Journal of Electroanalytical Chemistry 808 (2018) 59–64

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Electrogenerated chemiluminescence of lucigenin at mesoporous platinum electrode and its biosensing application to superoxide dismutase

Sungju Nam, Won-Yong Lee*

Center for Nanomedicine, Institute for Basic Science (IBS), Seoul 03722, Republic of Korea
Department of Chemistry, Yonsei University, Seoul 03722, Republic of Korea

ARTICLE INFO

Keywords:
Electrogenerated chemiluminescence
Lucigenin
Mesoporous platinum electrode
Superoxide anion
Superoxide dismutase

ABSTRACT

The electrogenerated chemiluminescence (ECL) behavior of lucigenin on a mesoporous platinum electrode has been studied in neutral aqueous solution (pH 7). The present mesoporous Pt electrode with highly enlarged surface area, with a roughness factor of 301, exhibits much larger electrochemical responses for the oxidation of water and the reduction of molecular oxygen, which leads to much larger lucigenin ECL responses related to the anodic and cathodic electrochemical reactions even in a neutral solution, compared to those at a bare Pt electrode. Based on the capability of superoxide dismutase (SOD) to inhibit lucigenin ECL by scavenging superoxide anions, a highly sensitive ECL bioassay for SOD has been developed with a linear dynamic range of 5.75×10^{-10} M–3.00×10^{-6} M and a detection limit of 3.49×10^{-10} M (S/N = 3), which is much lower than those obtained with other detection methods.

1. Introduction

Electrogenerated chemiluminescence (ECL) of lucigenin (*N*,*N*′-dimethyl-9,9′-biacridinium dinitrate) has been first reported in 1939 by Tammamushi and Akiyama [1]. They observed ECL in alkaline solution when lucigenin is reduced at a platinum (Pt) electrode along the path of hydrogen gas. After that, extensive electrochemical and spectral studies on the ECL of lucigenin have been made at a mercury electrode in aqueous system and at a Pt electrode in non-aqueous system in 1969 by Legg and Hercules [2]. They observed strong ECL from the reaction of lucigenin with electrochemically generated superoxide from oxygen in relatively polar nonaqueous solvents. Since then, several studies on the ECL of lucigenin have been carried out in aqueous solutions at different electrodes.

Most reported studies on analytical applications of lucigenin ECL in aqueous solutions utilize cathodic ECL of lucigenin [3–11], many of which involve the addition of materials to the system or further modification of the electrode surface with carbon materials or other means of signal enhancement in order to amplify the electrochemical signal. For instance, detection of glutathione through the inhibition reaction of cathodic lucigenin ECL with MnO_2 nanosheets in alkaline solution at glassy carbon electrode has been reported [4]. Only a few reports have been published on the electrochemical analysis using the anodic ECL of lucigenin, including the detection of SOD through inhibition of anodic lucigenin ECL [12]. However, it involves modification of the electrode surface with carbon materials, and it was performed in alkaline solution since ECL was not observed in acidic or neutral conditions.

A mesoporous Pt electrode provides significant enhancement of the electrochemical signals for kinetic-controlled reactions since they are affected by the microscopic surface area of the electrode. For a diffusion-controlled reaction, however, the electrochemical signal is identical to that of a bare Pt electrode since reactants are depleted inside the mesopores [13]. Since lucigenin ECL involves the oxidation of water and the reduction of molecular oxygen, such characteristics of a mesoporous Pt electrode can provide highly enhanced signal for the lucigenin ECL in neutral aqueous system without the use of additional modifications to the electrode or further addition of other materials for signal enhancement.

Superoxide dismutase (SOD) is an enzyme that plays an important role in nearly all living cells as it provides defence against oxidative damage from superoxide free radicals. In humans, three types of SOD isozymes have been identified: SOD1, SOD2, and SOD3. Numerous studies have shown overexpression of SOD2 in multiple cancer cells such as breast cancer, pancreatic adenocarcinoma, papilloma, and prostate carcinoma cancer cells [14–17]. Also, it has been reported that the overexpression of SOD1 is associated with symptoms of Down's syndrome [18,19]. In skin disorders, SOD1 is found to increase in quantity to protect cells against oxidative stress due to the exposure of radiation and thalassemia [20,21]. Quantitative analysis of SOD is crucial for accurate diagnosis of such diseases.

