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Use of glycosylated horseradish peroxidase to improve the stability of an amperometric enzyme-based biosensor

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One of the drawbacks of enzyme-based sensors is the rapid loss of activity because enzymes are very sensitive to their environment and thermodynamically intrinsically unstable. Several studies have shown that covalent modification of enzymes with glycans (Gly) can prevent activity loss and improve their long-term stability. To address this problem we glycosylate horseradish peroxidase (HRP) with lactose to construct a simple and low cost amperometric horseradish peroxidase biosensor based on a 4-mercaptobenzoic acid self-assembled monolayer on gold nano-particles (AuNPs) at the surface of a glassy carbon (GC) electrode. We compared the sensitivity, linear range, and detection limit (LOD) of the biosensor using the native and glycosylated form of the enzyme for this biosensor design. Circular dichroism (CD) spectra showed no change as result of glycosylation with lactose. The highest sensitivity of the biosensor to hydroquinone was obtained for the Lac-HRP-4-MBA-AuNPs-GC biosensor with 370 $nA\mu M^{-1}cm^{-2}$ compared to 308 $nA\mu M^{-1}cm^{-2}$ for the HRP-4-MBA-AuNPs-GC biosensor; showing that the glycosylated form of the enzyme catalyzed the reduction of hydroquinone more rapidly and efficiently than the native form of the enzyme. The LOD observed for the native HRP biosensor was 0.74 and 0.83 for the Lac-HRP biosensor respectively. The linear range for both biosensors was from 5- 300 μM. The E_a value obtained for native HRP biosensor was 3.49 $kJmol^{-1}$ and 1.20 $kJmol^{-1}$ for Lac-HRP biosensor. From this study we found that glycosylation of HRP does not affect the catalytic property of the enzyme. Also, the glycosylated HRP showed an improvement on the biosensor sensitivity for detection of hydroquinone.

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