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A novel sensing platform for sensitive cholesterol detection by using positively charged gold nanoparticles

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ABSTRACT

This work described a label-free platform for sensitive cholesterol detection upon the precipitation of cetyltrimethyl ammonium bromide (CTAB) stabilized gold nanoparticles (AuNPs). Modifying with CTAB, the stability of AuNPs was greatly improved compared to conventional used AuNPs, and the surface of them was positively charged, which could effectively adsorb with negatively charged DNA detection probe (DP) based on electrostatic adsorption, and inducing the precipitation of them. Tremendous H_2O_2 would produce based on the reaction of cholesterol with oxygen by using cholesterol oxidase (ChO_x) as the catalyzer, and followed the happen of Fenton reaction in the presence of Fe^{2+} to generate $OH^{\bullet}$, inducing the cleavage of DP into DNA fragments. As a result, the adsorption ability of AuNPs decreased, accompanying with a decrease of AuNPs precipitation. Then, based on detecting the absorption spectra change of AuNPs in supernatant along with the increase of cholesterol concentration, sensitive and simple visual-based cholesterol detection was realized with a detection limit of 335 pM. Such method showed high selectivity for the detection of cholesterol. Moreover, the high stability of such AuNPs in solution that contained salt, protein or other metal ions, making our sensing method have a potential application in bioanalysis.

1. Introduction

Cholesterol is of great importance in the cell of life, the level of it is directly associated with several diseases [1]. For example, low level of cholesterol is related with anemia, septicemia, hyperthyrea, hepatopathy and so on, while high level of it is symptom of hypertension, arteriosclerosis, brain thrombosis etc. [2,3]. Thus, detection of cholesterol plays an essential role for the early diagnosis of some related diseases. Due to the important biological function of cholesterol, a number of strategies including electrochemical [4–6], colorimetric [2,7], surface plasmon resonant [8], electrochemiluminescent [9,10] and fluorescent [11,12] have been developed for the detection of cholesterol.

Over the past decades, electrochemical [13–18], colorimetric [19–21] and fluorescent [22,23] biosensors have been widely used for the detection of biomolecules. Thereinto, colorimetric method is extremely attractive owing to its merits such as rapidity, simplicity, cost-effectiveness. Moreover, it can be read out easily by the naked eye without needing expensive analytical instruments.

Signal amplification strategies that used the nanoparticles is crucial to improve the sensitivity of the sensor [17,18]. Among which, gold nanoparticles (AuNPs) are widely used as nanoprobes in colorimetric biosensors preparation up to date because of the high extinction coefficients and unique distance-dependent optical properties of them [24,25], which is based on monitoring the visible color change of AuNPs from red to blue or from blue to red that induced by the assembly or disassembly of AuNPs [26]. Almost all of these colorimetric strategies mainly use the negatively charged AuNPs ((−)AuNPs) based on the cross-linking assembly-disassembly or salt-induced assembly of (−)AuNPs [27–29]. However, the (−)AuNPs based colorimetric methods suffer from weaknesses such as time-consuming due to the modification of AuNPs with DNA, or susceptible to the sensing environments that contained salt, protein and metal ions, which limit the application of such strategies in biological assay. Recently, positively charged AuNPs ((+)AuNPs) were introduced for colorimetric-based biosensors preparation based on the properties of (+)AuNPs that can adsorb onto the negatively charged surface of DNA. For example, Li's group developed a naked-eye sensitive detection of nuclease activity using (+)AuNPs as colori-

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metric probes [30]; Su and coworkers reported a visual detection of lysozyme based on (+)AuNPs [31]. Such label-free methods are relatively simple and low-cost, without needing the modification of substrate with AuNPs.

Herein, a novel and label-free sensor for cholesterol detection was proposed based on observing the precipitation of CTAB stabilized (+)AuNPs. To construct such a sensor, oligonucleotide with the sequence 5′-TCTGCCTTGCTAGGTATGGA TGGTATGCTC-3′ was designed as a detection probe (DP), and (+)AuNPs can adsorb with DP via electrostatic interaction and led to the precipitation of (+)AuNPs. Then, tremendous H_2O_2 would produce based on the reaction of cholesterol and oxygen by using cholesterol oxidase as the catalyzer, and Fenton reaction would occur in the presence of Fe^{2+} to generate $OH^{\bullet}$, which could directly cleave the DP into DNA fragments. As a result, the adsorption ability of AuNPs was gradually decreased along with the increase of cholesterol concentration, accordingly accompanied a decrease of AuNPs precipitation degree. Thus, cholesterol could be measured simply based on detecting the concentration of dispersed (+)AuNPs in supernatant (Fig. 1). Such strategy was similar to magnetic based sensors with the help of an external magnet, but simpler than those methods. In addition, (+)AuNPs used in such strategies showed good stability in solution that contained salt, protein or other metal ions because of the modification of AuNPs surface with CTAB as a common surfactant, which make our sensing method more tolerant to the sensing environments, and expected to have potential applications for biomolecules determination in real samples.

