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Colorimetric detection of cholesterol based on enzyme modified gold nanoparticles

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ABSTRACT

We develop a simple colorimetric method for determination of free cholesterol in aqueous solution based on functionalized gold nanoparticles with cholesterol oxidase. Functionalized gold nanoparticles interact with free cholesterol to produce H_2O_2 in proportion to the level of cholesterol visually is being detected. The quenching in optical properties and agglomeration of functionalized gold nanoparticles play a key role in cholesterol sensing due to the electron accepting property of H_2O_2. While the lower ranges of cholesterol (lower detection limit i.e. 0.2 mg/dL) can be effectively detected using fluorescence study, the absorption study attests evident visual color change which becomes effective for detection of higher ranges of cholesterol (lower detection limit i.e. 19 mg/dL). The shades of red gradually change to blue/purple as the level of cholesterol detected (as evident at 100 mg/dL) using unaided eye without the use of expensive instruments. The potential of the proposed method to be applied in the field is shown by the proposed cholesterol measuring color wheel.

1. Introduction

Due to inappropriate diet and unhealthy lifestyle leading to fatal heart attacks from cardiovascular diseases are a major concern in the last decades [1]. The arteries of blood vessels which prevent the blood circulation due to excess level of cholesterol in blood serum forms plaques and cause cardiovascular diseases [2]. In a healthy human, a typical cholesterol level required in serum is 200 mg/dL [3]. Therefore, demanding easy to use, cheaper and reliable detection method for routine monitoring of cholesterol in blood and food. The conventional colorimetric methods are used for detecting DNA [4,5], proteins [6–11], metal ions [12–14] and glucose [15], biothiol [16] and cancer biomarker [17]. However, the range of cholesterol detection offered by visual colorimetric sensing, especially the lower detection limit is poor from color discrimination, when compared to those offered by fluorescent detection techniques [18,19]. Thus, the present work aims to propose a system which could simultaneously take the advantage of both techniques for a much enhancement cholesterol detection.

Different conventional methods such as colorimetric and fluorescent detection techniques, electrochemical and molecular imprinting technology are used for cholesterol determination. But denaturation of the enzyme is the major challenge for all these methods [20,21,19,22]. Thus, constant focus of the researchers has been to find substrates which not only support easy immobilization of enzymes but also prevents its denaturation and provides enhanced enzymatic activity for the particular kind of detection technique being applied [23–25]. Along with this line, metallic nanoparticles, especially gold nanostructures based system have shown immense potential in biosensing owing to their properties like high surface area, non-toxicity, excellent chemical stability, biocompatibility, surface plasmon resonance effect and unique catalytic activity [26].

Gold nanoparticles are widely implemented in colorimetric biosensing for clinically important biomolecules due to having the wide opportunities it offers in the design of easy to perform methods [27–29]. Its large surface results in increased enzyme loading per unit mass of nanoparticles, while the multipoint attachment of enzyme molecules to nanomaterial surfaces reduces protein unfolding (enzyme denaturation), resulting in the enhanced stability of the enzyme attached to the nanoparticle surfaces. Additionally, these noble nanoparticles also offer interesting fluorescence properties. Although the majority of gold nanoparticles emits fluorescence in the visible spectral range with a very low (about 0.3%) quantum yield (QY), on the contrary, small nanoparticles emit fluorescence with little higher QY [30]. The tuning of unique fluorescence properties through functionalization of nanoparticles (GNPs) are stable and non-degradable fluorescence [31–34]

In addition to the above-mentioned properties, GNPs provide their potential in optical sensing as well. The plasmon resonance absorption

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exhibited by nanoparticles has been used with a view to developing analytical tools in clinical diagnosis [26]. It is well known that the gold nanoparticles display surface plasmon resonance band (SPRB) absorption at a specific wavelength (~519 nm) [35]. The change in SPRB depends on the size and shape of the gold nanoparticle as well as on the dielectric constant of the gold itself and of the medium surrounding [36]. On aggregation of the gold nanoparticles, the absorption maxima shifts to longer wavelength resulting in the color change of the gold colloidal solution from wine red to purple due to mutually induced dipoles that depend on inter-particle distance and aggregate size [37,38].

Thus realizing change in optical properties of GNPs which depend on the level of aggregation and size of nanoparticles being used, along with the good stability and immobilization advantages offered by GNPs, we adapt a system of cholesterol oxidase immobilized gold nanoparticles for simultaneous fluorescent and colorimetric detection of free cholesterol in aqueous solution based on the H_2O_2-induced aggregation of GNPs through cascade reactions using ChOx.

2. Materials and Methods

2.1. Materials

We have used all pure analytical grade reagents, gold chloride ($HAuCl_4$), trisodium citrate, Tween 80, 11-mecaptoundecanoic acid (MUDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), cholesterol oxidase (ChOx) and cholesterol were obtained from Sigma-Aldrich, Bangalore, India.

