S4-D.9

Electrochemical, Photoelectrochemical and Piezoelectric Analysis of Tyrosinase Activity by Functionalized Nanoparticles

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Abstract— The electrochemical and photoelectrochemical detection of tyrosinase (TR) activity (an indicative marker for melanoma cancer cells) is reported, using Pt nanoparticles (NPs) or CdS NPs as electrocatalytic labels or photoelectrochemical reporter units. The Pt Nps or CdS NPs are modified with tyrosine methyl ester, capping layer. Oxidation of the capping layer by TR/O_2 yields the respective L-DOPA and dopaquinone products. The reduction of the resulting mixture of products with citric acids yields the L-DOPA derivative, as a single product. The association of the L-DOPA derivative funtionalized Pt NPs or CdS NPs to a boronic acid monolayer-modified electrode enables the electrochemical transduction of H_2O_2 or the photoelectrochemical transduction of TR activity by the generation of photocurrents in the presence of triethanolamine as a sacrificial electron donor. The detection limits for analyzing TR corresponds to 1 U and 0.1 U by the electrochemical and photoelectrochemical methods, respectively. The association of the Pt NPs or CdS NPs to the functionalized monolayer electrode is followed by quartz crystal microbalance measurements.

Tyrosinase is a copper containing protein that catalyzes the oxidation of phenol derivatives, such as tyrosine or tyramine, in the presence of O_2 to the respective catechol derivatives, e.g., L-DOPA or dopamine, that are further oxidized by the enzyme to the respective quinone products. Elevated amounts of tyrosinase were detected in a melanoma cancer cellsi and the enzyme is considered as an indicative marker for this type of malignant cells[1]. Also it was reported that the loss of dopamine in neurons cause disease such as Parkinson disease[2]. Similarly, dopamine is a central neurotransmitter, and its sensitive detection, particularly with integrated miniaturized devices, could be valuable for the invasive monitoring of neural response.

Several recent reports addressed different optical and electrochemical methods to detect tyrosinase activity. The luminescence of semiconductor quantum dots modified by a tyrosine derivative was quenched by the tyrosinase-mediated oxidation of the tyrosine residues to the respective quinone units, thus providing an optical assay for enzyme[3]. The biocatalytic oxidation of a tyramine monolayer-modified Au electrode by tyrosinase to the respective dopamine monolayer, followed by the association of ferrocene boronic acid, acting as a redox label, to the resulting catechol monolayer, was used to develop an electrochemical sensor that follows tyrosine activities[4]. Also the tyrosinase-stimulated oxidation, and consequently the accompanying potential changes of the tyramine- or dopamine-functionalized gate surfaces of field-effect transistor devices, was used to electronically monitor tyrosinase and its activity[5]. Metallic nanoparticles or semiconductor quantum dots are often used as catalytic[6] or optical[7] labels for biosensing events.

In this study, it has been introduced different methods to analyze the activity of tyrosinase through the biocatalalyzed oxidation of the tyrosine methyl ester functionalized Pt or CdS nanoparticles. The oxidation of amidated-L-tyrosine methyl ester-modified Pt nanoparticles and the subsequent coordination of the resulting catechol-amidated L-DOPA methyl ester-functionalized Pt nanoparticlesto the boronic acid monolayer functionalized electrode enabled the amplified analyses tyrosinase activities by the electrocatalyzed reduction of H2O2 by the Pt nanoparticle labels. Similarly, it has been introduced the use of the amidated-L-tyrosine methyl ester ---

-modified CdS nanoparticles for the photoelectrochemical analysis of tyrosinase through the association of the enzyme generated-amidated L-DOPA methyl ester-functionalized CdS nanoparticles to the boronic acid monolayer-functionalized electrode. Illumination of the CdS nanoparticles-functionalized electrode generated photocurrents in the presence of triethanolamine (TEOA) as a sacrificial electron donor. The quartz crystal microbalance (QCM) method provided a complementary tool to characterize the association of the Pt nanoparticles and CdS nanoparticles to the boronic acid monolayer-functionalized Au electrode. Besides the quantitative characterization of the surface coverage of the nanoparticles on the piezoelectric crystal, the frequency change, and the accompanying mass change on the crystal, yielded an additional readout signal for the activity of tyrosinase. Last of all, comparison of detection limits of the two analytical methos in this study with other electrochemical and optical procedures to detect tyrosinase

H_2 molecules, two linking Ti-atom can absorb only 4 H_2 totaling to 14 H_2 per dimer. The gravimetric density for dimmer is ~10 wt %. The complex can also form stable paramagnetic polymer which can store hydrogen more than 6 wt %.

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