A boron-doped diamond electrode decorated with hemoglobin-modified platinum nanoparticles as a biosensor for acrylamide detection

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Abstract. Acrylamide (AA) is a neurotoxin and potential carcinogen. It has been found in various thermally processed foods, e.g., potato chips and biscuits. Thus, simple, rapid, and sensitive methods for AA detection are needed to ensure food safety. Herein, the fabrication of a highly stable AA biosensor is presented. A boron-doped diamond (BDD) was modified by Pt and hemoglobin. In the first step, platinum nanoparticles (Pt NPs) were chemically seeded onto the BDD surface using NaBH₄ as a reducing agent. The electrochemical overgrowth of these Pt NP seeds was conducted at a constant potential of −0.2 V in a 1 mM Pt solution. Then, rapid thermal annealing (RTA) of the BDD/Pt NP composite was conducted at 700 °C under N₂ atmosphere to enhance its stability. After RTA, BDD/Pt NP was electrochemically activated between −0.5 and 1.5 V. Then, further overgrowth was performed using a deposition voltage of −0.2 V to renew the BDD/Pt NP surface. Finally, 0.15-mM hemoglobin was used to modify BDD/Pt NP. The characterization of the resulting surface was performed using scanning electron microscopy. The biosensor exhibited an optimal response (limit of detection = 0.012 nM) at pH 4.9 in a 0.2-M acetate buffer solution.

Keywords: BDD, platinum, hemoglobin, acrylamide, biosensor

1. Introduction

Recently, multiple studies have focused on food safety, most of which are concerned with the monitoring of harmful substances, e.g., acrylamide (AA), in food products and ingredients. AA is a neurotoxin and a potential carcinogen [1] that is materialized in reactions between reducing sugars, e.g., glucose and asparagine. The Maillard reaction mechanism is responsible for its formation in high-content starch food during cooking process at high temperatures (>120 °C) [2]. The standard methods employed for the detection and determination of AA levels in food products are GC-MS/MS [3], LC-MS/MS [4], and HPLC-MS [5]. However, these methods are laborious and expensive [1]. Accordingly, inexpensive biosensors for AA that can be easily produced and used have recently been developed. Garabagiau and MihaiElesc [1] developed a simple hemoglobin (Hb)/Au nanoparticle (NP)-modified indium tin oxide glass electrode for the amperometric detection of AA [1]. Krajewska et al. [6] developed an electrochemical biosensor for AA, which comprised Hb that was deposited on single-walled carbon nanotubes. Li et al. [7] developed a facile electrocatalytic Hb redox system using flower-like Au NPs supported on a boron-doped diamond (BDD) surface. Umam et al. [8] developed a
biosensor comprising a Hb/Au NP-modified BDD electrode for the detection of AA with a limit of detection (LOD) of 5.1 μM. However, this biosensor was found to be unstable.

Many researchers have focused their attention on enhancing the electron transfer properties of Hb using nanostructured metals and alloys, such as Ag, Au, Pt, Pd, Ir, Rh, Ag/Au, Pt/Ag, and Pt/Pd, as mediators or promoters [9,10]. However, electrochemical deposition of metal NPs on BDD substrates was found to show poor stability due to its sp coordination [11,12]. In addition, their electrochemical activities vary with different substrates and deposition methods [13]. On the other hand, Gao et al. [10] reported wet-chemical seeding method to deposit highly stable Pt NPs on diamonds.

In this study, the wet-chemical seeding method was employed to prepare the Pt NPs-modified BDD [10]. This work presents highly stable and active Pt NPs on BDD, which is coated with Hb molecules to promote electron transfer from the electrolyte to the electrode, for the determination of AA via the formation of its Hb adduct.

2. Experimental

2.1 Materials and instruments

BDD was obtained from Keio University, Japan, with the specifications of 0.1% B/C ration and 1000-μm thicknesses H₂PtCl₆·6H₂O was purchased from Wako, Japan. Human Hb H7379 and AA were purchased from Sigma-Aldrich. All reagents were used as received without further purification.

Electrochemical experiments were conducted using an eDAQ potentiostat (Bioelectrochemistry Main Laboratory, Universitas Indonesia). An electrochemical containing of Pt and Ag/AgCl was used as the counter and reference electrodes, while the BDD modified with Pt NPs and Hb as the working electrode. Scanning electron microscopy (SEM) images were recorded using an EVO MA 10 instrument (Carl Zeiss Microscopy Gmbh, Germany).

2.2 BDD electrode modification with Pt NPs

The deposition of Pt NPs on BDD was performed with 1-M NaBH₄ in 0.1-M NaOH and 1-mM H₂PtCl₆ solution. First, 10 μL of the NaBH₄ solution was dropped onto a BDD electrode. Then, 40 μL of the 1-mM H₂PtCl₆ solution was added onto the sample for a reaction with adsorbed NaBH₄, generating Pt NPs. The electrode was then washed with water and dried under N₂ flow. Once the seeding process completed, the electrochemical overgrowth of Pt seeds was performed in 5 mL of 1.0-mM H₂PtCl₆ solution for 15 min under a fixed potential of −0.2 V. The Pt NPs-modified BDD was then subjected to rapid thermal annealing (RTA) at 700 °C for 5 min under N₂ atmosphere before activated by cyclic voltammetry in the potential range of −0.5 to +1.5 V at 200 mV/s scan rate for 100 cycles. Subsequently, forth overgrowth was conducted at −0.2 V deposition voltage for 15 min to renew the Pt NP surface.