2.2. Instrumental Details

The investigation of surface modification of gold nanoparticles is monitored by using a Perkin-Elmer Lamda-25 spectrophotometer operated at a resolution of 1 nm. LS55 fluorescence spectrometer Perkin-Elmer is used for the measurement of fluorescence spectra. We have investigated of surface functionalization through Spectrum 100 FTIR Spectrometer, Perkin-Elmer. The structural morphological changes were examined by employing a scanning electron microscope [FE-SEM (Zeiss, Merlin)] instrument operated at an accelerating voltage 20 V to 30 kV and Samples for SEM were prepared Si/SiO_2 substrates, and FEI Tecnai-G^2 transmission electron microscopy (TEM).

2.3. Preparation of Gold Nanoparticles

We have used modified methods reported by Turkevich et al. for the synthesis of gold nanoparticles (GNPs) [39]. In briefly, first we took 2 mL of trisodium citrate dehydrate (1%) was added to the boiling solution of 20 mL of $HAuCl_4$ (1.0 mM) under constant stirring. The solution is removed from the hot plate when the color changed from colorless to wine red. The solution was cooled and kept under refrigeration for further use.

2.4. Functionalization of Gold Nanoparticles

The gold nanoparticles have functionalized with 11-Mercaptoundecanoic acid (MUDA) through chemisorption. In briefly, we took equal volumes of GNPs colloidal solution and Tween 20 (2 mg/mL in phosphate buffer, pH 7.0) that were gently mixed under constant stirring for 20 min [27]. For the preparation of 11-Mercaptoundecanoic acid (1 mM) solution was dissolved in ethanol solution and added to the above gold solution and incubated for 24 h schematically represented in Fig. 1. Finally we remove excess Tween 20 and 11-mercaptoundecanoic acid (MUDA) by centrifugation (6000 rpm for 20 min).

Well known carbodiimide coupling chemistry (EDC/NHS) used for the functionalization of gold nanoparticles with cholesterol oxidase shown in Fig. 1. The activation of carboxylic functional groups presence over MUDA-Fn-GNPs have been done through just adding of 5 mL of a mixture of EDC and NHS (PBS, 5 mM, pH 7.0) into 2 mL of functionalized GNPs (MUDA-Fn-GNPs) and incubated at room temperature for 1 h. Finally, we took 1 mL of cholesterol oxidase (1 mg/mL, PBS, pH 7.0) was added into the above mixture and incubated at room temperature for 2 h. Unbound cholesterol oxidase was remove by centrifugation at 14000 rpm, and re-suspended in 3 mL of phosphate buffer (pH 7.0) and kept refrigerated until use.

2.5. Preparation of Cholesterol Solution

We measured desirable concentrations of cholesterol was dissolved in 1 mL Triton followed by mild heating for dissolving of cholesterol. After complete dissolution of cholesterol, then finally we added 9 mL of distilled water to get a transparent solution.

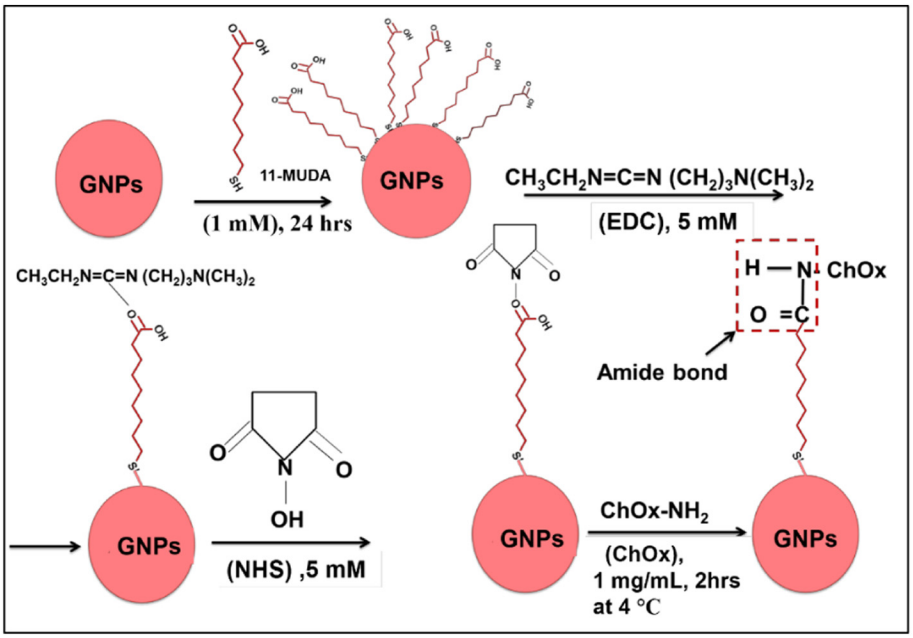

Fig. 1. Schematic representation of surface functionalization of GNPs.