Talanta 144 (2015) 704–709

Contents lists available at ScienceDirect

Talanta

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

A gold nanocluster-based sensor for sensitive uric acid detection

Pingping Xu, Ruiping Li, Yifeng Tu, Jilin Yan *

The Key Lab of Health Chemistry and Molecular Diagnosis of Suzhou College of Chemistry, Chemical Engineering and Materials Science, Suzhou University, 199 Ren'ai Road, Industrial Park, Suzhou 215123, China

ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form
5 July 2015
Accepted 8 July 2015
Available online 10 July 2015

Keywords:
Uric acid
Hydrogen peroxide
Urate oxidase
Fluorescence quenching
Gold nanoclusters
Iodide

ABSTRACT

In this report, we developed a highly sensitive and selective sensor for the detection of uric acid. Gold nanoclusters were synthesized with bovine serum albumin as the template material. Under the catalysis of urate oxidase, hydrogen peroxide was generated, which quenched the fluorescence from the gold nanoclusters. Furthermore, excessive iodide was found capable of enhancing this quenching effect, which significantly improved the sensitivity of the detection. Under an optimized condition, the extent of quenching was found linearly related to uric acid concentration in the range of 0.7–80 μM, and uric acid as low as 120 nM could be detected. With simple dilutions, blood samples could be analyzed, and satisfactory recoveries were obtained.

1. Introduction

Uric acid (UA) is a product of the metabolite of purine nucleotides. For its low solubility in aqueous solution (approximately 60 mg/L), UA may accumulate in human body, and excessive amount in body fluid may form solid state urate and lead to gout or kidney stones [1]. High concentration of uric acid in blood may also relate to the deterioration of renal function and other disorders. Monitoring the level of uric acid in body fluid is of significant importance, which has been adopted as a factor for the evaluation of health status [2]. For the analysis, UA usually can be measured through two types of methods, non-enzymatic analyses and enzymatic ones, especially the latter. One popular strategy is the combination of an enzymatic reaction with an electrochemical detection: under the catalysis of related enzyme, urate oxidase (UOx), UA degrades and produces hydrogen peroxide, which is measured subsequently through an electroanalytical process [1,3–5]. Electrochemical methods provide compact detection systems, but since the signals come from the interfacial interactions, they usually suffer from unsatisfying reproducibility [6–8]. Other analytical methods include HPLC and spectroscopic analyses, which are cumbersome to used because of limited sensitivity or complicated instrumentations [9–11].

In recent years, noble metal nanoclusters (NCs) have been intensively reported for the construction of sensing systems [12–14]. These clusters consist of a few to tens of atoms, and can give out intense luminescence under suitable irradiation. This luminescence is strongly dependent on the status of the nanoclusters, and interaction from other species could alter the fluorescence properties. This phenomenon has been adopted for the detection of various targets, including ions, small molecules and large entities as oligonucleotides or proteins [14,15]. Among these target analyte, hydrogen peroxide (H_2O_2) is an interesting one. It plays an important role in biological processes, and is the product of a series of enzymatic reactions. This compound was reported being capable of quenching the fluorescence from gold nanoclusters [16,17]. Unfortunately, this kind of quenching was not significant, and only limited sensitivity could be provided. In the work here, the interaction between hydrogen peroxide and gold nanolcuter was adopted. It was found that some extra additive could enhance this quenching, and hydrogen peroxide could be detected at a much lower level. This effect was then coupled with the catalysis of urate oxidase for uric acid detection. The developed sensor was found sensitive and selective, and could be easily applied for real sample analysis.

2. Experimental sections

2.1. Reagents and materials

Uric acid, chloroauric acid ($HAuCl_4 \cdot 4H_2O$), hydrogen peroxide and potassium iodide were obtained from Sinopharm Chemical

* Corresponding author. Fax: +86 51262930812.
E-mail address: yanjl@suda.edu.cn (J. Yan).

http://dx.doi.org/10.1016/j.talanta.2015.07.027
0039-9140/

Reagent Co., (Shanghai, China). Urate oxidase and dialysis tubing with molecular weight cut-off (MWCO) of 14 kD were purchase from Solarbio Technology Co., (Beijing, 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

Absorption spectra of the samples were measured with a TU-1901 UV–vis spectrophotometer (Purkinje General Instrument Co., China). Fluorescence measurements were performed on an F-2500 spectrofluorophotometer (Hitachi, Japan). Elemental analyses were conducted with an ICP-2000 inductively coupled plasma atomic emission spectrometer (ICP–AES, Skyray Instrument Co., China). Microscopic images were obtained with a Tecnai G220 transmission electron microscope (TEM, 200 kV) (FEI, USA). Fluorescence images of the samples were taken with a Cannon SD1100 digital camera (Cannon, Japan) under the irradiation of a 365 nm UV lamp (Cnlight Co., China).

2.3. Synthesis of gold nanoclusters

Gold nanoclusters were synthesized through a popular method as previously reported [18,19]. An aliquot of 5 mL 0.01 M $HAuCl_4$ was mixed with 5 mL 50 mg mL^{-1} BSA solution under vigorous stirring. After 2 min, 0.5 mL 1 M NaOH solution was added, and the mixture was incubated on a 37 °C water bath for 12 h with continuous stirring. After that the reaction stopped and the mixture was stored at 4 °C in dark before use.