* Corresponding author at: Center for Nanomedicine, Institute for Basic Science (IBS), Seoul 03722, Republic of Korea.
E-mail address: wylee@yonsei.ac.kr (W.-Y. Lee).

https://doi.org/10.1016/j.jelechem.2017.11.056
Received 27 September 2017; Received in revised form 21 November 2017; Accepted 21 November 2017
Available online 23 November 2017
1572-6657/

Sensors for bioanalytical applications are required to perform in neutral aqueous solutions. Moreover, in order to acquire high sensitivity, enhancement of the electrochemical signal is essential, especially when ECL quenching reaction is involved in the analysis. The use of mesoporous Pt electrode allows ECL sensors to be applicable for bioanalysis without compromising their distinctive merits of high sensitivity and selectivity, and enhanced temporal and spatial control. Based on the capability of SOD to inhibit the anodic ECL of lucigenin by its inherent ability to catalyze the reduction of superoxide anions, a simple and sensitive ECL bioassay for the detection of SOD has been developed.

2. Experimental

2.1. Materials and reagents

Hydrogen hexachloroplatinate (IV) hexahydrate, octaethylene glycol monohexadecyl ether ($C_{16}EO_8$), superoxide dismutase from bovine erythrocytes, lucigenin (*N*,*N*′-dimethyl-9,9′-biacridinium dinitrate), and plasma from human were purchased from Sigma-Aldrich Corporation, and were used without further purification.

2.2. Instrumentation

Cyclic voltammetric (CV) experiments were performed with an EG&G 263A potentiostat/galvanostat (Oak Ridge, TN). ECL measurements were performed with a Hamamatsu Photonics HC 135-02 photon counting module (Hamamatsu city, Japan). A conventional three-electrode system with a 5 mL electrochemical cell was used for all CV and ECL measurements. A platinum wire and Ag/AgCl (3 M NaCl) were used as counter and reference electrodes, respectively. A conventional gold electrode (0.0314 cm^2, Bioanalytical Systems, West Lafayette, IN) was used as a substrate for the mesoporous Pt film. The electrochemical cell was placed directly in front of the photomultiplier tube (PMT) for the ECL measurements, and the entire ECL system was enclosed in a light-tight box.

2.3. Preparation of the mesoporous Pt electrode

Fabrication of the mesoporous Pt electrode was performed using a previously reported method by Park et al. [13]. A mixture of $C_{16}EO_8$, distilled water, and hydrogen hexachloroplatinate (IV) hexahydrate was heated to 80 °C until a transparent homogenous mixture was obtained. The temperature of the mixture was then lowered to room temperature. The electrodeposition of Pt was carried out on a polished gold electrode (0.0314 cm^2) at constant potential of − 0.06 V *vs.* Ag/AgCl (3 M NaCl). The resulting mesoporous Pt electrode was placed in distilled water for 1 h to extract the $C_{16}EO_8$, in which the process was repeated 3–4 times. The electrode was then electrochemically cleansed in 1 M sulfuric acid using cycling potential between − 0.26 and + 1.3 V until reproducible cyclic voltammograms were obtained. After all experiments, the electrode was cleansed with the same method described above and stored in distilled water until next use.

3. Results and discussion

3.1. Voltammetric behavior of mesoporous Pt electrode

A gold electrode with identical geometrical surface area as the control bare disk Pt electrode (0.0314 cm^2) was used as a substrate for the mesoporous Pt deposition. The microscopic surface area of bare and the fabricated mesoporous Pt electrodes were compared with cyclic voltammograms obtained in 1.0 M sulfuric acid. As shown in Fig. 1, the curves corresponding to the formation and removal of platinum hydride were observed in the potential range from *ca.* + 0.1 V to *ca.* − 0.25 V for both bare and the fabricated mesoporous Pt electrodes, but the peak currents of the mesoporous Pt electrode were much more distinct with more than 100-fold enhancement on the current signals.

