Glucose Sensor Based on Au-Pt Black Electrode
-Preparation of Functionally Different Sites on Electrode Surface

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An enzyme sensor (glucose sensor) based on a composite metal (Au and Pt) black electrode, which was fabricated by simultaneously codepositing gold and platinum electrode, has been developed. In this sensor, enzyme was immobilized at the gold sites on the electrode surface, and the enzymatic product was oxidized at platinum sites. The largest current response was obtained when the electrode was prepared in an electrodepositional solution containing 40:60 mol% gold and platinum complexes. The existence of such an optimal mixing ratio suggested functionally different roles of gold and platinum sites on the electrode.

Key Words: Platinum, Gold, Electrodeposition, Glucose Sensor

1 Introduction

In the past decade, nanoscale technology has been attracting people in the various fields. Compliant with this trend, molecular level regulation of 2-D or 3-D structure at the electrode surface has become an exiting area in electrochemistry.

Especially, in the case of biosensor, molecular level arrangement of biomolecules at the surface of electrode is one of the current topics. For such studies, gold electrode provides suitable surface, where molecular monolayer is self-assembled via melcaptide-bonding. Other studies reported three-dimensional structure of enzymes. For instance, Hoshi et al. proposed sequential construction of enzyme multilayer with avidin/biotin pair as a binding agent. Similarly, Yoon and Kim constructed multilayered enzyme film on a gold surface by stepwise stacking of periodate-oxidized glucose oxidase and fourth-generation (G4) poly (amidoamine) dendrimers. These experiments resulted linear correlation between the number of layers and the electrode current upon the application of enzyme substrate.

The above approaches are the arrangement of molecules in the perpendicular direction to electrode surface. Although it is not in the case of enzyme assembly, Gradner et al. reported lateral arrangement of functionalized molecules (they call orthogonal self-assembly). They fabricated Au and ITO pattern simultaneously on a single Si3N4 substrate, and they applied thiols and functionalized carboxylic acids to this substrate. They demonstrated selective assembly of thiols and carboxylic acids on the Au and ITO microstructures.

Although their approach provides an attracting method to arrange molecules in the parallel direction with the electrode surface, we cannot regard it a di-dimensional molecular scale structuring, since the resolution of Au and ITO pattern was µm level.

Recently, we reported a nanostructured gold-platinum electrode (Au-Pt black electrode). Such gold-platinum structure was fabricated by electrochemically codepositing Au and Pt on a platinum electrode. We believe Au and Pt are dispersed on the electrode surface with an atomic scale. In the present article, an enzyme sensor (glucose sensor) based on a gold-platinum codeposited electrode is reported.

Goorde et al. fabricated a glucose sensor where a platinum layer was electrodeposited on an enzyme immobilized gold surface. They suggested that gold surface is suitable to immobilize enzymes, while platinum surface has highly catalytic surface for the oxidation of enzyme products. However, their sensor has some uncertainty with regard to the structure of the platinum layer. It must have pores to permeate substrate to the enzyme layer, and it must have contact points with the basal gold electrode to conduct electrons. Their sensor has a practical drawback, that it takes 3–10 minutes to obtain steady current, probably due to the diffusion of substrate through the platinum layer.

Our idea is to prepare functionally different sites with an atomic or molecular scale in the lateral direction to the electrode surface. We designed enzyme molecules immobilized at the gold sites on electrode surface, and the enzymatic product catalyzed at the platinum sites; so that the sensor response would be larger than the gold black-based sensor, more stable than the platinum black-based sensor, and it would be as fast as both sensors.

2 Experimental

Hexachloroplatinate, tetrachloroaurate, and lead ace-
ate were purchased from Wako Pure Chemical Industries. Aminoethanethiol was obtained from Tokyo-kasei. Glucose oxidase (123 U/mg, from Aspergillus sp.) was obtained from Toyobo. Phosphate buffer solution (100 mM) was prepared with potassium dihydrogenphosphate (Nacalai tesque) and di-sodium hydrogenphosphate (Nacalai tesque), and the pH value was found 6.8. Water was distilled and passed through a Mill-Q purification system (Millipore, Bedford, MA). All other chemicals and solvents were of analytical-reagent grade.

In all electrochemical experiments, i.e. electrodeposition, anodic treatment, and evaluation of sensor response were performed with a three-electrode system which had a Pt counter electrode and a Ag/AgCl reference electrode. The apparatuses used in these electrochemical experiments were Toho Giken Potentiostat/Galvanostat 2090 and Rika Denki R-302 x-t recorder.

