



## Research paper

# Direct electron transfer and sensing performance for catechin of nano-gold particles-polymer nano-composite with immobilized Laccase



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## ABSTRACT

An electrochemical sensor for catechin detection was prepared. This sensor based on the electrode covered by nano-composite with Laccase. As-prepared nano-composite was made up of 4-mercaptobenzoic acid functionalized nano-gold particles and polymer. Site-directed adsorption of Lac in matrix led to stable immobilization of Lac and improved its direct electrochemistry. The Lac-based electrode was sensitive to catechin with high selectivity and low detection limit (16 nM). This novel sensor exhibited excellent reproducibility, long-term stability and high tolerance to enzyme inhibitor. Result of test in industrial sewage convinced the liability of Lac-based sensor characterized with high performance liquid chromatography and electrochemical method.

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## 1. Introduction

Phenol is one class of the important raw materials and by-products resulting from chemical production with high toxicity. It is hard to be eliminated through the method of organisms or abiotic degradation. Emissions in the industrial production of phenol-like compounds if directly discharging into the water, would pose a serious threat to residents' drinking water and agricultural irrigation water. So the real-time monitoring of the concentration of phenols in aqueous system is the first step in detection and processing of city industrial and domestic water pollutants. At present commonly used methods for the phenols determination including spectrophotometry, gas chromatography, high performance liquid chromatography (HPLC) and so on, expose their disadvantages such as: inconvenience in on-line detection, expensive instrument, and long testing period. All these shortcomings limit their application in field detection for economic underdeveloped regions, especially for remote areas. Enzyme based sensor serves as a new type of phenol-like compounds detector, with the preferences of simple preparation, low cost, high selectivity, quick response and continuous online testing, shows its inferiority in susceptibility to environmental impact resulting in response performance attenuation and short service life [1].

Laccase (Lac) is a typical class of blue multi-copper oxidase with efficient catalysis for aromatic compounds and oxygen molecules

[2,3]. However, Lac as sensitive primitive of electrochemical sensor essentially requests that direct electron transfer be achieved between attached Lac molecules on the electrode surface and conductive support. Due to complicated structure of active centers in Lac molecules, any illustrated case has not included that rapid direct electron transfer between enzyme and electrode achieves through reconstitution of apo-enzyme on the interface of conductive matrix [4,5] so far. Numerous reports provide examples of functional nano-device modified electrodes with Lac as electrochemical sensors to detect phenol-like compounds, aromatic amine compounds and oxygen molecules [6–8]. Thermal stability, acid base tolerance, durability and reproducibility of those Lac based detectors should be improved further.

Nano-gold particles display such advantages as good conductivity, controllable particle size, large specific surface area and favorable bio-compatibility. They have been widely used as matrix to accommodate bio-macromolecules such as protein, DNA and lipid [9]. When nano-gold particles tend to come into direct contact with the enzyme molecule, coordination configuration distortion and valence state change of active center in Lac molecules might appear, causing the loss of catalytic activity and the failure of direct electrochemistry of enzyme-based electrode [10]. Previous reports [7,9] indicate that nano-device modified with macro  $\pi$ -conjugated systems, i.e.: aromatic rings or aromatic heterocyclic rings, may not only immobilize protein molecules firmly on the surface of matrix on the premise that inherent activity of enzyme be retained but also achieve direct electron transfer between enzyme and basal supporting electrode.  $\pi$ - $\pi$  stacking effect