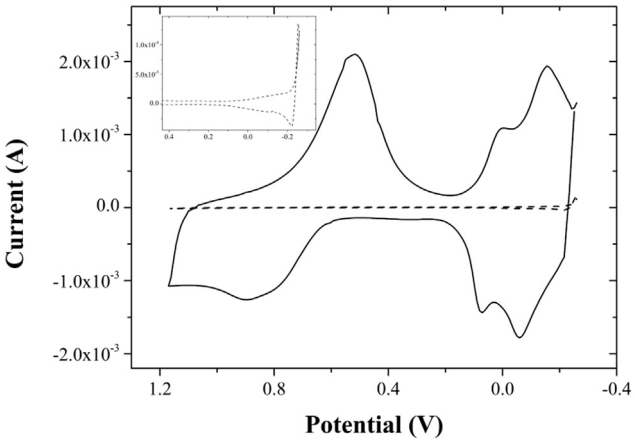

Fig. 1. Cyclic voltammograms of 1.0 M H_2SO_4 at mesoporous Pt electrode (solid line) and bare Pt electrode (dashed line). Scan rate: 200 mV/s, scan range: from − 0.26 V to 1.3 V. Inset: Enlarged CV at bare Pt electrode under identical conditions.

The roughness factors, which represent the ratio of the microscopic area to the geometric area, of both electrodes were calculated from the curves in Fig. 1 using a conversion factor of 210 μC cm^{-2} [22], and were found to be 1.48 for bare and 301 for mesoporous Pt electrode. This confirmed that the microscopic area of the fabricated mesoporous Pt electrode was enlarged greatly compared to a bare Pt electrode with identical geometrical surface area.

Cyclic voltammograms at a bare Pt electrode in the absence and presence of 0.5 mM lucigenin, prepared in pH 7 phosphate buffer solution (PBS), were compared with those at a mesoporous Pt electrode, as shown in Fig. 2. Three cathodic peaks were observed at − 0.03 V, − 0.50 V, and − 0.86 V at mesoporous Pt electrode, which correspond to the reduction of platinum oxide, reduction of O_2, and adsorption of H_2 on the platinum surface, respectively. The results were similar to those reported by Han et al. [23]. The reduction potential for O_2 reduction was significantly more positive at the mesoporous Pt electrode (− 0.50 V) compared to bare Pt electrode (− 0.78 V), and the current signal was also larger at the mesoporous Pt electrode. This behavior was in agreement with previously reported results for mesoporous Pt electrode by Birkin et al. [24].

3.2. ECL behavior of lucigenin

The potential-ECL profiles of 0.5 mM lucigenin at a bare and a mesoporous Pt electrode were obtained by cycling the electrode potential from − 1.0 V to + 1.5 V at 100 mV/s in pH 7 PBS, as shown in Fig. 2. For a bare Pt electrode (Fig. 2A), one anodic ECL peak at *ca.* + 1.5 V and one cathodic ECL peak at *ca.* − 1.0 V were observed. Similarly, for a mesoporous Pt electrode (Fig. 2B), two anodic ECL peaks at *ca.* + 1.0 V (ECL-1) and + 1.5 V (ECL-2) and two cathodic peaks at *ca.* − 0.5 V (ECL-3) and − 1.0 V (ECL-4) were observed. In comparison with the bare Pt electrode, ECL-2 was significantly enhanced by *ca.* 15-fold due to the enlarged surface area of the mesoporous Pt electrode, and a slight signal enhancement of ECL-3 by *ca.* 2-fold was observed.

It has been reported that two different mechanisms exist for the anodic ECL of lucigenin that involve the generation of $O_2^{\cdot -}$: one proposed by Qiu et al. [12] where hydroxide ion is oxidized to produce $O_2^{\cdot -}$, and another proposed by Su et al. [25] where water is oxidized to produce highly reactive OH radicals, which further oxidizes to produce $O_2^{\cdot -}$. It is highly probable that the separation of the anodic ECL of lucigenin into two ECL peaks (ECL-1 and ECL-2) at mesoporous Pt electrode is due to the existence of both of the mentioned reactions occurring at the surface of the mesoporous Pt electrode. Both reactions result in ECL emission from NMA*, the excited state of *N*-methylacridone, which is produced from the reaction between the generated $O_2^{\cdot -}$ and lucigenin in solution.