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Turn-on fluorescence detection of ascorbic acid with gold nanolcusters

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ABSTRACT

In this report, a sensitive fluorescent detection of ascorbic acid was developed with protein-stabilized gold nanoclusters. The fluorescence signal from the gold nanoclusters could be effectively quenched by elemental iodine. This kind of quenching could be inhibited by ascorbic acid through a corresponding reduction process, thus a turn-on response toward ascorbic acid was obtained. The validity and performances of the detection scheme were evaluated. The fluorescence responses were linearly related to ascorbic acid in the concentration range 0.1–10 μM, and the method was capable of detecting ascorbic acid down to 22 nM. The high performances of the developed scheme greatly simplified the sample preparation step, and the method was successfully applied for real sample analyses.

1. Introduction

Chemical oxidations are common in living bodies. With the proceeding of metabolic processes, a lot of reactive oxygen species (ROS), such as hydroxyl radical, singlet oxygen, etc. are generated [1]. Antioxidants are a type of species that inhibit the oxidation. They react with ROS inside the body, thus maintain some kind of balance in related biological processes, which is crucial for the proper functioning of the living organism [2]. One well-known example of these antioxidants is ascorbic acid (AA, also known as vitamin C or Vc). Because primates as human cannot obtain this species through their own biosynthesis, AA should be taken from external sources as a necessary nutrient [3]. This species exists in a variety of foods and drinks, and it is frequently detected for the evaluation of these sources. For its analysis, quite a few types of methods have been developed, including electrochemistry [4,5], luminescence [6–8], absorption [9,10], high performance liquid chromatography [11,12] etc. However, most of these methods suffer from low efficiency, limited sensitivity or complicated procedure, and facile and sensitive detections for this purpose are still of interest.

With the development of nanotechnology, nano-sized materials have been found useful in the field of analytical chemistry [13]. Some of these are capable of providing improved analytical performances, such as higher sensitivity, better selectivity, faster responses etc. Within these nanomaterials, gold nanoclusters (AuNCs), ultra-small entities consisting a few to tens of gold atoms in their metallic core, have also been intensively reported for analytical purposes [14]. Various targets, such as such as metal ions (Hg^{2+}, Cu^{2+}, Fe^{3+}), small molecules (as hydrogen peroxide, thiols) and large ones such as proteins, have been detected with this kind of method [15–23]. This material has also been applied for ascorbic acid analysis through related interactions [8,21,24]. However, as limited by the design of related detection schemes, only limited sensitivity was rendered. In this work, the reductive ascorbic acid was combined with the fluorescence property of the gold nanoclusters. It was found that ascorbic acid could prevent the AuNC fluorescence from being quenched by a related etchant. Through the selection of a powerful etchant, the amount of AA required for the recovery was significantly reduced, and thus a sensitive turn-on detection of AA could be achieved. This method was then successfully applied for real sample analyses.

2. Experimental section

2.1. Chemicals and reagents

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), iodine and ascorbic acid were obtained from Sinopharm Chemical Reagent Co., (Shanghai, China). Bovine serum albumin was from Suzhou Yacoo Chemical Co. (Suzhou, China). All other chemicals were of analytical grade and used as received. Double-distilled water was used throughout the experiments.

2.2. Instruments and apparatuses

Fluorescence signals were obtained on an F4600 fluorospectrophotometer (Hitachi, Japan) with a xenon lamp for the excitation. Ultraviolet-visible absorption spectra were measured with a TU-1901

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UV–vis spectrophotometer (PGeneral Instruments, China). Microscopic images of the nanoclusters were obtained with a Tecnai G220 transmission electron microscope (TEM, 200 kV) (FEI, USA).

2.3. Synthesis of bovine serum albumin-stabilized gold nanoclusters

Gold nanoclusters were synthesized as previously reported, in which a highly abundant protein, bovine serum albumin, served as both the reducing agent and the stabilizer [16,17]. All glassware used was first immersed in a chromic acid solution overnight, then thoroughly rinsed with water. Under vigorous stirring, 5 mL 0.01 M $HAuCl_4$ was added into 5 mL 50 mg mL^{-1} BSA solution. After that 0.5 mL 1 M NaOH solution was added and the obtained mixture was incubated on a 37 ℃ water bath for 12 h with continuous stirring. The reaction was then stopped and the mixture was kept at 4 ℃ in dark before use.

2.4. Fluorescence detections

Citrate-phosphate buffers at 20 mM were used for controlling the pH. The stock solution of the AuNCs was diluted 300-fold for applications. First ascorbic acid samples were mixed with the diluted AuNCs for a 40-min incubation under pH 7.0. The mixture was then transferred into a fluorescence cuvette and the fluorescence signals were recorded with the spectrofluorometer for further analysis.

2.5. Real sample measurement

Fruit or beverage samples were first centrifugated at 6000 rpm for 20 min. After that, the supernatant was collected and filtered with 0.22 μm membranes, and diluted with the buffer (1,800-fold dilution for fresh lemon juice and orange juice, 600-fold for orange-grape mixed juice and 1,000-fold dilution for a vitamin C drink). The samples were then analysis with the steps mentioned above.

