Pt-Au interdigitated array electrodes for biosensing applications

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Simple and robust properties are the important aspects for biosensing chip. We have fabricated Pt and Au interdigitated array electrodes (Pt-Au IDEs) for amperometric biosensor. A spontaneous electrical current generation was observed depending on H₂O₂ concentration. The glucose oxidase (GOx) immobilized Pt-Au IDEs exhibited a glucose sensor action in which H₂O₂ produced by the enzymatic reaction of GOx was detected through Pt-Au IDEs. The phenomenon demonstrated that Pt-Au IDEs can provide a simple amperometric biosensor without an external potential source nor a reference electrode.

Key words: interdigitated array electrodes, amperometric, biosensor, glucose, enzyme

1. INTRODUCTION

An amperometric biosensor based on an enzymatic reaction generally requires a potentiostat instrument to control the applying voltage, since enzymatic activity is transformed to the electrical current signal by applying a constant voltage [1,2]. The typical measurement system of enzyme based sensor involves a potentiostat, a reference electrode, and a pair of working and counter electrodes. Accordingly the total measurement set can be a costly and complex for personal use. If it is possible to build a simple enzyme based biosensor working with a patterned working-counter electrodes and an ammeter, such simple sensor system will have a great advantage for easy and low cost measurement.

H₂O₂ is a common product of general oxidative enzymes. Most of biosensors detect H₂O₂ production to monitor the enzymatic activity [2]. Now, if H₂O₂ itself can generate electrical current without applying potential, the above mentioned simple biosensor system would be built. For this purpose, we focused on the phenomenon found in the nano-size bimetallic nanorod, a nano scale object of combined two kind of metals at each end, that shows a self-propelled motion by taking energy from the environment [3,4,5]. The mechanism is based on an electrocatalytic conversion of H₂O₂ to H₂O and O₂ on

each surface. It was estimated that a current generation was occurred inside the nanorod. The reaction on both surfaces have been extensively studied and was estimated to be the followings: (anode) H₂O₂ → O₂ + 2H⁺ + 2e⁻, (cathode) H₂O₂ + 2H⁺ + 2e⁻ → 2H₂O (Fig.1) [3]. At the anode side, a proton and an electron are generated by oxidation of H₂O₂ and they are consumed by the reductive reaction of H₂O₂ on the cathode side. Therefore, as shown in Fig. 1, the electrons move from the anode to cathode inside the nanorod, and the protons flow in the same direction on the nanorod surface.

To develop a new device based on this principle, we fabricated the Pt-Au interdigitated array electrodes (Pt-Au IDEs) which has arranged platinum and gold electrodes with a constant interval of micro scale. It is expected that the very narrow interval IDE can reproduce the same reactions occurring on the bimetallic nanorod. In the short-circuiting circuit between both electrodes, an electrometer is connected to detect the current generation accompanying the oxidation-reduction of H₂O₂. In fact, Paxton et al made a current measurement on the similar short-circuiting between Pt-Au with an electrometer, and succeeded in observation of the current generation [4]. However, they have not confirmed the sensing performance of this principle.

In this study, we fabricated Pt-Au IDEs and characterized the H₂O₂ sensor performance then examined the application to enzyme based biosensor. The advantages of Pt-Au IDEs biosensor are the simple measurement system of non-requirement of external potential source nor reference electrode together with the robust nature of Pt-Au noble metal electrodes. To demonstrate the applicability of biosensors, we have fabricated a glucose sensor combined with glucose oxidase enzyme (GOx) and Pt-Au IDEs. The GOx is immobilized with layer-by-layer (LbL) method on the Pt-Au IDEs [6]. It is expected that Pt-Au IDEs generates the electric current linearly depending on glucose concentration through H₂O₂ production of GOx activity.

Fig. 1 Electrochemical mechanism of current generation in bimetallic nanorod through H₂O₂ decomposition.

2. EXPERIMENTAL

2.1 Chemicals

A 30% H_2O_2 aqueous solution and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were purchased from Wako Pure Chemical Industries. An aqueous solution (20%) of poly(allylamine) hydrochloride (PAA), GOx from *Aspergillus* (E.C. 1.1.3.4) and D-(+)-glucose were purchased from Sigma-Aldrich. All reagents were of analytical grade and used as received without further purification. Water was purified using a Millipore water purification system. HEPES buffer was prepared by adding 5.6 g in 500 mL of deionized water. Then, the pH of the HEPES buffer was adjusted with 100 mM KOH or HCl.

2.2 Sample preparation

Pt-Au IDEs were fabricated on a quartz glass plate (1 cm $\times$ 2 cm $\times$ 0.05 cm, Daico, Japan) using photolithography technique (Fig.2). Ti/Au layers and Ti/Pt layers (thickness 400/2000 Å) were deposited on the SiO_2 surface of the quartz plate and were patterned through lift-off process. The Pt-Au IDEs with 175 pairs of fingers (7 mm length, 10 μm width) were separated 10 μm gap between adjacent fingers. To clean the surface, Pt-Au IDEs were pretreated in piranha solution (3:1 mixture of H_2SO_4 and H_2O_2) for 10 min. then performed cyclic voltammetry (CV) measurement between -0.2 and 1.5 V at a scan rate of 0.05 V/s (vs. Ag/AgCl) until a stable voltammetric response of clean Au or Pt surface was obtained [7, 8].

