Sensitive and selective electrochemical sensor using silver nanoparticles modified glassy carbon electrode for determination of cholesterol in bovine serum

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A B S T R A C T

For the first time, a newly sensitive and simple method for the determination of cholesterol based on coupling of enzymatic assay and electrochemical detection has been developed. Silver nanoparticles modified glassy carbon electrode (AgNPs/GCE) was fabricated by electrochemical deposition technique and used as the working electrode. The electrochemical performances were investigated by cyclic voltammetry and chronoamperometry. Under the optimized conditions, a linear relationship between the reduction current and cholesterol concentration was found in the range of 3.9 mg/dL to 773.4 mg/dL with a detection limit of 0.99 mg/dL. The proposed method was applied to determine cholesterol in bovine serum. The recoveries obtained were within the range of 99.6–100.7%, which indicated that the presented method is applicable to determine cholesterol in bovine serum. In addition this electrochemical sensor displayed very high specificity to cholesterol with no observed interference from easily oxidizable species such as ascorbic acid and uric acid. All these excellent performances of the developed sensor indicated that this sensing platform could be easily extended to the detection of other important biomarkers.
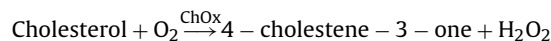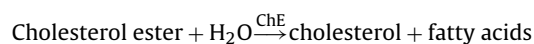
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1. Introduction

Cholesterol is an important biomarker for various diseases. It acts as a major structural constituent of plasma membranes and is a precursor of biological substances, such as bile acid, vitamin D and steroid hormones [1]. High cholesterol level in blood serum is a major factor for several diseases, such as coronary heart disease, myocardial infarction and arteriosclerosis hypertension, while low cholesterol level may result in hypolipoproteinemia, anemia, septicemia, malnutrition, hyperthyrea and hepatopathy [2]. The normal total plasma cholesterol for an individual is less than 5.2 mM (200 mg/dL), with a high level being considered as more than 6.2 mM (240 mg/dL) [3]. Hence, accurate, sensitive and fast monitoring of cholesterol levels is of great importance in clinical analysis/diagnosis. In addition, the development of quantitative methodology for determination of cholesterol is significant and challenging to develop reliable cholesterol sensors.

The analytical methods for cholesterol determination can be classified into two groups: nonenzymatic and enzymatic methods.

In general, the enzymatic method provides more selectivity than nonenzymatic method and has been employed in routine clinical laboratory. The determination of cholesterol by enzymatic method is based on the following mechanism [4];

$$Cholesterol\ ester + H_2O \xrightarrow{ChE} cholesterol + fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{ChOx} 4-cholestene-3-one + H_2O_2$$

The cholesterol ester will be hydrolyzed by cholesterol esterase (ChE) to produce free cholesterol and fatty acids. Then free cholesterol is oxidized by oxygen in the presence of cholesterol oxidase (ChOx) to produce 4-cholestene-3-one and hydrogen peroxide (H_2O_2). The measurement of H_2O_2 can be used for the indirect quantification of cholesterol.

Up to now, several cholesterol determination techniques have been published including colorimetric [5], spectrophotometric [6], high performances liquid chromatography (HPLC) [7] and electrochemical method [8,9]. Electrochemical method is the most frequency applied for cholesterol determination via the monitoring of oxygen consumption or the production of H_2O_2 during the enzymatic reactions. Amperometric measurement of H_2O_2 is the most frequently monitored and proposed. However, the suffering from

some easily oxidized species such as ascorbic acid and uric acid at high overvoltage will be occurred at the same time. Therefore, the accuracy of method is limited. To overcome the weak point, the possible interference from easily oxidizable species in biological samples can be minimized using modified working electrode with selective catalyst or using the reduction of H_2O_2 at low applied potential instead of oxidation of H_2O_2.

In recent years, metal nanoparticles have been used in wide applications in electrochemical sensors. Among the metal nanoparticles, silver nanoparticles (AgNPs) are one of the most well-developed materials and have been used to modify the surface of working electrodes because they are inexpensive in relative comparison with those other materials, possess good chemical and physical properties, providing excellent electron transfer rates, and greatly decrease the overpotential of oxidizing or reducing agents produced from enzymatic products. In previous studied, AgNPs were shown excellent electrocatalytic activity for H_2O_2 and size distribution of AgNPs played an important role in their electrocatalytic activity [10–13].

Electrochemical cholesterol biosensor based on electron transfer between an electrode and immobilized cholesterol oxidase has been focused for cholesterol research because they show advantages such as high sensitivity, selectivity and suitable for real time detection [14,15]. However the use of enzyme immobilized on electrode surface had limitations because the enzyme is easily denature during its immobilization procedure. In addition, the reduction of substrate–enzyme complex formation due to steric hindrance and high cost of the supporting material or its immobilized procedure were reported. Moreover, the activity of enzyme is often to be affected by pH, temperature, humidity and toxic chemicals [16].