3. Results and discussions

3.1. Gold nanoclusters and their response toward ascorbic acid

Gold nanoclusters were obtained through a facile route with a highly abundant protein bovine serum albumin (BSA), where BSA functioned as both the reducing agent and the stabilizer [16]. These AuNCs were found with diameter around 2 nm under the transmission electron microscope (Fig. 1A). They absorbed radiation in the UV region and gave out intense red luminescence with emission maximum at 639 nm (Fig. 1B).

For the structure of the gold nanoclusters, interaction with either the metallic core, the stabilizer, or the linkage between these two, might result in change of their luminescence behavior, and these interactions have been applied for the construction of various sensing systems [18], including the ones toward ascorbic acid here. One strategy for the AA detection is directly based its interaction with gold core of the AuNCs: as a strong reducing agent, AA is capable of reducing Au(I) on the gold core into Au(0), thus brings in a quenching effect. [24]. This interaction renders a "turn-off" signal, which is usually related to compromised reproducibility and limited linear range. Alternatively, an indirect scheme is developed on AA's functioning as an antioxidant. Strong oxidants breaks up the bonding between the gold core and the stabilizer, and brings in a fluorescence quenching [16]. The addition of a reducing agent, such as AA, inhibits related oxidation, thus prevents the AuNCs from subsequent quenching. [8]. For example, the AuNC fluorescence could be quenched by highly oxidative hydrogen peroxide [25], and AA's reaction towards hydrogen peroxide inhibited related quenching [8]. This scheme is favorable for the "turn-on" signaling. Unfortunately, it is usually limited by the low quenching ability from common oxidants, and high concentration of the oxidant is required to give enough quenching. This means in order to counteract the oxidant added, correspondingly large amount of AA (as the antioxidant) should be added, thus limits the sensitivity of the detection scheme.

The kernel part of this work is to introduce a new efficient oxidant as an alternative to the commonly used hydrogen peroxide. If this oxidant could render a significant quenching even under low concentration, then possibly in its corresponding removal step, only low amount of AA (antioxidant) would be required, and thus the detection performances could be improved. The difficulty in this idea lies in the fact that gold is one of the most inert metal in the periodic table, and it may remain intact with most oxidants. One reagent that is capable of reacting vigorously with gold, *aqua regia* (which is formed by concentrated hydrochloric acid and nitric acid with a volumetric ratio of 3:1), is highly acidic and corrosive, and thus incompatible with most analyses [26]. Fortunately, there do exist some formulas that could react with gold under non-extreme conditions. One example is the iodine/iodide combination, a low corrosivity solution, which is capable of etching gold with efficient reaction. In one of our previous studies, it was found that this mixture etched the gold core of the AuNCs and rendered significant quenching of the AuNC fluorescence [26]. In this reaction, although the I_2/I^- couple does not come with a high oxidative capability (0.54 V vs. SHE), the extra iodide has a strong binding with the gold ion produced (auric or aurous ions) ($lg\beta(AuI_4^-)$=44.7) and thus reduces the activity of free gold ion, which makes the etching proceed efficiently [27]. This interaction has been applied for the detection of elemental iodine down to 64 nM [26]. Thus it is possible that antioxidants (such as AA) that counteract elemental iodine in the

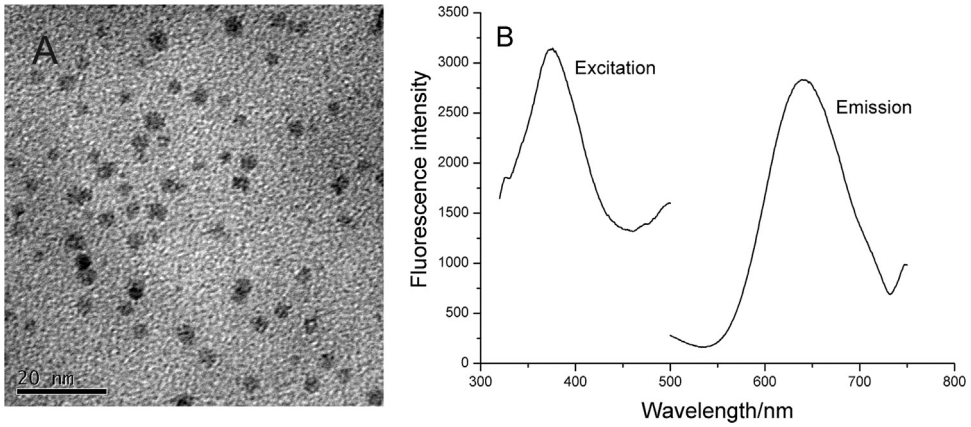

Fig. 1. (A) The transmission electron microscopic image of the synthesized gold nanoclusters. (B) The fluorescence excitation and emission spectra of the gold nanoclusters. The spectra were collected with 639 nm emission and 370 nm excitation respectively.