A Glucose Biosensor Using Au Nanoparticle Modified Glucose Oxidase

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ABSTRACT

The construction of glucose biosensor by immobilization of glucose oxidase (GOD) on the surface of gold nanoparticle is reported. Data of the current response of enzyme electrodes to glucose are obtained. The experiments show that the gold nanoparticle could significantly enhance the GOD electrode response sensitivity. A set of experimental results indicate that the current response for the enzyme electrode containing hydrophobic Au nanoparticle increased from 0.385 μA to 9.8 μA in the solution of 22.2 mmol/L β-D glucose. The time reaching the steady-state response reduced from more than 60 seconds to 20 seconds, three times less than those without Au nanoparticle involved. The measurement limit is less than 0.2 mmol/l. A possible mechanism for the behavior of the different kind of Au nanoparticles is proposed. The experiments and theory give proof of the important function of Au nanoparticles in immobilizing enzyme.

Keyword: glucose oxidase, Au nanoparticle, and immobilization enzyme

1 INTRODUCTION

With the improving standard of living and increasing aging population, diabetes attacks more and more people, which has heightened the need for the diagnosis and treatment of diabetes. The glucose biosensor can accurately, rapidly and precisely measure glucose levels, which is essential to the appreciate administration of diabetes therapy, so the electrochemical biosensor is the focus of much research. The development of enzyme-based biosensor technology for chemical, environmental and medical analysis has been very rapid. According to the mechanism of the electron transfer between enzyme and electrode surface, the amperometric biosensors consist of three generations [1].

Efficient electrical communication between an immobilized enzyme and a solid electrode surface is necessary for monitored a current in an external circuit of amperometric enzyme biosensor. Such electron transfer requires electrical contact between the protein’s redox center and the electrode interface. In most of instances this is inhibited due to that the protein matrix separates the redox-site from the electrode surface and practically insulates the active center from electrical contact with electrode interfaces. The first generation biosensors are dependent on the concentration of dissolved oxygen in the bulk solution. In order to overcome these problems, the concept of using artificial electron acceptors, evolved in the second generation biosensors, which can avoid the reduction of oxygen. Artificial electron mediators as charge transporters and/or promoter molecules have been employed in order to improve the transfer of electrons between the active redox center of the enzyme and the electrode surface [2-4]. Some electrodes have been developed which can directly oxidize the reduced enzyme and do not require any exogenous mediator [5-7]. These have been called third generation biosensors. Such enzyme electrodes can be prepared by the coating of the electronic conductors (conducting salts) and are stable for several months. Usually the rate of reaction is a diffusion controlled phenomenon where external membranes are used, therefore the current produced is proportional to the analyte concentration and independent of both the enzyme and electrochemical kinetics.

One of the most exciting developments in enzyme chemistry was the recent discovery of the possibility to immobilize biomolecules by entrapment within gold nanoparticle. The nanostructural materials often have quite different chemical, structural, electric and magnetic properties with many potential applications in modern society from their corresponding bulk solid. Efficient electrical communication between an enzyme-colloidal gold nanoparticle and a solid electrode surface is illustrated by the direct (non-mediated) electron transfer between the enzyme and an electrode surface. Reports, however, on the improvement of electrode properties by this method have been very limited to date [6]. And almost all of the research focused on the biocompatibility of colloidal gold, and the diameter of the gold nanoparticles used is about 30-50 nanometers [6,8-11].

