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Copper nanocluster-based fluorescent sensors for sensitive and selective detection of kojic acid in food stuff

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ABSTRACT

We utilized bovine serum albumin-capped copper nanoclusters (CuNCs) to develop a new fluorometric method for the identification and determination of kojic acid (KA) with good sensitivity and selectivity. The fluorescence of CuNCs was quenched effectively by KA, but not by the possible coexisting substances. Based on multiple spectroscopic studies, we propose that selective binding of KA to copper ions of CuNCs promotes the formation of copper kojate on the surface of CuNCs, which statically quenches CuNCs fluorescence. The as-proposed biosensor allows selective determination of KA in the range from 0.2 μM to 50 μM, with a detection limit of 0.07 μM at a signal-to-noise ratio of 3. The CuNC-based fluorescent sensor provides a simple, rapid, cost-effective, and label-free platform for KA detection. Furthermore, we demonstrated this fluorescent sensor can be used for KA detection in food samples.

1. Introduction

Kojic acid (KA), 5-hydroxy-2-(hydroxymethyl)-4-pyrone, a fungal metabolite commonly produced by species of *Aspergillus*, is globally used in the production of a number of foodstuff, including soy bean paste, soy sauce and sake [1–3]. KA has gained increasing attention because of its various effective uses as an antioxidant, a preservative, a food additive to inhibit tyrosinase, an antimicrobial agent in fermented food, and an inhibitor of nitrosopyrrolidine formation in fried food [2]. However, safety assessment for KA remains controversial because of its potential carcinogenicity, embryotoxicity, and teratogenicity [4,5]. The Japanese Ministry of Health, Labor, and Welfare re-evaluated the use of KA in the food and personal care industries because of its possible carcinogenicity [6]. Furthermore, KA is restricted for use as an over-the-counter pharmaceutical product by the US Food and Drug Administration [5]. Since KA may severely harm human health, the monitoring of KA is very important in food safety and quality control. Thus, methods of detecting KA levels in food products to find out those containing relatively higher concentrations of KA

To date, gravimetric [7], colorimetric [8], electrometric [9–13], and chromatographic [14–16] techniques have been employed for the identification and determination of KA. Among these methods, electrochemical measurement suffers from the interference of coexisting electroactive substances, whereas chromatographic analysis often requires hours of work, skilled labor, and complicated procedures. The colorimetric method [8] that detects KA by the intense red coloration produced when KA is treated with ferric chloride has been used in many cases, but it is not specific for KA. As for the gravimetric detection method, it is limited by sensitivity, using which only more than millimolar concentrations of KA are detectable [7]. Thus, a simple and efficient sensing system for detecting KA directly with high sensitivity and selectivity should be developed.

Fluorescent sensors have received widespread attention in chemical and biological detection applications because of their high sensitivity, simplicity, and rapid implementation. Among the many fluorescent materials, transition metal nanoclusters (MNCs) with sizes comparable to the Fermi wavelength of the electron exhibit molecule-like properties, such as quantized charging and photoluminescence. MNCs are promising fluorescent probes because of their ultra-small size and water solubility. MNCs can detect the changes in fluorescence based on the specific interaction between the analyte and nanoclusters. In recent years, the synthesis of gold and silver nanoclusters have been extensively studied, as well as their application in biological labeling, imaging and sensing [17–23]. In contrast to gold and silver nanoclusters, there are few

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studies on the synthesis and optical properties of copper nanoclusters with much lower cost [24–26]. Recently, fluorescent copper nanoclusters (CuNCs) have been successfully prepared by Pradeep et al. using bovine serum albumin (BSA) as a template [26], and have shown great potential for lead ion sensing.

Inspired by the gravimetric detection method of precipitating KA with copper salts for the determination of KA [7], we present a fluorescent sensing system utilizing CuNCs for KA detection, which is the first example of a fluorescence sensing system for recognition and determination of KA that relies on the fluorescence quenching of CuNCs in the presence of KA. This method has the combined benefits of nanocluster and fluorescence detection, and offers the following advantages: (i) the sensor is designed with a simple, inexpensive, green, and one-pot synthetic route in aqueous solution, which minimizes cost and avoids the use of toxic reagents; (ii) the fluorescence behavior of CuNCs is highly sensitive to KA; (iii) the detection is time saving, easy, low cost, simple and reliable.

2. Materials and methods

2.1. Materials

KA (98%) was obtained from Alfa Aesar (Ward Hill, MA USA). Bovine serum albumin (BSA) was purchased from Sigma–Aldrich Chemical Co. (USA). Copper sulfate ($CuSO_4$) and other metal salts were of analytical reagent grade and used as received. Ultrapure water from a Milli-Q ultrapure water purification system (Millipore, Billerica, USA) was used throughout the experiments.

