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A colorimetric sensor for the detection of hydrogen peroxide using DNA-modified gold nanoparticles

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ABSTRACT

Reactive oxygen species (ROS) are byproducts of biological reactions of energy generation, and depending on their concentration, ROS can be beneficial or harmful to cells and tissues. However, excessive ROS is hazardous. In this study, we used DNA-modified gold nanoparticles to develop colorimetric detection for hydrogen peroxide (H_2O_2). The assay has two steps. In the first step, ferrous ions are added into samples to react with hydrogen peroxide producing hydroxyl radicals ($^{\cdot}OH$) based on the Fenton reaction. In the second step, DNA-modified gold nanoparticles and sodium chloride are added. When hydrogen peroxide is present the color of the solution changes from red to purple because the hydroxyl radicals break the phosphodiester bond in the DNA and decrease the quantity of DNA on the surface of gold nanoparticles causing gold nanoparticle aggregation. All the operating steps could be completed in 15 min and the limit of detection (LOD) was 1 μM. This rapid colorimetric assay has potential for application in detection of other ROS.

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1. Introduction

Reactive oxygen species (ROS) are essential for human health [1]. An appropriate amount of ROS is crucial for living organisms; however; the species are highly hazardous if their concentration exceeds a limit [2]. Hence, detection and quantification of these species in food, the environment and living cells are important.

Among the various ROS including hydrogen peroxide (H_2O_2), the hydroxyl radical ($^{\cdot}OH$), hypochlorite, nitric oxide, and peroxynitrite, H_2O_2 is an important molecule in living cells and is produced during the activity of almost all oxidase enzymes [2]. H_2O_2 accumulation in the cell causes oxidative stress which can induce damage to proteins and DNA and is associated with aging and serious diseases [3]. The concentration of H_2O_2 in living organisms is extremely low [4] and the detection of H_2O_2 is important in food and environmental monitoring, diagnosis, and biological studies [5]. Therefore, it is essential to design a highly sensitive method to detect H_2O_2 for biological samples.

Over the last few years, various methods have been developed for H_2O_2 detection, such as fluorimetry [6], chemiluminescence [7], fluorescence [8], spectrophotometry [9], chromatography [10], electrochemical sensing [4,11] and surface enhanced Raman scattering [3]. Nevertheless, some of these methods are toxic or even cause cell destruction, which conflicts with the *in-situ* monitoring of H_2O_2 in live biological samples [12,13]. Others are sensitive and specific; however, complex procedures, time-consumption, high equipment cost and personnel training, limit their use in resource-limited laboratories.

Compared with the above analytical assays, colorimetric biosensors using modified gold nanoparticles have attracted considerable attention due to their rapidness and sensitivity. Gold nanoparticles (AuNPs) have been extensively used in different applications over the past decades, because of their easily controllable size distribution, long-term stability, ease of synthesis and surface functionalization by proteins and nucleotides [14]. For instance, Mirkin and co-workers developed a DNA analysis method by using two different single-stranded DNA (ssDNA) probe sequences, which binds two batches of AuNPs and mixes them with target

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DNA [15]. If the target DNA sequences are complementary to the sequences of both probes, the nanoparticles cross-link together leading to particle aggregation, resulting in a color change of the solution from red to purple and the peak wavelength of the solution shifts from 525 nm to 560 nm [15]. Additionally, Mirkin et al. founded that the bare AuNPs undergo irreversible particle aggregation in a solution of high salt concentration [15]. In contrast, DNA-modified AuNPs are stable because their DNA-modified surfaces protect them from NaCl-induced aggregation. Gold nanoparticle probes linked with ssDNA have emerged as an attractive alternative to molecular probes for many assays, including single-nucleotide polymorphisms [16,17], pathogens [18,19], mercury [20], and other small biomolecules [21].

It has been reported that the AuNP solution was stabilized by the citrate anions as their repulsion prevented the AuNPs from aggregating [22]. With the addition of salt, it would neutralize the negative charge of citrate, leading to the AuNP aggregation [22]. In addition, ssDNA with a random coil structure could adsorb onto the surface of AuNPs because of the coordination interaction between the nitrogen atoms of the exposed bases and the AuNPs. The negative charges of ssDNA on the surface of AuNPs prevent the salt-induced AuNP aggregation [23,24]. The aggregation of AuNP results in a distinct red shift of the surface plasmon resonance (SPR) [25]. It causes the color change from red to blue, observed by naked eye. In recent years, the peak shift of AuNP absorption spectra and the color change have been applied to colorimetric rapid screening test for the detection of various analytes [22,25,26].

More than a century ago, Henry John Horstman Fenton showed that a mixture of H_2O_2 and ferrous ions, called Fenton's reagent, had very strong oxidizing properties. A hydroxyl radical and a hydroxide ion are produced when a ferrous ion is oxidized by H_2O_2 to a ferric ion [27]. It is well known that hydroxyl radicals are highly reactive. In recent years, Fenton's reagent was used in several colorimetric sensors to detect various analytes. Liu et al. developed a colorimetric assay for blood glucose based on glucose oxidase and Fenton reaction to induce etching of gold nanorods [28]. Furthermore, Lai et al. designed a colorimetric immunoassay for detection of brevetoxin B using enzyme-controlled Fenton reaction with 3,3′,5,5′-tetramethylbenzidine-based colored system [27]. Xia et al. developed a colorimetric sensor containing Ag nanoprisms, glucose oxidase and Fenton's reagent for blood glucose detection [29].

