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Development of neuraminidase detection using gold nanoparticles boron-doped diamond electrodes

Wulan T. Wahyuni [a], Tribidasari A. Ivandini [a, *], Endang Saepudin [a], Yasuaki Einaga [b, c, **]

[a] Department of Chemistry, Faculty of Mathematics and Sciences, University of Indonesia, Kampus UI Depok, Jakarta 16424, Indonesia
[b] Department of Chemistry, Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan
[c] JST ACCEL, Yokohama 223-8522, Japan

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ABSTRACT

Gold nanoparticles-modified boron-doped diamond (AuNPs–BDD) electrodes, which were prepared with a self-assembly deposition of AuNPs at amine-terminated boron-doped diamond, were examined for voltammetric detection of neuraminidase (NA). The detection method was performed based on the difference of electrochemical responses of zanamivir at gold surface before and after the reaction with NA in phosphate buffer solution (PBS, pH 5.5). A linear calibration curve for zanamivir in 0.1 M PBS in the absence of NA was achieved in the concentration range of 1×10^{-6} to 1×10^{-5} M ($R^2 = 0.99$) with an estimated limit of detection (LOD) of 2.29×10^{-6} M. Furthermore, using its reaction with 1.00×10^{-5} M zanamivir, a linear calibration curve of NA can be obtained in the concentration range of 0–12 mU ($R^2 = 0.99$) with an estimated LOD of 0.12 mU. High reproducibility was shown with a relative standard deviation (RSD) of 1.14% ($n = 30$). These performances could be maintained when the detection was performed in mucin matrix. Comparison performed using gold-modified BDD (Au–BDD) electrodes suggested that the good performance of the detection method is due to the stability of the gold particles position at the BDD surface.

Neuraminidase (NA) detection is substantial in controlling the disease caused by related virus and microbes because NA is an important enzyme in the pathogenic viruses and microbes spreading. Common detection methods of NA, such as reverse transcription polymerase chain reaction (RT–PCR), enzyme-linked immunosorbent assay (ELISA), and enzymatic reaction [1–3], require several types of chemical reagents, specific instruments, and highly skilled operators. Therefore, a more simple and practical method is urgently required. Accordingly, an electrochemical detection method of NA based on its reaction with zanamivir was developed using gold and gold-modified boron-doped diamond (Au–BDD) as the working electrodes [4].

Zanamivir (Fig. 1A) is an NA inhibitor. On the other hand, zanamivir decreases the current peaks of the oxidation and reduction of gold, which is proposed due to its adsorption on the electrode surfaces [4]. It is presumable that the same active sites that inhibit NA are used to adsorb zanamivir at the gold surface [4]. Therefore, if NA was mixed with zanamivir before the electrochemical measurement is performed, zanamivir will bind to NA and decrease its adsorption at the gold surface. Consequently, when electrochemical measurements are performed, the currents of gold oxidation and reduction increase (Fig. 1B). Furthermore, better detection performance was achieved at an Au–BDD than at a bulk gold electrode due to its low background current, which induces the higher signal currents [4].

On the other hand, boron-doped diamond (BDD) has been successfully used in electrochemical detections for some

Abbreviations: NA, neuraminidase; ELISA, enzyme-linked immunosorbent assay; Au–BDD, gold-modified boron-doped diamond; BDD, boron-doped diamond; AuNPs, gold nanoparticles; LOD, limit of detection; SEM, scanning electron microscopy; UV–vis, ultraviolet–visible; TEM, transmission electron microscopy; XPS, X-ray photoelectron spectroscopy; PBS, phosphate buffer solution; EDX, energy dispersive X-ray spectroscopy; CV, cyclic voltammogram; RSD, relative standard deviation.

* Corresponding author. Department of Chemistry, Faculty of Mathematics and Sciences, University of Indonesia, Kampus UI Depok, Jakarta 16424, Indonesia.
** Corresponding author. Department of Chemistry, Faculty of Science and Technology, Keio University, Yokohama 223-8522, Japan.
E-mail addresses: ivandini.tri@ui.ac.id (T.A. Ivandini), einaga@chem.keio.ac.jp (Y. Einaga).