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together with complexation occurred between  $\text{Cu}^{2+}$  in Lac and hetero-atoms of tethered functional groups in polymer units helps to secure goals mentioned previously. As long as the direct electron transfer rate is fast enough and difference in electrocatalytic reduction potential between quinine and di-oxygen molecules is big enough, both of them might be detected without interference under variable applied potential, respectively. In this letter, we demonstrate nano-composite through hybrid of Chitosan-*g*-*N*-carboxymethyl-2-sulfo-4, 5-2H imidazolinone and 4-mercaptobenzoic acid surface-tailored nano-gold particle, is used as enzyme carrier to prepare glassy carbon electrode with entrapped Lac. Applied performance of this Lac based electrochemical sensor for catechin detection is evaluated by electrochemical means (cyclic voltammetry and chronoamperometry) and result of test is compared with other Lac-based electrochemical sensors (its liability is confirmed with traditional chromatography (HPLC)). Results from tests prove this Lac-based electrochemical sensor shows its favorable sensor function to catechin monitoring and potential appliance value in industrial pollutants monitoring and control.

## 2. Experiment

### 2.1. Reagents and apparatus

Tetrachloroauric acid tetrahydrate ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ), 4-mercaptobenzoic acid (4-MBA), *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxy succinimide (NHS) are purchased from Aladin reagent Co., Ltd (Shanghai, China). Lac from *Trametes Versicolor* ( $M_w$ : 68 000), 2,6-dimethoxyphenol (DMP) and polyethylenimine (PEI,  $M_w$ :  $7.5 \times 10^5$ ) are obtained from Sigma reagent Co., Ltd (USA). Chitosan (CTS, degree of deacetylation:  $\geq 90\%$ ,  $M_w$ : 250 000) is purchased from Shanghai Shengyao Biotech Co., Ltd (China). Other commonly used chemicals are of analytical grade without extra explanation obtained from sinopharm Co., Ltd (China). Used buffer solution in tests is 0.2 M potassium dihydrogen phosphate (PBS). All solutions are prepared with Milli-Q ultrapure water.  $\text{N}_2$  and  $\text{O}_2$  are provided by Nanjing special gas Co, Ltd (China).

### 2.2. Materials and methods

#### 2.2.1. Preparation and characterization of Lac-based electrode

4-Mercaptobenzoic acid surface-tailored nano-gold particle (denoted as 4-MBA@GNP) and chitosan-*g*-*N*-carboxymethyl-2-sulfo-4,5-2H imidazolinone (denoted as CTS-*g*-*N*-CSIDZ) were synthesized and characterized according to previous Refs. [11,12], respectively. Preparation of 4-MBA@GNP was briefly summarized as following: tetrachloroauric acid and 4-MBA were co-dissolved in 35 mL of mixed solution (volume ratio of methanol to acetic acid: 6:1) with molar ratio: 1:3. 15 mL solution of methanol containing 0.3 g  $\text{NaBH}_4$  was added into mixture mentioned previously with rapid stirring. Produced black suspension was refluxed for 30 min and kept stirring for another 30 min after it was cooled down to room temperature. Product was then transferred to centrifuge tube and the centrifugal sedimentation of disperse phase was performed for 10 min at rotating rate: 8000 round/min. Black product was washed three times with diethyl ether and dried in  $\text{N}_2$  stream subsequently. Final product was collected for use in the future. Gold disk electrode (diameter: 1 mm) as basal support was pretreated as undermentioned procedure: basal electrode was polished on 3500# emery paper with 1.0 and 0.5  $\mu\text{m}$  alumina slurry consecutively and was rinsed ultra-sonically twice with acetone and distilled water, respectively. Firstly basal gold disk electrode (obtained from Aida Hengsheng industrial commerce Co., Ltd, Tianjin in China) was immersed into dendrimer polymer PEI solution

