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Modified gold surfaces by 6-(ferrocenyl)hexanethiol/dendrimer/gold nanoparticles as a platform for the mediated biosensing applications

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ABSTRACT

An electrochemical biosensor mediated by using 6-(Ferrocenyl) hexanethiol (FcSH) was fabricated by construction of gold nanoparticles (AuNPs) on the surface of polyamidoamine dendrimer (PAMAM) modified gold electrode. Glucose oxidase (GOx) was used as a model enzyme and was immobilized onto the gold surface forming a self assembled monolayer via FcSH and cysteamine. Cyclic voltammetry and amperometry were used for the characterization of electrochemical response towards glucose substrate. Following the optimization of medium pH, enzyme loading, AuNP and FcSH amount, the linear range for the glucose was studied and found as 1.0 to 5.0 mM with the detection limit (LOD) of 0.6 mM according to S/N = 3. Finally, the proposed Au/AuNP/(FcSH + Cyst)/PAMAM/GOx biosensor was successfully applied for the glucose analysis in beverages, and the results were compared with those obtained by HPLC.

1. Introduction

Glucose oxidase (GOx; β-D-glucose:oxygen 1-oxidoreductase; EC 1.1.3.4) is a dimeric protein containing one tightly bound flavin adenine dinucleotide (FAD) per monomer as cofactor, and catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide using molecular oxygen as the electron acceptor [1]. However, the thick protein layer surrounding flavin redox centre results in difficulties on direct electron transfer between FAD and electrodes [2,3]. It has taken about half a century and tremendous amount of research to overcome this problem and to obtain better electron transfer between the enzyme and electrodes such as adding redox mediators [4], conducting polymers [5], nanoparticles [6] and dendrimers [7]. The surface quality is an important factor affecting results obtained with electrochemical methods especially when self assembled monolayers (SAMs) are applied to build a recognition layer with various functional groups. SAMs of alkanethiols bearing different functional groups have been widely used in studies of the intelligent modification of solid surfaces. Alkanethiol SAMs having terminal electro-active groups were considered to be ideal for understanding the fundamentals of the electron transfer process at an electrode/solution interface and received a great deal of attention [8,9]. Among these electroactive groups, the ferrocenylalkanethiol monolayer has been used as a model system to study the electron exchange between ferrocenyl groups and gold electrodes, because of the simple and good electrochemical characters of the ferrocene groups [9]. 6-Ferrocenyl-1–hexanethiol ($Fc(CH_2)_6SH$; will be referred as FcSH) has been previously investigated as SAM to meet such purposes [8].

Nanoscale materials combined with biological components hold the potential of revolutionary changes in fields of science and technology [10]. There is rapidly increasing interest in nanotechnology in analytical chemistry [11] and clinical diagnosis [12,13]. The high surface to mass ratio makes nanomaterials exhibit unique physical and chemical properties [10,11,14,15]. Among these unique characteristics of nanomaterials, electronic properties are widely used to construct electrochemical biosensors. Gold nanoparticles (AuNPs) are mostly used metal-nanoparticles in the construction of enzymatic sensors [15]. Such works carried out by Sun and co-workers, have been reported to increase bioelectrocatalytic response in glucose biosensors [6,16,17]. Beside metallic nanoparticles, dendrimers as immobilization materials are also being used widely because of advantages such as biocompatibility, stability, porous structure and enlarging surface [18]. Polyamidoamine (PAMAM) provides abundant amine groups available for biomolecules immobilization.

Here, we described a mediated glucose biosensor based on Au/AuNP/(FcSH + Cyst)/PAMAM/GOx system and investigated the effect of the presence of AuNPs and FcSH. After optimization studies, analytical characterization experiments were carried out by amperometric measurements.

2. Materials and methods

2.1. Materials

Glucose oxidase (GOx; EC 1.1.3.4, 21200 units/g, type II-S, from *Aspergillus niger*), β-D-glucose (99.5%) purchased from Sigma (USA). 6-ferrocenyl-1-hexanethiol and polyamidoamine (PAMAM

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G4 dendrimers in methanol solution) are obtained from Aldrich Chem. Co (USA). Sodiumborohydride (99%), glutaraldehyde solution (Grade II, 25%) and gold colloidal solution (10 nm, 0.75A_{520} units/mL, Concentration: ~0.01% as $HAuCl_4$) are purchased from Sigma-Aldrich. Cysteamine hydrochloride was purchased from Fluka. All other chemicals were analytical grade. Distilled and de-ionized water was used during experiments.

2.2. Apparatus

The electrochemical measurements were performed with a potentiostat (PalmSens, The Netherlands). A conventional three electrode system was used in experiments, namely: Ag/AgCl electrode as a reference (Metrohm, Switzerland), a platinum wire as an auxiliary electrode (BAS; United Kingdom, USA), and a gold electrode (BAS; United Kingdom, USA) as working electrode. During amperometric determinations, a magnetic stirrer and a stirring bar provided the desired convective transport in the reaction cell. Cyclic voltammetry was performed in quiescent solutions.

High-performance liquid chromatography (HP1100, Hewlett and Packard, Santa Clara, CA, USA) with a refractive index detector (RI) controlled by a HP-Chemstation from Agilent (Karlsruhe, Germany) was used as reference method for sample applications.

The morphology of modified gold surfaces with AuNP and AuNP/FcSH were imaged by Atomic Force Microscopy (AFM, NanoMagnetics Instruments, UK), at ambient temperature in non-contact mode.