2.2. Apparatus

Fluorescence measurements were carried out using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Inc., USA) in a 1 cm × 1 cm quartz cell. The emission spectra were recorded in the wavelength of 350–900 nm upon excitation at 330 nm. The slit widths for excitation and emission were 10 nm. The UV–vis absorption spectra were recorded using a TU-1810 spectrophotometer (Pgeneral, China).

2.3. Preparation of BSA-capped fluorescent CuNCs

Fluorescent BSA-capped CuNCs were synthesized in aqueous solution according to a one-pot method with slight modification. Aqueous $CuSO_4$ solution (1 mL, 20 mM) was added to BSA solution (5 mL at 15 mg mL^{-1}) with vigorous stirring at room temperature. After 2 min, NaOH solution was introduced to reach a pH value of 12. The mixture was incubated at 55 °C for 8 h. Finally, the solution was dialyzed against distilled water using a 3 kDa cut-off dialysis bag for 36 h to remove the unreacted small molecules, and the final solution was stored at 4 °C. The as-prepared BSA-capped CuNCs were stable for more than a month.

Size information of the CuNCs can be obtained using transmission electron microscopy (TEM). As shown in Fig. S1 (Supporting information), the TEM image indicated that the average diameter of the CuNCs was 2.7 ± 0.8 nm which is similar to the result (2.8 ± 0.5 nm) observed by Goswami et al. [26]. The concentration of CuNCs was approximately 12.05 μM based on the average size of CuNCs (2.7 ± 0.8 nm). The as-prepared CuNCs solution was 10 times diluted with 20 mM acetate buffer (pH 7.0) for subsequent experiments. At this concentration, a strong fluorescence signal can be obtained and the possible self-absorption effect in the fluorescence measurements can be minimized.

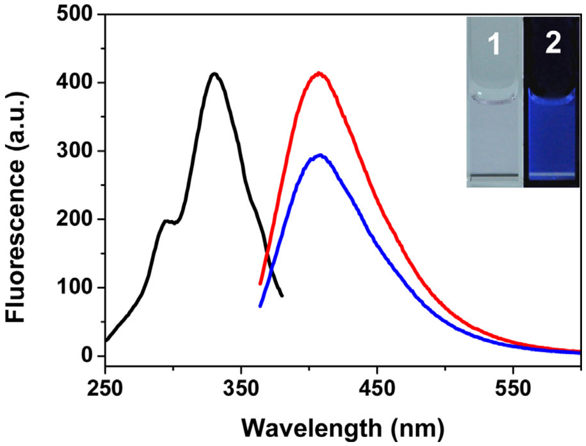

Fig. 1. Fluorescence excitation (black) and emission spectra of CuNCs in the absence (red) and presence (blue) of 50 μM KA. The inset displays the photograph of the CuNCs under visible and UV light. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.4. Fluorescence detection of KA

A stock solution of KA (0.1 M) was prepared, and various concentrations were obtained by serial dilution of the stock solution. KA solutions at different concentrations were added into the CuNC solution. The mixture solution was equilibrated at a specified temperature for 3 min before fluorescence measurement.

2.5. Selectivity and interference measurements for KA

To investigate the selectivity of CuNCs to KA over other coexisting substances, the following substances were used including potassium nitrate, sodium chloride, calcium nitrate, magnesium sulfate, ethanol, glucose, sodium benzoate, and sodium citrate. A 10 mM stock solution of the interfering substance was prepared. The solution was mixed with CuNCs at an appropriate volume in the absence or presence of KA. The solution was subsequently equilibrated for 5 min before the spectral measurements.

2.6. Detection of KA in real samples

Real samples were analyzed to test the practical use of CuNCs for KA detection. Samples of sauce and vinegar were obtained from the local market. A sample of 1 mL was added to 9 mL H_2O, and the solution was adjusted to pH 7.0 with 0.5 M sodium hydroxide. Subsequently, the solution was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through a 0.22 μm pore size syringe filter for further analysis.

3. Results and discussion

3.1. Spectral characteristics of the BSA-capped CuNCs

The prepared aqueous solution of BSA-stabilized CuNCs exhibited violet fluorescence under UV light (the inset pictures in Fig. 1), showing the successful preparation of fluorescent CuNCs. The fluorescence properties of the as-prepared CuNCs were further examined. As shown in Fig. 1, the fluorescent CuNCs had the excitation and emission peaks at 330 and 407 nm, respectively. The emission originates from the electronic transitions between the “sp” conduction band and the filled “d^{10}” band of CuNCs [26]. In this study, BSA-stabilized CuNCs were synthesized based on the capability of BSA to sequester and reduce Cu precursors *in situ* and further provide scaffold for the formed nanoclusters. BSA has