Talanta 143 (2015) 157–161

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Colorimetric cholesterol sensor based on peroxidase like activity of zinc oxide nanoparticles incorporated carbon nanotubes

Akhtar Hayat [a,*], Waqar Haider [b], Yousuf Raza [b], Jean Louis Marty [c]

[a] Interdisciplinary Research Centre in Biomedical Materials (IRCBM), COMSATS Institute of Information Technology (CIIT), Lahore, Pakistan
[b] Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan
[c] BAE: Biocapteurs-Analyses-Environnement, Universite de Perpignan Via Domitia, 52 Avenue Paul Alduy, Perpignan Cedex 66860, France

ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form
19 May 2015
Accepted 22 May 2015
Available online 27 May 2015

Keywords:
ZnO incorporated carbon nanotubes
Synergic effect
Enzyme mimic
Colorimetric assays
Cholesterol detection

ABSTRACT

A sensitive and selective colorimetric method based on the incorporation of zinc oxide nanoparticles (ZnO NPs) on the surface of carbon nanotubes (CNTs) was shown to posses synergistic peroxidase like activity for the detection of cholesterol. The proposed nanocomposite catalyzed the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) in the presence of hydrogen peroxide (H_2O_2) to produce a green colored product which can be monitored at 405 nm. H_2O_2 is the oxidative product of cholesterol in the presence of cholesterol oxidase. Therefore, the oxidation of cholesterol can be quantitatively related to the colorimetric response by combining these two reactions. Under the optimal experimental conditions, the colorimetric response was proportional to the concentration of cholesterol in the range of 0.5–500 nmol/L, with a detection limit of 0.2 nmol/L. The applicability of the proposed assays was demonstrated for the determination of cholesterol in milk powder samples with good recovery results.

1. Introduction

Cholesterol is a construction unit of hormonal system of mammals and an important component of cell membrane. It plays a vital role in the synthesis of several vitamins, steroid hormones and bile acids. Cholesterol is also related to the immune system, brain synapses and protection from cancer. The abnormal concentration of cholesterol can result in certain diseases such as brain thrombosis, anemia, hypolipoproteinemia, malnutrition hypertension, septicemia and arteriosclerosis [1,2]. Therefore, the concentration of cholesterol is monitored most often in food and clinical samples. Several methods are reported for the determination of cholesterol such as high performance liquid chromatography [3], electrochemical methods [4,5] and electrogenerated chemiluminescence [6]. However, most of these methods undergo the problems of low sensitivity, selectivity and expensive instrumentation.

The alternative approach for the determination of cholesterol level can be a simple colorimetric method with added advantages such as rapid analysis, good sensitivity and low background singles. Recently, artificial enzymes are gaining vital importance over the natural enzymes for catalysis of colorimetric reactions due to ease of their preparation, low cost and stability under harsh reaction conditions. Although natural enzymes have impressive catalytic activity and substrate specificity, but their expensive and long preparation time, purification and storage conditions critically limit their real time applications. Therefore, the recent research has focused on the exploration of new materials that can be used as artificial enzymes mimics. These materials included hematin [7], hemin [8,9], cyclodextrin [10], DNAzyme [11], and porphyrin [12]. In this context, nanomaterials have been emerged as the most attractive artificial enzymes due to their low cost, easy preparation and large surface area. The synergistic catalytic effect of different nanocomposites has been investigated in biochemical reactions by combing the catalytic activities of two different materials of known properties and structures. The synergistic catalytic phenomenon is based on the integration of two or more catalytic materials into a single nanocomposite in such a way that all the integrated materials retain their catalytic properties, and finally obtained nanocomposite is characterized with enhanced/added catalytic activity. Various novel combinations of inorganic nano-hybrids such as PtIr/CNT [13] and Fe_3O_4/graphene oxide [14] have been employed for divers applications. Thus, there is immense scope to explore new nanocomposites having enzymatic activities. Carbon nanotubes have fascinating feature of good catalytic activity even without catalytic factors [15]. The peroxides like activity of CNTs have been explored in the field of biosensors and biofuel cells. Similarly, ZnO NPs are known to have high catalytic activity due to their large surface area and high adsorption

* Corresponding author.
E-mail address: akhtarhayat@ciitlahore.edu.pk (A. Hayat).

http://dx.doi.org/10.1016/j.talanta.2015.05.051
0039-9140/

ability. The tremendous electrical and optical properties, high chemical stability and the reduced nontoxicity make them the most suitable candidate for sensing applications [16,17]. Keeping in view the above mentioned exciting features of CNTs and ZnO NPs, the catalytic activity of ZnO incorporated CNTs was explored for the detection of cholesterol. Herein, we have reported a novel, simple and sensitive assay for the colorimetric detection of cholesterol via H_2O_2, during which 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) was oxidized by H_2O_2 to produce a green colored product using a new combination of ZnO and CNTs to replace the commonly used natural enzyme.