The main purpose of the experiment reported here was to find a new immobilization support material, which is of one of the possible future alternatives for organic conducting salts, which is the usual support material in preparing the direct electron transfer electrode. In this paper, Au nanoparticles, which were less than 10 nanometers, were used to immobilize glucose oxidase. The Au nanoparticles prepared in the aqueous solution are named hydrophilic Au nanoparticles in this paper, while those prepared in reversed micelles are named hydrophobic Au nanoparticles. The properties of the enzyme electrodes, which contain Au nanoparticle, were investigated. The current response of the glucose biosensor containing hydrophobic Au sol increased from 0.385 μA to 9.8 μA in the solution of 22.2 mmol/L
β-D glucose. The time reaching the steady-state response reduced from more than 60 seconds to 20 seconds, three times less than those without Au nanoparticle involved. The measurement limit is less than 0.2 mmol/l. The influence of the microenvironment of the colloidal Au nanoparticle on enzyme activity was studied, and a possible electron transfer mechanism between the GOD molecule and electrode surface was proposed.

2 MATERIALS AND METHODS

2.1 Chemicals and reagent

Glucose oxidase was extracted from Aspergillus niger (Toyobo company 100U/mg). Sodium 1,2-bis-(ethylhexyloxy carbonyl)-1-ethane sulfonate (AOT) was provided by Nacalai Tesque, INC. Kyoto, Japan. β-D Glucose was obtained from Sigma.

All other chemicals used in this work were of analytical reagent grade where available and without further purification. All solutions were prepared with re-distilled water.

2.2 Preparation of Au Nanoparticles

The hydrophilic gold particle was prepared by reducing gold chloride tetrahydrate with citric acid at 80°C for half an hour. The preparation of the hydrophobic gold nanoparticles was carried out by mixing two sets of reversed micelle solutions containing equal gold chloride tetrahydrate and citric acid. To control particle size, \( R_e \) (molar ratio of gold chloride tetrahydrate and citric acid, respectively to surfactant) and \( W_e \) (molar ratio of water to surfactant) were varied. These reversed micelles were then mixed by thorough stirring.

2.3 Preparation of Enzyme Electrode

Platinum wire with a diameter of 1 mm was boiled in nitric acid for 10 min, washed in redistilled water and boiled in redistilled water for 5 min. 12 U GOD solution was added to 400 μL Au particles to form mixtures. Several minutes later, 2ml of polyvinyl butyral (PVB) solution (2%) in anhydrous alcohol and appropriate glutaraldehyde solution were added and the contents were stirred evenly by the platinum wire. After the precipitation had dissolved, the platinum wire was dipped into the solution to a depth of 1 cm for 8 min. The electrode was stored in a refrigerator at 4°C.

2.4 Amperometric Measurement

A two-electrode cell was constructed to measure the electrical current response of the GOD electrode. An Ag/AgCl electrode served as the reference. A fixed potential of 0.4V was applied to this electronic cell as described in reference [12]. A pH 6.86 phosphate buffer with the concentrations of the β-D glucose solution from 2.7 to 33 mmol/l was used.

3 RESULTS AND DISCUSSION

Fig. 1 consists of two electron micrographs showing the typical nanostructures of Au nanoparticles synthesized by the method described above. Fig. 1 (a) is an image of hydrophilic Au nanoparticles taken by a transmission electron microscope. It shows that the size of the nanoparticles is rather uniform with an average diameter of 8.2 nm. Fig. 1 (b) shows an image of hydrophobic Au nanoparticles taken from a transmission electron microscope. In sample (b), the average diameter of the Au nanoparticles is 4 nm.

The effect of experimental conditions, such as the amount of nanoparticles, the concentration of membrane matrix PVB, the electrode dipping time and the working potential were investigated to optimize testing performance of the
glucose biosensor. It was found that 6 U GOD, 100 µl glutaraldehyde solution (1%) in 2ml of PVB solution (2%), are optimal parameters for the fabrication of the biosensor. And the appreciate working potential was found to be 0.4 V (Vs. Ag/AgCl). As a consequence, the optimal experiment conditions were used for later biosensor preparation and response determination.