2.3. Preparation of Au/AuNP/(FcSH + Cyst)/PAMAM/GOx Biosensor

The gold electrode surface was initially polished with alumina powder (Gamma, 0.05 lm) and was conditioned in 0.5 M H_2SO_4 solution by cycling the potential between 0 and +1.5 V until a reproducible voltammetric response was obtained. Then, the modification of the electrode was carried out by following the steps given in a previous study [19]. Briefly, the cleaned gold surface was immersed in cysteamine (100 mM) and 5.0 μL (1.98 μmol) of 6-ferrocenyl-1-hexanethiol solution in the mixture of acetonitrile and ethanol (ACN: EtOH; 1:1, v/v) for 30 min and washed with distilled water to get rid of physically adsorbed molecules. Then, dipped into glutaraldehyde solution (5.0%, v/v, in potassium phosphate buffer, pH 7.0; 50 mM) for 30 min and again washed with distilled water. After that, exposed to the solution of PAMAM dendrimer (1.0%, v/v) for 1 h, treated with 5.0 mM $NaBH_4$ for 30 min, and washed with distilled water [19]. 42 U of GOx (2.0 mg in 5.0 μL potassium phosphate buffer, pH 7.0; 50 mM), 5.0 μL of AuNPs solution and 5.0 μL of glutaraldehyde (1.0%, v/v) were added to electrode surface respectively. Then, surface was allowed to dry at 25 °C for about 30 min. The modified electrode was stored in contact with the working buffer solution when not in use. Schematic representation of thme preparation of Au/AuNP/(FcSH + Cyst)/PAMAM/GOx biosensor was shown in Scheme 1.

2.4. Electrochemical measurements

All amperometric measurements were carried out by applying a potential +0.35 V (vs. Ag/AgCl reference and Pt wire as counter electrode) at ambient temperature. 10.0 mL of working buffer solution (50 mM, pH 4.0 Na-Acetate buffer) was used in the voltammetric cell. The cell was rinsed with distilled water and the working buffer solution respectively after each measurement. In all amperometric measurements, the error bars on each plot represent the standard deviation of three replicates of the related measurement.

In order to investigate electron transfer properties of proposed system, cyclic voltammetry experiments were carried out in the presence and absence of AuNPs and FcSH. Obtained results from these different electrodes compared to characterize the effects of mediator and nanoparticles in the electrode configuration.

2.5. Sample application

In order to verify the performance and feasibility of the proposed Au/AuNP/(FcSH + Cyst)/PAMAM/GOx biosensor for analysis of glucose in real samples, the biosensor was applied to glucose analysis in some beverages such as cherry juice and fizzy with orange. The samples were analyzed without any dilution or treatment and added directly to the reaction cell described above (2.4 Electrochemical Measurements). Results were compared with HPLC as a reference method. HPLC column (GL Sciences Inc. Inertsil NH_2 5.0 μm (4.6 I.D × 250 mm), Japan) was used for the chromatographic separation of monosaccharides at 30 °C. Injection volume was 20 μL. The mobile phase was acetonitrile:water (3:1 v/v). The flow rate was 1.0 mL/min). Initially, a calibration curve for glucose was constructed in a concentration range of 0.5–5.0 mg/mL. After dilution with mobile phase and filtration through membrane filter (pore size: 0.20 μM) samples were applied to the column. Then glucose levels were calculated using the calibration plot.

3. Results and discussion

3.1. Characterization

There have been a number of demonstrations of the applications of SAMs based biosensors. Silanes and alkanethiols have been mostly used to prepare DNA and enzyme sensors based on SAMs. In addition, dendrimers of different numbers of branches were bound onto a gold surface via cysteamine to construct SAMs based enzyme sensor for the detection of different analytes such as glucose [18] and pesticides [20]. In this study, FcSH as a mediator and GOx were immobilized by means of PAMAM via construction of SAM via cysteamine. The use of glutaraldehyde as the cross-linker resulted in more stable enzyme immobilization. The enzymatic process occurs according to the following reaction:

$$\mathrm{Glucose} + \mathrm{GOx(FAD)} \rightarrow \mathrm{Gluconolactone} + \mathrm{GOx(FADH_2)}$$

$$\mathrm{GOx(FADH_2)} + 2\mathrm{AuNP/Cyst-HS-Fc^+} \rightarrow \mathrm{GOx(FAD)} + 2\mathrm{AuNP/Cyst-HS-Fc} + 2\mathrm{H^+}$$

$$\mathrm{AuNP/Cyst-HS-Fc} \rightarrow \mathrm{AuNP/Cyst-HS-Fc^+} + 2\mathrm{e^-}$$

where Fc represents reduced and oxidized forms in the redox layer, GOx(FAD) and GOx($FADH_2$) are the oxidized and reduced forms of GOx.

The electrochemical behaviour of the enzyme electrode was studied using cyclic voltammetry. Fig. 1 shows the cyclic voltammograms of the Au/AuNP/(FcSH + Cyst)/PAMAM/GOx in working buffer solution at different scan rates.

The peak at +0.35 V corresponds to the oxidation peak of FcSH. No reverse peak was obtained indicating the irreversible character of the electron transfer mechanism. The dependence of the peak currents on the scan rate as can be seen in Fig. 1 (inset). Peak currents linearly changes with the square root of scan rate, in the range from 5 to 100 mV/s, linear regression equation; $y = 0.123x - 0.059$, $R^2 = 0.998$ which indicates diffusion-controlled electrode process. It could be expected to obtain surface controlled system for monolayer immobilized material [21]. However, the transport of the substrate is controlled by diffusion from the solution. The presence of the dendrimer and bio-active layers upon an immobilized mediator might hinder this characteristic. Similar results have also been obtained in our previous work [22], which does not contain immobilized mediator. In the case of immobilized mediator forming SAMs, reversible character of PAMAM/enzyme layers is lost.