In this study, a rapid and reliable colorimetric assay for ROS was developed. We used H_2O_2 as a model molecule and DNA-modified AuNPs as a probe for ROS detection. DNA-modified AuNPs could protect the AuNPs against NaCl-induced aggregation. H_2O_2 is also an essential reagent in the Fenton reaction, in which the ferrous ion reacts with H_2O_2 to form a hydroxyl radical [30]. The hydroxyl radical breaks the DNA and decreases the amount of DNA on the surface of AuNPs, resulting in NaCl-induced aggregation. This assay for detection of H_2O_2 could be observed by monitoring the color change of the DNA-modified AuNPs even with the naked eye.

2. Experimental section

2.1. Reagents and buffers

Gold nanoparticles (Nano Gold-40) were purchased from TANBead Taiwan. Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, E5134), sodium phosphate dibasic heptahydrate (S9390), and ethanol absolute (32224) were purchased from Sigma Aldrich. Sodium phosphate monobasic monohydrate crystal (3818-01), hydrochloric acid (9535-01), and TRIS (base) (4109-02) were purchased from J.T. Baker. Hydrogen peroxide (H_2O_2, 202460025), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 36383-0000000-14AC) were purchased from Acros Organics. Iron (II) Chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 0909-1150), sodium chloride (0241), and the DNA probes (5′TGTGGATGGGTGGTGAAAAAAAAAA-$(C)_6$-S-S-3′) were purchased from Showa Chemical, Amresco and BioBasic Inc.

2.2. Procedure of thiol modified oligo disulfide reduction

The lyophilized oligonucleotide was dissolved with 100 µl sterile deionized-distilled water. 50 µl of 100 µM oligonucleotide were mixed with 50 µl reduction solution (4 mg TCEP in 100 µl of 0.1 M pH 8.5 Tris-HCl) at room temperature for 3 h. The solution was then added in succession to 100 µl of 6% acetic acid, 25 µl of 3 M sodium acetate and 800 µl of absolute ethanol at −20 °C for 1 h. The mixture was centrifuged at 4 °C for 5 min and then the ethanol was decanted. The pellet was washed with 1000 µl of 70% ethanol, repeatedly centrifuged at 4 °C for 5 min twice and air dried for 10 min. Then the pellet was dissolved with 50 µl of deionized-distilled water and stored at −20 °C.

2.3. Preparation of thiol-DNA modified gold nanoparticles

The synthesis protocol of DNA-conjugated gold nanoparticles was modified from our previously described method [17,18]. Gold nanoparticles (500 µl) were mixed with 10 µl thiol-modified oligonucleotide for 16 h in the dark. The solution was then added to 56 µl of 0.1 M phosphate buffer (PB: NaH_2PO_4/Na_2HPO_4, pH 7.4) and 15 µl of 2 M NaCl. After 8 h, the colloidal dispersions were added in succession to 14.26 µl, 32.52 µl and 36.34 µl of 2 M NaCl solution for 8 h. The mixture was centrifuged at 4 °C for 20 min and then the supernatant solution was removed, the oily precipitate was washed with 500 µl deionized-distilled water, repeatedly centrifuged and suspended twice then re-suspended in 500 µl deionized-distilled water and stored at 4 °C until use.

2.4. H_2O_2 detection procedure

The H_2O_2 detection procedure comprised two steps. The first step was the reaction of ferrous ions with H_2O_2 to produce hydroxyl radical (·OH) based on the Fenton reaction. The different concentrations of 36 µl H_2O_2 (0, 1, 3, 5, 10, 50 µM) were mixed with 4 µl of 2 mM $FeCl_2$ for 5 min. The second step was the detection of H_2O_2 by adding DNA modified gold nanoparticles and sodium chloride. Then 60 µl deionized-distilled water, 100 (O.D. X µl) DNA-modified gold nanoparticles and 10 µl of 1 M sodium chloride were added in succession and mixed for 10 min. The solution was measured by UV–vis spectrophotometer.

3. Results and discussion

3.1. Mechanism of the colorimetric assay for the detection of H_2O_2

In this study, we developed a colorimetric assay that uses DNA-modified AuNPs to detect H_2O_2 (Fig. 1). The DNA-modified AuNPs were prepared as mentioned in the Materials and Methods and displayed red color. In this assay, H_2O_2, the analyte, could react with ferrous ions to generate hydroxyl radicals as the Fenton reaction. When H_2O_2 molecules were absent, following the addition of ferrous ion, the color of DNA-modified AuNPs in salt solution was still red because the negatively charged DNA could protect AuNPs from NaCl-induced aggregation. Whereas, in the presence of H_2O_2 and ferrous ions, the hydroxyl radicals broke the DNA on the surface of AuNPs, causing NaCl-induced aggregation, with the color changing from red to purple.

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