To overcome those problems, the use of coupling between the enzymatic assay and electrochemical detection has been demonstrated [15]. ChOx were added in the solutions instead of the immobilization on electrode surface to catalyst the reaction of cholesterol and generated H_2O_2 will be recorded immediately. In this work, we report a sensitive, selective and simple method using silver nanoparticles modified glassy carbon electrode (AgNPs/GCE) to achieve a new nanosensor for the determination of cholesterol. AgNPs possess the catalytic activity of H_2O_2 reduction occurred at low overpotential. The proposed method showed high selectivity for cholesterol detection without disturbance from interference such as ascorbic acid and uric acid.

To the best our knowledge, there is no record for the using of AgNPs/GCE to detect cholesterol in real samples. The results show AgNPs/GCE displayed excellent performance, wide linear range, and low detection limit of cholesterol detection.

2. Material and methods

2.1. Apparatus

All electrochemical experiments were performed with a model PGSTAT 101 Autolab Electrochemical System controlled with the NOVA software package (Kanaalweg 29-G 3526 KM Utrecht, The Netherlands). Three-electrode system was used, where an Ag/AgCl (3 M KCl) electrode served as the reference electrode, a platinum wire electrode served as the auxiliary electrode and AgNPs/GC electrode served as working electrode.

2.2. Reagents and solutions

Cholesterol, cholesterol oxidase (ChOx) from *Streptomyces* sp. (25 U/mg) and lipid cholesterol rich from adult bovine were purchased from Sigma (St. Louis, MO). Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Carlo ErbaReagenti-SDS (Val de Reuil, France). Hydrogen peroxide (H_2O_2), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl) and boric acid (H_3BO_3) were purchased from Merck (Darmstadi, Germany). Silver nitrate ($AgNO_3$) was purchased from POCH S.A. (Poland). Glacial acetic acid (CH_3COOH) was purchased from Fisher Scientific (Pittsburgh, PA). Phosphoric acid (H_3PO_4, 85%) was purchased from Carlo Erba (Rodano, MI, USA). Stock solution of cholesterol was daily prepared by dissolving cholesterol in the mixture of triton X-100 and isopropanol. This stock solution was further diluted to make difference concentrations of cholesterol in 0.05 M phosphate buffer pH 7.4 containing 0.1 M KCl. The solution was stirred with magnetic bar at 60 °C to obtain a homogeneous solution. Stock solution of ChOx was prepared freshly by dissolving in 0.05 M phosphate buffer (pH 7.4). All standard and sample solutions were prepared by using high purity water from MilliQ Water System (Millipore, USA, $R \geq 18.2\,M\Omega\,cm$). All chemical were of analytical reagent grade and used without further purification.

2.3. Preparation of AgNPs/GC electrode

The procedure for preparation of silver nanoparticles modified glassy carbon electrode was adapted from Ref. [17]. Prior to use, glassy carbon electrode (GCE, diameter 3 mm) was polished with 1.0 and 0.2 μm alumina powder, respectively, and sonicated in ethanol and then in deionized water for 1 min each. Silver nanoparticles (AgNPs) have been deposited onto glassy carbon electrode surface from a solution of $AgNO_3$ in Britton–Robinson (pH 2.0) which prepared by mixing of 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M glacial acetic acid and adjust with 0.2 M sodium hydroxide. Electrodeposition was carried out by applying an accumulation potential during a time with stirring.

2.4. Electrochemical measurements

Cholesterol oxidase was pipetted into the electrochemical cell containing 1 mL standard/sample solution and stirred for 1 min. Three-electrode system was immersed in the solution. Then, the measurement using chronoamperometry at −0.5 V for 50 s was performed. All experiments were performed at room temperature.

2.5. Bovine serum analysis

The labeled concentration of cholesterol in adult bovine serum was used to represent the biological sample. The serum sample was prepared by dissolving of adult bovine serum powder in triton X-100 and isopropanol, and then diluted with 0.05 M phosphate buffer pH 7.4 containing 0.1 M KCl. The solution was stirred with magnetic bar at 60 °C.

3. Results and discussion

3.1. Electrochemical detection of standard H_2O_2 and standard cholesterol

For an electrochemical determination of cholesterol, the measurement of the H_2O_2 produced from cholesterol oxidation can be used for the indirect quantification of cholesterol [18]. In this study, silver nanoparticles modified glassy carbon electrode (AgNPs/GCE) was fabricated by deposition of silver at −0.6 V for 200 s using solution of 5 mM $AgNO_3$ in Britton–Robinson (pH 2.0). The experiment was start from using 1 mM standard H_2O_2 in 0.05 M phosphate buffer pH 7.4 containing 0.1 M KCl to investigate the electrochemical response of H_2O_2 using cyclic voltammetry. The cyclic voltammograms of standard H_2O_2 measured on the AgNPs/GCE