH 7.0 phosphate buffer solution. Figure 2 shows the biosensor scheme.
In this work have been also investigated the electrochemical characterization, the kinetics and the electrocatalytic properties of modified electrodes in the reduction of hydrogen peroxide and the direct electrochemistry of HRP. The optimal applied potential to direct reduction of HRP was -0.2 V (vs. SCE). The obtained apparent overload heterogeneous constant was 43.1 μA/mM and the apparent Michaelis-Menten constant was 1.84 mM. Both values are indicative of the high enzymatic efficiency of the bioelectrochemical system.
Also the analytical properties of the new biosensor were studied at E= -0.2 V, and a linear range from 0 to 120 μM was obtained with the detection limit of 0.32 μM and sensitivity of 526 nA/μM cm^2.
This biosensor was also applied to determination of other organic peroxides [3]. The obtained sensitivities for the determination of cumene and tert-butyl hydroperoxides were 457 nA/μM cm^2 and 33 nA/μM cm^2 respectively.
Finally, this developed biosensor was successfully applied to determine hydrogen peroxide in real samples of a contact lens cleaning solution.

References

[1] J. Losada, M.P. García Armada, I. Cuadrado, B. Alonso, B. González, C.M. Casado, J. Zhang, Journal of Organometallic Chemistry, **689** (2004) 2799–2807.
[2] Q. Wan, H. Song, H. Shu, Z. Wang, J. Zou, N. Yang, Colloids and surfaces B: Biointerfaces **104** (2013) 181-185.
[3] M.P. Garcia Armada, J. Losada, I. Cuadrado, B. Alonso, B. González, C. M. Casado, J. Zhang, Sensors and Actuators B **101** (2004) 143-149.

Figure 1. UHSEM micrographs of Pt wires modified with the cylotetrasiloxane polymer and AuNPs.

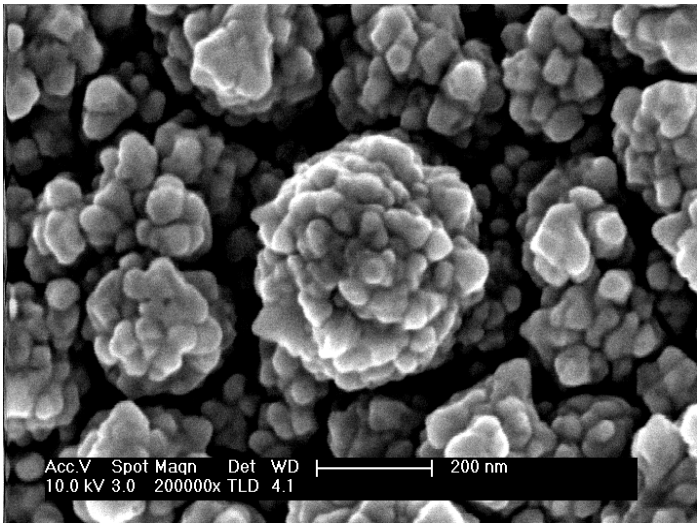

Figure 2. Scheme of the developed peroxide sensor.