2.3 Modification of Pt/BDD electrodes with Hb

A 10-μL drop of 0.2-M acetate buffer solution (ABS) solution (pH 4.9) containing 0.15-mM Hb was placed onto a Pt/BDD electrode for 24 h to obtain a Hb/Pt NP-modified BDD electrode. The electrode was rinsed with Milli-Q water and dried in a stream of N₂ before use. The modified electrodes thus obtained were kept in air at 4 °C.

2.4 Detection of AA standard

AA solutions of various concentrations (0.01, 0.02, 0.03, 0.04, and 0.05 nM) were prepared in 0.2 M ABS (pH 4.9). Samples were then analysed using cyclic voltammetry with the Hb/Pt NP/BDD electrode between −0.5 V and 1.5 V at a scan rate of 100 mV/s.

3. Results and discussion

3.1 BDD electrode modification process

BDD provides positively charged C–H dipole layers on the surface [10], therefore, enables NaBH₄ adsorption in the process of metal particles wet chemical seeding. The major functional group in NaBH₄ plays a role in the adsorption process is non-polarized BH₄⁻. Accordingly, an attractive force is generated, which enhances the adsorption of NaBH₄. [14], NaBH₄, and H₂PtCl₆ chemical reaction generates Pt NPs, the reaction is highly familiar and commonly used [15–17]:

NaBH₄ + 3H₂O + H₂PtCl₆ → Pt + H₂BO₂⁻ + 5HCl + NaCl + 2H₂↑
Figure 1. Scanning electron microscopy (SEM) images of Pt/BDD (a) before and (b) after rapid thermal annealing (RTA) and their related EDX traces (d and e, respectively). Inset of (a) shows the surface of the unmodified BDD.

Figure 2. Cyclic voltammograms for Pt nanoparticle (NP)-coated BDD electrodes in 0.1-M HSO₄, at a scan rate of 100 mV/s. (a) As-deposited scenario, (b) scenario after RTA, and (c) scenario after RTA and electrochemical activation.

The key to a successfully seeded surface is the adsorption of NaBH₄ before the Pt NPs growth, leading to the supersaturation of the surface. It is also possible to generate similar supersaturation via pulse deposition at a constant potential of −0.2 V. In this way, the nucleation density on the BDD electrode surface is enhanced [14].

RTA helps to form an epitaxial lattice structure between BDD and Pt NPs. Accordingly, RTA of Pt NP-coated BDD was conducted at 700 °C under N₂ atmosphere for 5 min. Figure 1a and figure 1b show the SEM images of Pt/BDD before and after RTA, respectively. The SEM of unmodified BDD
3.2 Response of the AA biosensor

AA forms an adduct with Hb as a result of the reaction with the NH group of its N-terminal valine. The formations of this adduct causes changes in the Hb structure [4,5]. This change generally decreases the current responses as the accessibility to the redox-active centre of Hb at the electrode surface was disturbed. Thus, Hb–AA adduct formation alters the electroactivity of Hb, generating the response of the biosensor. Figure 3a shows an SEM image of Hb/Pt/BDD, and figure 3b shows the CV measurements for the Pt/BDD with and without immobilization of Hb.

Figure 3. (a) SEM image and (b) cyclic voltammogram response of Pt/BDD electrodes toward hemoglobin (Hb) at different concentration of 0.15 mM.

Figure 4. (a) Cyclic voltammograms showing the response of the Hb/Pt/BDD electrodes toward AA at different concentrations (0, 0.01, 0.02, 0.03, 0.04, and 0.05 nM). (b) Linearity of the response of the acrylamide (AA) biosensor to AA concentration.

was shown in the inset of figure 1a for comparison. The SEM images show that Pt distributed more homogeneously on the BDD surface, after RTA. Figures 1c and figure 1d show that the EDX spectra of Pt/BDD before RTA and that after RTA have almost identical elemental composition.

Figure 2 shows the cyclic voltammetry (CV) results for Pt NP-coated BDD electrodes in 0.1-M HSO4 before RTA, after RTA, and after electrochemical activation. Before RTA, a pair couple of peaks were noticeably observed for hydrogen adsorption/desorption, suggesting that the Pt NPs are electrochemically active. Upon completion of the RTA, the peaks disappeared and the background current similar to that of bare BDD was the only one observed, showing a reactivity loss by Pt NPs toward hydrogen adsorption/desorption. This phenomenon could be caused by the passivation film formation, for the carbon dissolves in Pt at high temperature and precipitate on metal surface upon cooling process [18].

To acquire an active Pt NPs, surface reactivation electrochemically was performed by cycling in the 0.1-M HSO4 solution. The strategy is to oxidize and reduce the Pt NPs surface repeatedly, that is refreshed the electrode surface [19]. As shown in figure 2, after RTA and electrochemical activation the hydrogen adsorption/desorption peaks reappeared after this treatment.

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The Hb/Pt/BDD electrode exhibits a quasi-reversible electrochemical reaction (Hb–Fe³+/Hb–Fe²⁺). As shown in figure 4, the current peak in the cyclic voltammogram decreases in parallel with an increase in the Hb–AA adduct concentration at the electrode surface. As a result, this biosensor exhibits an electrochemical response toward AA in the linear range 0.01–0.05 nM. The results confirm that an AA biosensor with a considerably low detection limit (0.012 nM) was successfully fabricated.

4. Conclusions
An AA biosensor was successfully fabricated via the Hb modification of Pt NPs attached to a BDD electrode. The resultant device exhibited relatively rapid response and a considerably low detection limit (0.012 nM).

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