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A Novel Electrochemical Enzyme Biosensor for Detection of 17β-Estradiol by Mediated Electron-transfer System.

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Abstract:

In this work, an extremely sensitive enzyme sensor for detection of 17β-estradiol based on electropolymerized L-lysine molecules on a glassy carbon electrode (GCE) modified with critic acid@graphene (CA-GR) and cross-linked with laccase enzyme has been developed in this work. The morphology of the enzyme modified electrode was characterized by TEM, SEM and FTIR. The electrochemical detection of 17β-Estradiol was successfully conducted by mediated electron-transfer system and detection limit of 17β-Estradiol was as low as 0.13 pM. The human urine samples analysis confirmed the application of this enzyme sensor to quantitative analysis of ultra-trace hormone.

Keywords: laccase enzyme, poly L-lysine, 17β-Estradiol, thionine, electrochemical sensor.

Introduction

17β-Estradiol (E2), a natural steroid with estrogenic activity, excessive E2 remained in animal food like meat and milk will cause women's fertility problem, increase the risk of exposure to ovarian and breast cancer [1]. Therefore, studying 17β-Estradiol is significant for clinical analysis/diagnosis. Conventionally, various of methods, such as HPLC, immunological methods, gas chromatography, and chemiluminescence, have been developed for detecting 17β-Estradiol [2]. However, these methods require complex precipitation procedures and it suffer from low sensitivity, time consumption and high cost [3]. Hence, it is extremely urgent to develop a simple, rapid and sensitive method for the determination of E2. Enzyme based on electrochemical sensor is one of the most promising sensors for ultra-trace detection in complex environment, due to its high specificity and excellent accurate.

In this work, a novel 17β-Estradiol sensor was fabricated by laccase loading with CA-GR and electro-polymerized L-lysine film modified glassy carbon electrode (Lac/PLLY/CA-GR/GCE) as sensing platform. The as-prepared sensor showed good stability and low detection limit (0.13pM) for the determination of 17β-Estradiol.

Characterization of Lac/Poly L-lysine/CA-GR/GCE

Fig. 1 is the morphology and microstructure of different modified materials investigated by SEM and TEM. Fig.1A shows that the highly polydisperse sphere-like laccase molecules of 150nm±50nm were coated on nanocomposite materials. Fig. 1 B, the CA-GR film presents the flake-like and crimple shapes structure. Fig.1(C), poly L-lysine nanoparticles loads on crimple shape of CA-GR. After cross-linking laccase, the surface

of GCE was covered with uniform laccase sphere which had been illustrated in Fig.1(D).

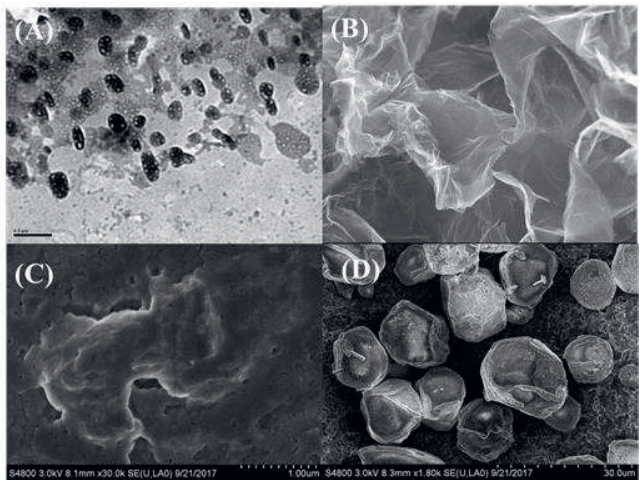

Fig.1. TEM images of (A) Lac/Plly/CA-GR/GCE and SEM images of (B) CA-GR/GCE, (C) Plly/CA-GR/GCE and (D) Lac/Plly/CA-GR/GCE

Fig. 2 (A) shows differential pulse voltammograms toward 0.5 pM 17β -estradiol at a series of modified electrods, the For Lac/Plly/CA-GR/GCE, the peak current became larger than bare GCE obviously. Fig. 2(B) shows the electrochemical impedance spectroscopy of different modified electrodes. the CA-GR/GCE shows a small semicircular, which represents faster electron-transfer kinetics of [Fe(CN)6]3- / 4- compared to bare electrode. However, after the poly L-lysine films produced on the modified electrode surface, the Rct (80.42 Ω) was enlarged. When the laccase anchored on the Poly L-lysine films, the Rct (1064.07 Ω) was obviously enlarged, that because of its high selectivity electrochemical catalysis, what's more, its large size impede electron transferring.

Experimental result

As the Fig. 2(C) showed, differential pulse voltammograms toward 0.5 pM 17β -estradiol in 0.1 M sodium phosphate buffer, pH 7.0 containing 1.2 mM thionine as electrochemical mediator at bare GCE, CA-GR/GCE, Poly L-ly/CA-GR/GCE, Poly L-ly/CA-GR/Lac/GCE. For Lac/Poly L-ly/CA-GR/GCE, the peak current became larger than others obviously. Fig. 2B shows the amperometric signal of 17β-estradiol at different concentration ranging from 0.4pM to 57pM on Lac/Poly L-ly/CA-GR/GCE. As shown in Fig. 2D,a linear regression equations: Ipa= 0.2364 + 0.197c (pM) (R2=0.992) was obtained. The detected limit was 0.13pM.

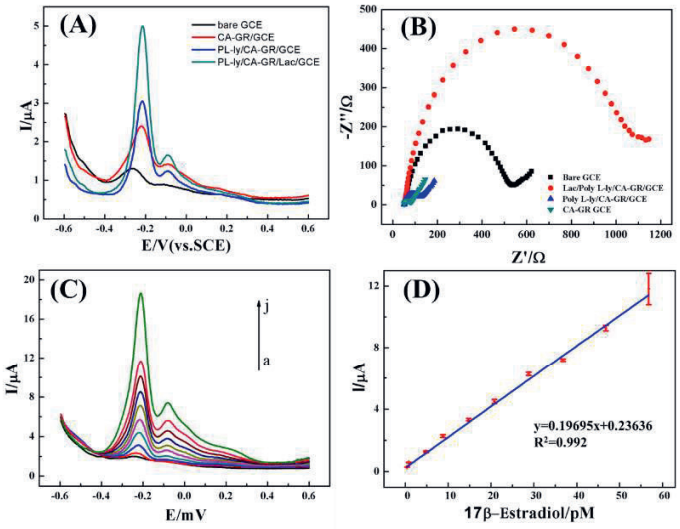

Fig. 2 (A) Electrochemical impedance spectra of different modified electrodes (B) DPV of 5pM 17β -estradiol in 0.1M PBS (pH 7.5) at bare GCE, CA-GR/GCE, Poly L-ly/CA-GR/GCE, Lac/Poly L-ly/CA-GR/GCE. (C) DPV recorded at the enzyme sensor for 17β -estradiol concentrations form bottom to top 0.4pM to 57 pM. (D) Calibration curve constructed for 17β -estradiol at the Lac/Poly L-ly/CA-GE/GCE.

Table. 1. Results of real sample analysis of Lac/PLLY/CA-GR/GCE

Samples	*Detected (μM)*	*Added (μM)*	*Found (μM)*	*Recovery*
Human urine	*-*	*14.5*	*14.9*	*102.8%*

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