Fig. 2 A schematic picture of Pt-Au interdigitated array electrodes.

To immobilize GOx on the Pt-Au IDEs, we employed LbL method. Here, PAA and GOx were dissolved separately in 0.05 M pH 7 HEPES buffer at a concentration of 5 mg/mL for LbL assembly. GOx is negatively charged at pH 7 because its isoelectric point is 4.3 [9]. LbL films were assembled by immersing the Pt-Au IDEs alternately into positively charged PAA solution for 5 min. and negatively charged GOx solutions for 5 min. respectively, interspersed by a washing step of 1 min in buffer to remove loosely adsorbed molecules. We repeated this procedure 6 times to deposit 6 layers of PAA/GOx films on Pt-Au IDEs to obtain (PAA/GOx)₆/Pt-Au IDEs. After the deposition finished, the sample was carefully dried with nitrogen gas flow.

2.3 Amperometric measurements

Amperometric experiments were performed with an electrometer at room temperature. All amperometric

experiments were carried out in 0.05 M pH 7 HEPES buffer. The buffer solution was stirred by a magnetic stirring bar (rpm = 100) during the amperometric experiments. The measurements were performed under the successive injection of a H_2O_2 solution. For glucose sensor measurements of GOx immobilized sample, a dense glucose solution was dropped instead of H_2O_2 solution.

3. RESULTS AND DISCUSSION

3.1 H_2O_2 sensor performance

Fig.3 shows the amperometric responses to H_2O_2 injections. The amount corresponded to 0.05 – 1 mM increase for each injection. The short-circuit current between Pt and Au electrodes increased at the each moment of H_2O_2 introduction, which indicates that the current was generated by the H_2O_2 . It was found that the response time to reach a steady state was around 10 s. The lowest limit of detection, where a signal-to-noise ratio becomes 3, was estimated to be 0.05 mM. Fig. 4 shows the analytical curve of the current versus the concentration of H_2O_2 . The figure exhibits that Pt-Au IDEs has a broad linear response range from 0.05 to 100 mM for H_2O_2 detection.

Fig. 3 Amperometric response of Pt-Au IDEs to H_2O_2 . The arrows show the moment of H_2O_2 injection whose amount corresponds to an increase of the indicated concentration.

Fig. 4 Analytical curve obtained for Pt-Au IDEs. For the vertical current value, the change in observed current from that of 0 mmol/L is plotted in this figure.

3.2 Glucose sensor application

In the above section, we have shown that Pt-Au IDEs can detect H_2O_2 through an amperometric measurement with an ammeter only. Here we examine to apply this phenomenon to build a simple amperometric biosensor system with a sensor chip and an ammeter. Since H_2O_2 is the common product of oxidative enzyme family, its electrochemical detection is a representative method to form a biosensor [10]. In order to examine this idea, we fabricated $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDEs in which GOx is immobilized on Pt-Au IDEs as layer-by-layer films with PAA. Note, GOx is a representative oxidase that catalyses the oxidation of glucose to H_2O_2 and gluconolactone.

The GOx immobilized $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDEs was immersed into HEPES buffer (pH 7.0) and examined the glucose sensor action through amperometric response. Current generation was monitored as successive injections of glucose. Fig. 5 shows the amperometric responses of $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDE to glucose injections. For each injection, an occurrence of current generation was clearly observed. This current generation indicates that H_2O_2 is produced through the enzymatic reaction of GOx with glucose, then the H_2O_2 generates the electric current through the reaction occurring on Pt-Au IDEs. Based on the result of amperometric response, the analytical curve of current versus glucose concentration was obtained (Fig. 6). The linear range of 1- 8 mM of glucose concentration was confirmed in this figure, which indicates that the $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDE performs as glucose sensor action in this range. In the higher concentration, the current production saturated and the nonlinear response appeared. The origin of such nonlinear behavior may be attributed to be the limited efficiency of GOx turnover described by Michaelis-Menten equation [11]. However, this point is not clear now.

Fig. 5 Amperometric response of $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDEs to glucose. The arrows show the moment of glucose injection whose amount corresponds to an increase of 1 mmol/L glucose concentration

Fig. 6 Analytical curve obtained for $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDEs

4. CONCLUSION

We have fabricated Pt-Au IDEs with 10 μm gap. Amperometric measurement revealed that an electric current appeared in the short-cut circuit between Pt and Au electrodes by H_2O_2 injection. The calibration plot demonstrates that Pt-Au IDEs has a wide linear response range in H_2O_2 concentration (0.05 to 100 mM). This phenomenon can be applied for building an simple and robust biosensor based on amperometry: no requirement of external potential source and the stable nature of noble metals. To demonstrate this idea, we have immobilized GOx on the Pt-Au IDEs with LbL method. It was observed that 6 layers immobilized $(\text{PAA}/\text{GOx})_6/\text{Pt-Au}$ IDEs exhibited a clear amperometric response to glucose injection. The linear range was confirmed below 8 mM of glucose. The result indicates that Pt-Au IDEs can provide a simple amperometric biosensor without potential source nor reference electrode.

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