2. Experimental

2.1. Materials

Cholesterol oxidase (ChOx) was purchased from Sigma-Aldrich (USA). Cholesterol, uric acid, glucose, phenol, cetyltrimethyl ammonium bromide (CTAB), chloroauric acid ($HAuCl_4$) and sodium borohydride ($NaBH_4$) were purchased from Aladdin Biotech CO. Ltd. (Beijing, China). Ultra-pure water was obtained from Heal Force Smart-Nultra-pure water system and used for all of the experiments. All other chemicals were analytical grade and used without further purification. Different lengths of DNA strands were purchased from Shanghai Sangon Biotech Co. Ltd and the sequences of them were shown in Table 1.

10.0 mM of tris-buffer (pH 7.4) was used for cholesterol detection. 10.0 mM of phosphate buffered solution (PBS, pH 7.4, with 50 mM of NaCl) was used for DNA dissolution.

Table 1
Aptamers sequences with different lengths.

Aptamers	Sequence
15 bases	5′- CTAGGTATGGATGGT-3′
20 bases	5′- TTGCTAGGTATGGATGGTAT-3′
25 bases	5′- CTGCCTTGCTAGGTATGGATGGTAT-3′
30 bases	5′- TCTGCCTTGCTAGGTATGGATGGTATGCTC-3′
35 bases	5′-CC TCTGCCTTGCTAGGTATGGATGGTATGCTCTTT-3′

2.2. Apparatus

UV–vis absorption spectra of (+)AuNPs were recorded on an evolution 60 spectrophotometer (Thermo Fisher Corporation, USA). The size distribution of (+)AuNPs was conducted by transmission electron microscope (TEM, JEM-2010HR, Japan). The zeta potential analysis of (+)AuNPs was investigated using particle size analyzer (MS2000, England).

2.3. Preparation of (+)AuNPs

(+)AuNPs were obtained by reducing 15 mL of $HAuCl_4$ (1.0 mM) with 2 mL of $NaBH_4$ (100 mM) in the presence of 2 mL CTAB (10 mM) according to our previous method [32]. Then, the excess CTAB was removed by centrifugation for three times. After that, the average size of (+)AuNPs estimated from transmission electron microscopy (TEM) analysis was about 4 nm (Fig. 3A(a)). In addition, zeta potential analysis illustrated that such nanoparticles were positively charged (Fig. 3B(a)).

2.4. Preparation of human serum samples

Human serum samples were collected from Xuzhou Central Hospital, and were pretreated with isopropyl alcohol and EDTA (20 mM) to precipitate and remove the proteins and metal ions. Subsequently, these human serum samples were diluted 5×10^3 times to repress the possible effect from its complex components. Then, 50 μL of such diluted samples were added into 450 μL of the mixture that contained 5.0 U of ChO_x, 80 μM of Fe^{2+} and 10 μM of DP.

2.5. Visual detection of cholesterol

Different concentration of cholesterol was mixed with 5.0 U of ChO_x, 80 μM of Fe^{2+} and 10 μM of DP separately, and incubated for 1 h at 37 °C. After that, 450 μL of (+)AuNPs (10.6 nM) was added and kept at room temperature for 30 min. Finally, the concentration

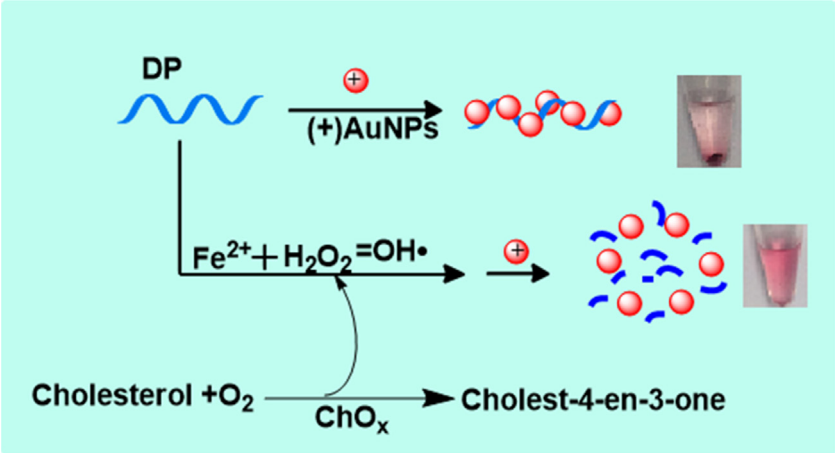

Fig. 1. The scheme for visual detection of cholesterol.