(Wt%: 0.5) for 8 h after pretreatment to secure amino group surface-tailored electrode. Subsequently  $\text{NH}_2$  functionalized gold disk electrode was dipped into methanol-acetic acid hybrid solution (ratio of volume: 10/1) with 0.1 M EDC, 0.02 M NHS and 0.5  $\text{g L}^{-1}$  4-MBA@GNP (mean size of nano-gold particles: 15 nm) for 3 h to obtain nano-gold particles tethered electrode. Batch of 20  $\mu\text{L}$  ether solution with 2.5  $\text{g L}^{-1}$  CTS-*g*-*N*-CSIDZ was dip-coated on the surface of nano-gold particles modified electrode and dried at ambient temperature at least for 15 min to acquire nano-composite thin film capped electrode. Finally 0.2 M PBS (pH = 6.0) containing 5.0  $\text{g L}^{-1}$  Lac was dipped onto the nano-composite modified electrode and the Lac-entrapped electrode was stored at 4 °C overnight. Dried electrode was rinsed with batches of 0.2 M PBS before use to remove unbound Lac molecules on the outer surface of electrode and was kept in refrigerator while not use. As-prepared Lac based electrode was denoted as Lac/CTS-*g*-*N*-CSIDZ-4-MBA@GNP/GD. Preparation of other controlled electrodes were similar to previous mentioned Lac based electrode except for a few omitted step or modification of over-coat layer on the surface of electrode: Preparation of nano-composite without enzyme modified electrode abbreviated the step of adding PBS with Lac or used CTS as film former (denoted as CTS-*g*-*N*-CSIDZ-4-MBA@GNP/GD and CTS-4-MBA@GNP/GD, respectively), while those of nano-gold particle with and without Lac were similar to Lac/CTS-*g*-*N*-CSIDZ-4-MBA@GNP/GD except for 4-MBA@GNP as conductive matrix and enzyme carrier alone instead of nano-composite (denoted as Lac/4-MBA@GNP/GD and 4-MBA@GNP/GD, respectively). In order to insure favorable reproducibility of result in tests, five electrodes capped by nano-composite with immobilized Lac molecules were prepared for each electrochemical experiment.

Leaching tests were performed as description in Ref. [9] with a small modification as following: as-prepared electrode over-coated by nano-composite with immobilized Lac was dipped into stirring PBS (pH = 4.4) for 30 min and was incubated at 4 °C for 8 h. Then the Lac based electrode was removed from PBS and the supernatant was mixed with 500  $\mu\text{L}$  10.0 mM DMP under magnetically stirring for 3 min to allow adequate chemical oxidation of DMP, followed by recording the absorbance change at 470 nm with time on the spectrophotometer. It should be noted that the absorbance changes were proportional to enzyme leaching amounts of nano-composite in the presence of shearing strength resulting from liquid flowing.

#### 2.2.2. Evaluation on performance of Lac-based sensor for catechin

All electrochemical measurements were performed in a routine three-electrode glass cell with volume of 25 mL, connected to a typical computerized electrochemical working station CHI-1140A (Chen-hua, Shang-hai in China). The Lac based electrode acted as working electrode, with Ag/AgCl (saturated KCl) electrode as the reference electrode and self-made platinum spiral wire as auxiliary electrode. 0.2 M PBS was used as electrolyte bubbled with  $\text{N}_2$  when studying direct electrochemical behavior of Lac-based electrode, while oxygen-saturated PBS was used when investigating the catalytic effect on oxygen reduction reaction (ORR). Catalytic effects on ORR of Lac-based electrodes were characterized by current difference at potential corresponding to limited diffusion current  $|i_{\text{Oxygen}} - i_{\text{Nitrogen}}|$ . Currents density ( $j$ ) was derived from normalization of current to active area ( $A_s$ ) of electrode (i.e.  $j = |i_{\text{Oxygen}} - i_{\text{Nitrogen}}|/A_s$ ) measured as Ref. [13] demonstrated previously, indicating  $\text{K}_3\text{Fe}(\text{CN})_6$  was used as electro-chemical probe and the active surface area of the electrode was derived from the slope of the fitted linear plot of anodic/cathodic peak currents vs square roots of scan rates. All potentials were relative to normal hydrogen electrode (NHE) without additional statement and all electrochemical experiments were performed at least three times under room temperature ( $25.0 \pm 0.4$  °C) without extra declaration. Cyclic voltammetry

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