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Multilayer membranes via layer-by-layer deposition of ascorbate oxidase and Au nanoparticles on the Pt electrode for reduction of oxidation current derived from ascorbate

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Abstract

A glass plate was alternately immersed in an Au colloid (10 nm φ) and an ascorbate oxidase (AO_x) solution (0.1 mg/mL, pH 6.8). Absorbance at 530 nm originating from the Au particles increased with the increasing number of depositions. The AO_x activity of the plate also increased as the plate was immersed in the AO_x solution. These results suggested that a multilayer membrane via the layer-by-layer deposition of AO_x and Au nanoparticles was formed on the glass plate. AO_x was also deposited on a Pt disk electrode using the same process. Using the $(AO_x/Au)_{10}$ modified electrode, the oxidation current of ascorbic acid (0.1 mM) decreased to 19% versus the unmodified electrode.
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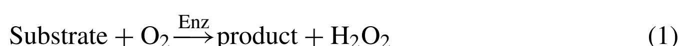
Keywords: Layer-by-layer deposition; Gold nanoparticle; Ascorbate oxidase; Ascorbic acid

1. Introduction

Amperometric detection of H_2O_2 has been widely used for enzyme sensors in which an oxidase is employed as a molecular recognition element (Eqs. (1) and (2)).



$$\text{Substrate} + O_2 \xrightarrow{\text{Enz}} \text{product} + H_2O_2 \qquad (1)$$

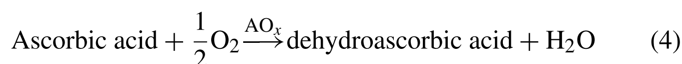
$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \qquad (2)$$

Though the operating principle is quite simple and available for most enzyme sensors using FAD enzymes, such as glucose oxidase (GO_x), lactate oxidase (LO_x), the current response of the sensors could be affected by some electrochemically active species which could be oxidized on the electrode surface at the operating potential (ca. +0.6 V versus Ag/AgCl). For application of the sensors with biological fluids, such as blood and urine, the oxidation current derived from the ascorbate is often greater than the signal current derived from H_2O_2 (Eq. (3)).

$$\text{Ascorbicacid} \rightarrow \text{dehydroascorbicacid} + 2e^- \qquad (3)$$

As a reducing reagent, ascorbate can also directly reduce H_2O_2. This reductive reaction would attenuate the signal current derived from H_2O_2. Moreover, because the concentration of ascorbate in biological fluids varies with daily meals, the magnitude of the interference varies from sample to sample.

Therefore, much attention has been devoted to solve the intrinsic problem of sensors based on the oxidative detection of H_2O_2. The best answer is the interception of the ascorbic acid molecules from reaching the electrode surface. There are two strategies: one is to cover the electrode surface with a permselective membrane; and the other is to modify the electrode with AO_x. As permselective membranes, Nafion, cellulose acetate, layer-by-layer membranes, and a polyion complex were often employed [1–3]. These membranes have a molecular sieve effect and/or negative net charge to repulse ascorbate by an electrostatic force. On the other hand, AO_x changes ascorbate to an electrochemical inactive species, dehydroascorbate (Eq. (4)).



$$\text{Ascorbic acid} + \frac{1}{2}O_2 \xrightarrow{AO_x} \text{dehydroascorbic acid} + H_2O \qquad (4)$$

Using the avidin-biotin method, biotinylated AO_x was alternately deposited with avidin on a glucose oxidase modified electrode and was found to effectively reduce the noise current derived from ascorbic acid [4,5]. AO_x was also incorporated in

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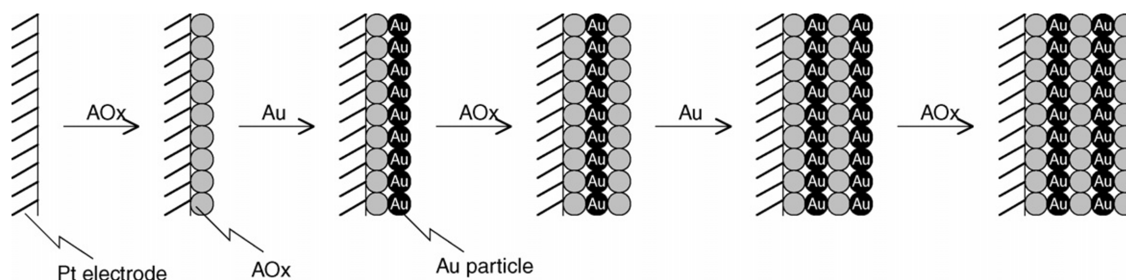

Fig. 1. Schematic representation of alternate deposition of Au nanoparticles and AO_x on a glass plate.

an avidin film prepared by an electrochemical method using a triangular wave form for use as a glucose sensor [6].