2.4. Detection gold nanocluster fluorescence

The stock solution of the nanocluster was diluted 300-fold and mixed with different samples. The pH value of the solutions was controlled with a 20 mM phosphate–citrate buffer. For enzyme-linked reactions, in every 1 mL of mixture, 3.6 U peroxidase was added and it was incubated with related substrates on a 40 °C water bath for 30 min; the solution was then mixed with the NCs. After that, related fluorescence spectra were recorded as the reporting signals.

2.5. Analysis of blood samples

Blood samples (from the First Affiliated Hospital of Soochow University) were first centrifuged at 3500 rpm for 5 min. After that, the up-layer serum was directly diluted 40-fold with a 20 mM phosphate–citrate buffer. The serum was incubated with UA for the degradation of uric acid and taken into the analysis.

3. Results and discussions

3.1. Nanocluster and its response toward hydrogen peroxide

For the nanocluster synthesis, a highly abundant protein, bovine serum albumin (BSA), was adopted as both the stabilizer and the reducing agent (BSA–AuNC). Since its development [18], this strategy has been widely adopted for nanoclusters with various template materials because of its convenience and effectiveness. With this method, clusters with sizes around 2 nm were obtained (from the TEM image in Fig. S1 in the supplementary material). These nanoclusters absorbed strongly in the UV region and gave out intense red luminescence with maximum around 610 nm (Fig. 1).

For the gold nanocluster-based sensing systems, most of them were based on the interactions with either the metallic core or the stabilizer [15]. As previously reported, hydrogen peroxide interacted with gold nanocluster and rendered related quenching. This quenching was proposed to come from the breaking down of the gold–sulfur (Au–S) bond and degradation of the nanoclusters [20]. Here the BSA–AuNC was tested with hydrogen peroxide as well. As expected, quenching of the fluorescence showed up. In order to confirm the degradation of the nanocluster, BSA–AuNCs were mixed with hydrogen peroxide at different concentrations and these samples were dialyzed against a pH 7 phosphate buffer. With membranes of 14 kD MWCO, small ions and molecules easily moved out of the membrane, and only gold atoms intercalated inside the cluster were retained and detected in the subsequent atomic emission analysis. The elemental measurements showed that as compared with intact gold nanocluster, 3% and 16% of loss of gold occurred with hydrogen peroxide at 200 and 330 μM, respectively. This indicated the occurrence of the degradations of the BSA–AuNCs during the interaction process, and larger amount of hydrogen peroxide was related to a greater loss of gold.

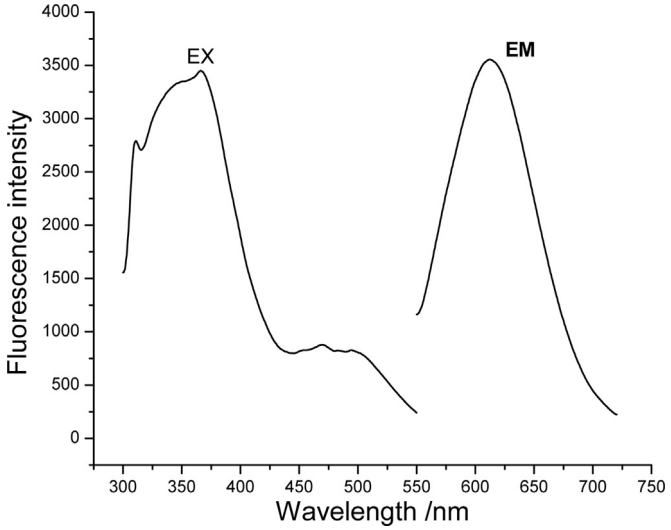

Fig. 1. Fluorescence excitation (a) and emission spectra and (b) of the BSA-stabilized gold nanocluster. The stock solution of the BSA–AuNC was diluted 40-fold with deionized water. The fluorescence excitation spectrum was measured with emission at 600 nm and the emission one was obtained with excitation at 365 nm.

In order to applied this quenching for the quantification of hydrogen peroxide, the extent of the quenching ($((F_0-F)/F_0)$, in which F_0 and F were the fluorescence intensities before and after the introduction of the hydrogen peroxide samples, respectively) was calculated. A Stern–Volmer plot was then constructed to relate the quenching and the concentration of hydrogen peroxide. It was found these two factors were linearly related in the H_2O_2 concentration of 3–1000 μM (Fig. S2). Also it was estimated that hydrogen peroxide could be detected as low as 0.9 μM (3 times of the standard deviation of the background noise, 3σ), the performances were similar to previous reports [17].

3.2. Enhanced fluorescence quenching with coexisting iodide

As previously reported, the quenching effect from hydrogen peroxide could be coupled with some oxidases for enzyme-based analyses. One example is the analysis of glucose with glucose oxidase [17]. It was possible that if the enzyme for uric acid, urate oxidase, was adopted, similar response for UA might be obtained. As for the much lower concentration of uric acid in body fluid (blood uric acid as 238–416 μmol/L for men and 178–357 μM for women) as compared with that of glucose (3.3–5.6 mM) [2], the sensitivity mentioned above might not be enough for uric acid analysis, especially when large-fold dilutions were about to be