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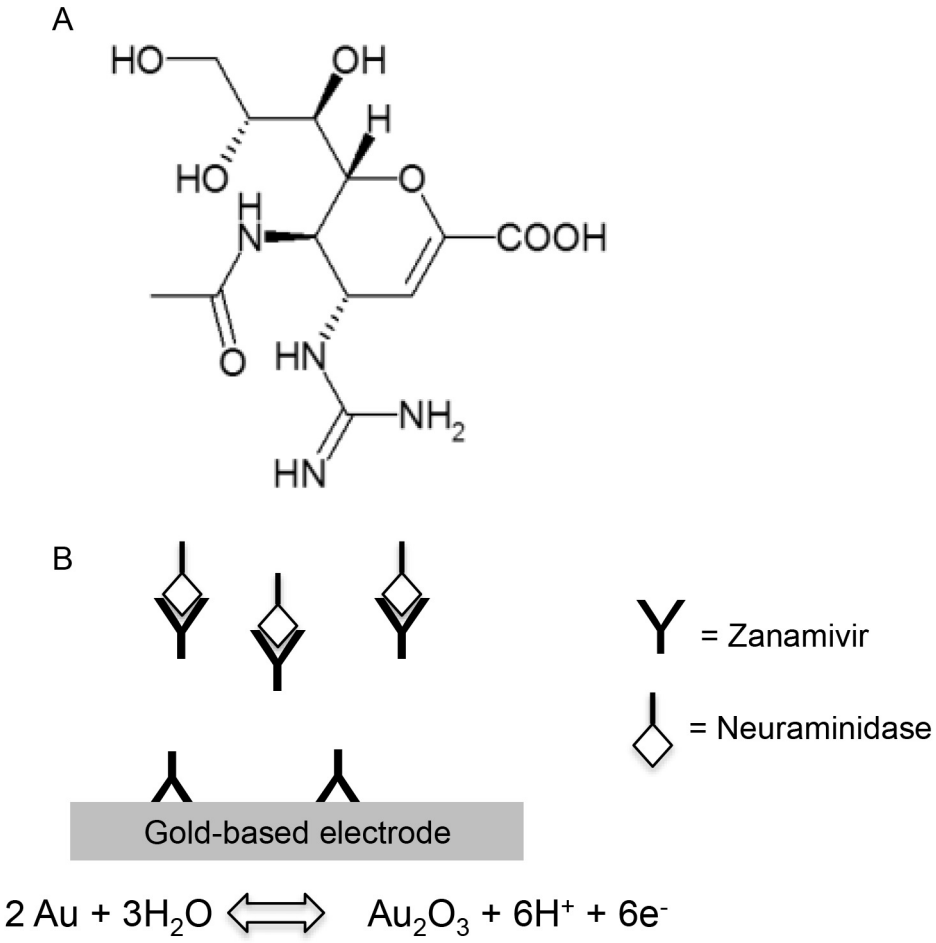

Fig. 1. (A) Molecular structure of zanamivir. (B) Pictorial design of the detection method of neuraminidase using zanamivir at a gold-based electrode.

important substances due to its superior properties, including low background current, wide potential window, and excellent physical and chemical stability [5–8]. However, it is reported that the stability limits its potency in sensing applications because it is difficult to modify the surface of BDD [5,9–11]. In the previous work of NA detection, Au–BDD was prepared by the electrochemical deposition method [4], which is very simple and easy to conduct. Unfortunately, this electrode results in less stability of the current responses because the physical interaction between gold particles and the BDD surface is not stable and easy to release [10,12]. Other methods to deposit AuNPs at the BDD surface include vacuum vapor deposition and sputtering [13–15]. Nevertheless, these methods are also based on physical interaction between gold and BDD. In this work, AuNPs–BDD prepared with a self-assembly deposition method of AuNPs at amine-terminated BDD was used to increase the analytical performance of NA detection. It is reported that better stability of the current responses could be performed by AuNPs–BDD due to the covalent interaction between AuNPs and the lone pairs of electrons from amine functional groups at the BDD surface [10,12]. Comparison was also made with gold-modified BDD prepared by the electrochemical deposition method (Au–BDD). The results showed that although the current sensitivity of AuNPs–BDD was lower than that of Au–BDD due to the amount of Au on the surface, a better limit of detection (LOD) and higher stability in the mucin matrix were observed, indicating that the electrodes are more suitable for application in a real sample.

Materials and methods

Materials

Zanamivir and allylamine were supplied by Tokyo Chemical Industry, whereas neuraminidase (from *Clostridium perfringens*) and mucin from bovine submaxillary glands were obtained from Sigma–Aldrich. $KAuCl_4 \cdot 4H_2O$ and other chemicals were supplied by Wako Chemicals (Japan). An Ag/AgCl (saturated KCl) system was obtained from BAS Chemicals, platinum wire was obtained from Nilaco (Japan), and high-purity water with maximum conductivity of 18 MΩ was obtained from a Simply Lab water system (Direct-Q 3 UV, Millipore).

Preparation of BDD films

Boron-doped diamond films were deposited on Si (100) wafers in a microwave plasma-assisted chemical vapor deposition (MPCVD) system (Cornes Technologies). Methane and trimethoxyborane (B/C ratio of 1:1000) were used as carbon and boron sources, respectively. Details of the preparation are described