Recently, a stable nanoscale gold colloid has been developed, and now the colloidal particles can be commercially obtained in sizes ranging from 1 nm to hundreds of nanometers. The gold particles can conjugate to various proteins and the interaction between the colloidal gold and the proteins could be used for the layer-by-layer deposition of proteins [7–9]. However, the conditions for the alternate deposition have not yet been clarified, since few studies have been reported.

In this study, we tried to alternately deposit AO_x with Au nanoparticles on a glass plate and a Pt electrode (Fig. 1). The residual activities of the deposited AO_x were then estimated by measuring the disappearance of ascorbic acid by spectrophotometry (Eq. (4)). The residual activities were also estimated by measuring the oxidation current of ascorbic acid at 600 mV versus Ag/AgCl using the AO_x modified Pt electrode (Eq. (3)).

2. Experimental

2.1. Chemicals

All solutions were prepared from ultrapure water (Simpli Lab-UV, Millipore, MA, USA). Avidin and GO_x (EC 1.1.3.4, Type X-S from *Aspergillus niger*) were purchased from Calzyme (CA, USA) and Sigma (MO, USA), respectively. These reagents were dissolved in phosphate buffer (pH 6.8, 50 mM) to form a 0.1 mg/mL solution. Ascorbate oxidase (EC 1.10.3.3, from *Cucumis* sp.) was obtained from Toyobo (Osaka, Japan). The chromic acid mixture was purchased from Wako (Osaka, Japan). An alumina suspension for fine polishing of the electrodes was obtained from Marumoto Struers (Tokyo, Japan). The gold colloid (10 nm φ, 5.7×10^{12} mL^{-1}) was purchased from BBInternational (Cardiff, UK). All other chemicals were of analytical grade and used as received.

2.2. Apparatus

The absorbance spectra were measured using a UV-3100PC spectrophotometer (Shimadzu, Kyoto, Japan). The amperometric measurements were performed using a NPGFZ-2501A (Nikko Keisoku, Atsugi, Japan) at room temperature with a conventional three-electrode system. A Pt disk electrode (3.0 mm φ) and a Pt wire electrode were used as the working electrode and the counter electrode, respectively. An Ag/AgCl electrode with an internal solution of KCl (3.33 M) saturated with AgCl was used as the reference electrode.

2.3. Preparation of a Au/protein modified glass plate

Two bathing solutions were employed for the alternate deposition of the Au nanoparticles and a protein: one was the as-received gold colloid, and the other was a 0.1 mg/mL solution of a protein. A quartz glass plate (50 mm × 9 mm × 1 mm) cleaned by a chromic acid mixture was immersed in a gold colloid for 30 min and washed in a working buffer for 5 min. The Au nanoparticle-modified glass plate was then immersed in the protein solution for 30 min to deposit the Au/protein bilayer. To deposit additional layers, this procedure was repeated several times.

2.4. Preparation of a Au/protein modified electrode

The Pt disk electrode was modified with Au and a protein using the same technique as described above. The Pt electrode was thoroughly polished using an alumina suspension and sonicated in water before use. The electrode was immersed in the protein solution for 30 min and rinsed in a working buffer for 5 min. The protein-modified electrode was then immersed in the Au colloid solution for 30 min to deposit the protein/Au bilayer. To deposit additional layers, this procedure was repeated several times.

2.5. Determination of AO_x activities

The relative enzyme activities of an AO_x-modified glass plate was determined by measuring the disappearance of ascorbic acid by spectrophotometry (Eq. (4)). A 10 mM solution of ascorbic acid containing EDTA (1.0 mM) and HCl (1.0 mM) was freshly diluted 10 times with the KH_2PO_4 solution (0.2 M) containing EDTA (1.0 mM). This solution (1.5 mL) was then mixed with an equal volume of Na_2HPO_4 solution (10 mM) in a quartz cuvette with a 10-mm path length. Actually, 10 min after the AO_x-modified glass plate was immersed in the cuvette, the plate was removed and the absorbance at 245 nm (A_{245}) originating from the ascorbic acid was measured.

3. Results and discussion

Au nanoparticles have a strong extinction (apparent absorption) around 530 nm. This is caused by Rayleigh scattering

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