![Graph showing current response of glucose biosensor](image)

**Fig.2** Enhancement effect of hydrophobic, hydrophilic Au nanoparticle on glucose biosensor.

To study the effect of the Au nanoparticle on the sensitivities of the glucose biosensor, enzyme electrodes coated with either hydrophobic or hydrophilic Au nanoparticles were tested. Fig. 2 shows the calibration curves of GOD electrodes without and with hydrophilic and hydrophobic Au nanoparticles. This result indicates that the current response of the electrode containing Au nanoparticles increases dramatically. In particular, the current response of the GOD electrode containing hydrophobic Au nanoparticles is much larger than that of the enzyme electrode containing hydrophilic ones. The reason of the enhancement effect by nanoparticle is the surface effect and volume effect of the nanoparticle, which lead to a very large ratio of surface to volume. Thus the nanoparticles provide an increased electrode surface area, which allows for increased enzyme loading on the electrode surface, i.e. the adsorbed concentration effect. Colloidal gold particles are known to provide a biocompatible surface for biomolecule immobilization [8]. The gold nanoparticles prepared were less than 10 nm, and almost the same magnitude as GOD molecule (about 7 nm [13]). GOD is a flexible and deformable amphiphilic species, and the small size of the colloidal gold particles may give the protein molecule more freedom in orientation, thus after GOD immobilizes on an Au nanoparticle surface, GOD might still retain its nature activity. In biosensor, Au nanoparticle can immobilize more active enzyme molecules. On the other hand, as gold is a good electrical conductor, gold nanoparticle act as an efficient electron mediator between the solid electrode and immobilization GOD, which can significantly enhance the current response. Therefore, Au nanoparticle can significantly enhance the current response of the glucose biosensor.

The reason of hydrophobic Au nanoparticle is more effective immobilization matrix than hydrophilic one is that the hydrophobic Au nanoparticle is in reversed micelles system. During the preparation of electrodes, we found that some precipitate was formed as soon as PVB contacted with water. In the hydrophobic Au nanoparticles reversed micelles system, solubilized water is located in the inner core of a micelle and forms a microdroplet separated from the organic solvent by a layer of surfactant (AOT) molecules [14]. Therefore, during the preparation of the electrode using the hydrophobic Au nanoparticle, the precipitate formed is less than those with the hydrophilic Au nanoparticle involved, which leads to less denaturation of the immobilization GOD. Since the most important property of hydrated reversed micelles is their ability to entrap molecules, which are dissolved in water, into their inner cavities. Some GOD molecules are entrapped spontaneously into the inner cavities of the reversed micelles by stirring the mixture of the GOD solution, and the hydrophobic Au nanoparticle sol. And the microenvironment of the inner cavity of reversed micelles provides a more natural surrounding for the entrapped enzyme than that of the bulk solution. Since hydrophilic Au sol cannot achieve this, the current response of the electrode containing hydrophilic Au nanoparticles is not as high as that of the electrode containing hydrophobic ones.

The result in Fig. 3 shows that the time reaching the steady-state response (95% of the highest response) reduced from more than 60 seconds to 20 seconds, three times less than those without Au particles involved. The measurement limit is less than 0.2 mmol/l. The stability of the modified platinum electrode was tested over 40 samples of glucose solution of 10 mmol/l. The results suggest that the degradation of the sensitivity of the sensor, during the
measurements, can be neglected.

The electrode prepared by about 7-8 nanometers Au nanoparticles, which is almost same size with GOD molecule, had the largest current response. It maybe due to that the GOD molecule and Au nanoparticles self-assemble into the special structure, which makes for quantum tunnel effect, which will improve the electron transfer. Further studies are still in progress.

4 CONCLUSION

In summary: High sensitivity glucose biosensors based on immobilization of enzyme in Au nanoparticles are presented. The enzyme electrode containing hydrophobic Au nanoparticles significantly enhances the response current compared with the electrode containing hydrophilic nanoparticles and with the electrodes containing no Au nanoparticles. The responding speed of the biosensors is also improved. The steady-state response time is three times less than those without Au nanoparticles involved.

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6 REFERENCES