2. Experimental

2.1. Reagents and instruments

Cholesterol, cholesterol oxidase (100 UN), hydrogen peroxide (H_2O_2), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), zinc acetate, Sodium hydroxide (NaOH) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich. The possible interfering compounds of the assay including phenol, histidine, uric acid, glucose, and absorbic acid were also purchased from Sigma. All purchased chemicals were of analytical grade, and used without further purification. The serial dilutions of stock solutions were prepared in order to obtain working solutions. Ultra-pure water was used to prepare stock solutions, as well as for all the dilutions. 96 Well Microplates were obtained from Greiner bio-one. The colorimetric measurements were carried out with Multiskan EX micro-titer plate reader. A Perkin-Elmer Lambda UV/vis spectrophotometer was used for the characterization of proposed assay.

2.2. Incorporation of ZnO on carbon nanotubes

To prepare the hybrid material, 5 mg of CNTs were dispersed in the neutral medium and subsequently sonicated for 2 h. Afterwards, the CNTs were mixed with zinc acetate (5 mM) and stirred for 3 h. NaOH was used to adjust the pH at 10. The obtained mixture was again stirred for 30 min. Subsequently, 20 mg of $NaBH_4$ was added with vigorous stirring for 30 min and then heated at 130 °C for 6 h. After completion of the reduction process, the final product was filtered and washed to remove the impurities [18]. The obtained nanocomposite was dispersed at a concentration of 0.5 mg/mL through the sonication process. The obtained ZnO incorporated CNTs were integrated in the construction of H_2O_2 and cholesterol colorimetric assays.

2.3. Procedure for the colorimetric detection of H_2O_2

The peroxidase like efficiency of ZnO incorporated CNTs determined by using ABTS solution. In the proposed assay, the reaction mixture of H_2O_2 and ABTS was incubated with 10 μL of nanocomposite. The intensity of green color of oxidized ABTS was determined by monitoring the absorbance at 405 nm. In reaction mixture, H_2O_2 was incubated in a concentration range between 0.1–37.5 μM in order to establish concentration dependence response and sensitivity of the nanocomposite. The calibration curve was obtained by plotting the values of absorbance against concentration. The kinetic parameters of the catalytic reaction were also performed by following the above described methodology.

2.4. Quantitative analysis of cholesterol

For the measurement of cholesterol, 65 μL of Cholesterol (the concentration range from 0.5 to 500 nmol), 25 μL of cholesterol oxidase (25 U/mL) and 162.5 μL of Phosphate buffer saline (PBS, PH 6.5) were incubated in the well of 96 microplate at room temperature for 10 min to obtain the testing sample solution. Afterwards, 20 μL of ABTS and 10 μL of ZnO incorporated CNTs were added to the reaction mixture. The reaction mixture was mixed well and the absorbance was measured after 20 min of incubation period at room temperature. The proposed assay was also applied to milk sample for the determination of cholesterol.

2.5. Selectivity of the proposed assay

The selectivity of the method was demonstrated by applying the same assay for the determination of glycine, uric acid, glucose, and absorbic acid which are the possible interfering compounds.

2.6. Preparation of milk powder sample

2.0 g of milk powder sample was dissolved in 10 mL of KOH/ethanol solution with subsequent sponification in a water bath for 1 h. Then, 10 mL of water and 20 mL of n-hexane were added to the solution and the reaction mixture was centrifuged for 5 min. The n-hexane was separated and solvent was evaporated under a steam of nitrogen. The residue was dissolved in isopropanol and triton.

3. Results and discussion

3.1. Principle for the colorimetric detection of cholesterol

The cholesterol sensor was developed by a colorimetric method during which cholesterol was oxidized in the presence of cholesterol oxidase to produce H_2O_2. The H_2O_2 formed during first step was systematically quantified by the oxidation of ABTS to give a green colored product that can be monitored at 405 nm by colorimetric analysis (see Eqs. (1) and (2)).The second step was catalyzed by the peroxidase like activity of ZnO incorporated CNTs. The overall depiction is provided in Scheme 1

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} H_2O_2 + \text{Cholest-4-en-3-one} \quad (1)$$

$$\text{ABTS} + H_2O_2 \xrightarrow{\text{ZnO NPs/CNTs}} \text{Oxidized ABTS} + H_2O \quad (2)$$

3.2. Colorimetric analysis under different experimental conditions

The peroxidase like catalytic activity of ZnO nanoparticles incorporated CNTs was demonstrated by the oxidation of ABTS in the presence and absence of H_2O_2. The oxidation rate of chromogenic substrate (ABTS) by H_2O_2 was significantly increased by the addition of catalyst, thus a green color was observed due to absorption of the radiations by the oxidized product in visible range (405 nm) (Fig. S1). A negligible green color was found in the presence of only ABTS and nanocomposite. Similarly the reaction did not proceed in the absence of ABTS. These results show that ZnO nanoparticles incorporated CNTs have excellent peroxidase like characteristics that can be used to mimic HRP enzyme for the colorimetric detection of H_2O_2.

Furthermore, ZnO nanoparticles incorporated CNTs were characterized by enzyme kinetic methodology for catalytic oxidation of ABTS by H_2O_2, which was employed to determine the kinetic parameters. To conduct the kinetic experiments, the concentration of one substrate was varied while the other was kept constant. The Lineweaver–Burk plots were used for the calculation of kinetic parameters including Michaelis–Menton (K_m) and maximum