Au-Pt black electrode was fabricated according to the following procedure. A platinum wire as a base electrode having a diameter of 0.2 mm (NilaCO) was sealed in a glass capillary tubing (Nichiden-Rika Glass), and its tip was polished with alumina powders. Then the electrode was immersed into 1 M H2SO4 and its surface was electrochemically refreshed by repeatedly sweeping potential between −200 to 1450 mV (500 mV/s). Electrodeposition of gold-platinum black was carried out in a solution containing hexachloroplatinate, tetrachloraurate, and lead acetate (1.6 mM). The total concentration of Au and Pt complexes was always 72 mM, but the concentration of each complex varied according to the aim of experiment (concentration ratio of these complexes is described in each time). Constant potential −80 mV was applied for 5 min during electrodeposition. The temperature was maintained at 4°C. After the deposition of metal black, the electrode was rinsed and then immersed into the phosphate buffer solution, and 1200 mV was applied for 15 min. We call it "anodic treatment".

To fabricate enzyme sensor, the electrode was immersed into 10 mM aminoethanethiol (in ethanol) for 60 min to introduce amino groups on the gold surface. Then it was immersed into 1% glutaraldehyde solution for 60 min, and successively into glucose oxidase solution (2460 U/ml) for 60 min.

The sensor response was evaluated in a 0.1 M phosphate buffer solution at an applied potential of 600 mV, under the constant temperature 30°C.

3 Results and Discussion

Figure 1 shows typical response of glucose sensor based on Au-Pt black electrode upon sequential addition of concentrated (1 M in the phosphate buffer) glucose solution. The concentration ratio of the Au and Pt complex in the electrodepositional solution was 40:60 mol%. The electrode current immediately increased after the addition of glucose and reached a steady state value within 10 s. The increase in the final current was proportional to the glucose concentration up to 30 mM.

In the case of enzyme sensor based on Pt black electrode, the anodic treatment is preferable to suppress nonspecific response to substrate and other ingredients in sample. In the present experiment, anodic treatment was also carried out on the Au-Pt black electrode before immobilizing enzyme. We discovered it is also effective to suppress nonspecific response. The anodic treatment drastically decreased nonspecific response to glucose, fructose, maltose, and urea (1 or 2 orders in the magnitude), while it was not effective to ascorbic acid and uric acid. In the case of Pt black-based sensor, nonspecific response to ascorbic acid was drastically suppressed by covering electrode with Nafion, and we think same method can be applicable to the Au-Pt black-based sensor.

The metal black electrode explained here has both gold and platinum sites on its surface. We can expect enzyme molecules tightly bind on the gold site. Then, roughly speaking, the sensor response might be proportional to the surface area shared by gold in the electrode surface. On the other hand, if the enzymatic product is catalyzed at the platinum site, sensor response might be proportional to the surface area of the electrode shared by platinum. By considering both relations, the sensor response should be proportional to the arithmetical product of the surface areas of these metals. Therefore, there must be an optimal ratio of gold and platinum sites to obtain maximum response. Under such expectation, we prepared some glucose sensors of which electrodes were fabricated by depositing Au-Pt black from the different solutions containing various concentrations of Au and Pt complexes.

Figure 2 shows response curves of these sensors. There was an optimal ratio of gold and platinum, supporting our expectation. The largest response was obtained
when the ratio of Au and Pt was 40:60 mol%. In our previous paper,\textsuperscript{11} we revealed that the deposition ratio of Au and Pt on the electrode was directly proportional to the metal complex ratio contained in the electrodepositional solution. Hence, the ratio of Au and Pt deposited on the electrode for the optimal sensitivity was also 40:60. This value differed from our anticipation. In our preliminary experiment, the catalytic activity of an platinum to hydrogen peroxide was more than 10 times larger than that of gold. Therefore, we thought only a small percentage of platinum would be required for the optimal response before the experiment in Fig. 2. This discrepancy is attributable to the noncovalent binding of enzyme to the electrode surface.

This hypothesis is also supported by another experiment. Figure 3 shows time course currents of glucose sensors. The sensor response rapidly decreased when the platinum content was large. The possible explanation is the dissociation of absorbed enzyme from the electrode surface. We can guess from Fig. 3 that the simple immersion of electrode into moderate solution takes extremely long hours to remove physically adsorbed enzyme from the surface. Therefore, our next challenge is to develop a more efficient method to remove these noncovaently adsorbed enzyme.

Furthermore, the sensor response was almost 100% after 23 days when the sensor was covered with an albumin membrane, also suggesting existence of noncovalent adsorption of enzyme on electrode surface. Although our purpose is to prepare molecularly controlled structure for enzyme electrode, this result also suggests albumin coating is a practical method to improve sensor stability.

4 Conclusion

In this paper, we proposed a new direction to construct electrode surface, where functionally distinguished sites, \textit{i.e.} enzyme-binding sites and catalytic sites, coexist. As an example, we fabricated a glucose sensor based on Au-Pt black electrode. At the electrode surface, Au and Pt played an enzyme-binding site and a product-catalysis site, respectively.

References