CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 43, Issue 12, December 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2015, 43(12), 1820–1828.

RESEARCH PAPER

Fluorescence "on-off" Responses of BSA-Cu System Towards Hydrogen Peroxide and L-Cysteine and Their Analysis Applications

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Abstract: The synthesis of copper nanoclusters (CuNCs) and its application as optical probe have attracted great attention. In this work, it was found that bovine serum albumin (BSA) reacted with copper ion (Cu^{2+}) in a base medium to form a stable BSA-Cu complex, and the introduction of hydrogen peroxide (H_2O_2) could remarkably accelerate the formation of CuNCs. At the same time, the fluorescence intensity increased rapidly. Based on the fluorescence "on" response of BSA-Cu system, a kinetics method was developed for the fluorescent detection of H_2O_2. The fluorescence intensity of BSA-Cu linearly increased with the increase of H_2O_2 concentration in the range from 1.0×10^{-6} M to 1.5×10^{-3} M with the detection limit of 3.1×10^{-7} M ($S/N = 3$). After that, the collected BSA-Cu solution was placed until its fluorescence intensity reached the maximal value, during which the Cu^{2+} ions were fully changed into CuNCs. The experiment results demonstrated that the addition of *L*-cysteine (*L*-cys) into the solution led to an obvious fluorescence quenching. Based on the fluorescence "off" response of BSA-Cu system, an analytical method was established for the fluorescent determination of *L*-cys. The fluorescence intensity linearly reduced with the increase of *L*-cys concentration in the range of 2.0×10^{-4}–1.0×10^{-2} M with the detection limit of 5.7×10^{-5} M ($S/N = 3$). Finally, the resulted BSA-Cu waste was treated by high temperature ashing and then dissolved with sulfuric acid, thus the CuNCs were turned into Cu^{2+} ions. The obtained Cu^{2+} solution continued to be used for the detection of H_2O_2 and *L*-cys in the next cycle. In this work, the cycle detection of H_2O_2 and *L*-cys and reuse of copper could be achieved through the conversion between Cu^{2+} and CuNCs. This method, with characteristics of high sensitivity, low cost and environment-friendliness, can be widely used for routine analysis of H_2O_2 and *L*-cys.

Key Words: Copper ions; Copper nanoclusters; Fluorescence probe; Hydrogen peroxide; *L*-Cysteine

1 Introduction

Metal nanoclusters, consisting of tens to hundreds of atoms, are one new type of nanomaterials. Different from metal atom, metal nanoparticle and metal substance, the size of metal nanoclusters is close to Fermi wavelength of electron. It can produce discontinuous electron energy levels and exhibits unique optical, electrical and chemical properties[1,2]. Compared to semiconductor quantum dots[3] and organic fluorescent dye[4], metal nanoclusters can not only generate size-dependent and tunable fluorescence, but also have many other excellent properties, including a relatively larger Stokes shift, high fluorescence quantum efficiency and good biocompatibility. To date, metal nanoclusters have been used in fluorescence detection, cell imaging and biologically labeling and other fields[5–7].

Copper is superior to noble metals in the conductivity and cost, thus the synthesis and application of copper nanoclusters (CuNCs) have received great attention in recent years. However, copper is highly chemically active and not stable in air and water, which make the preparation of water-soluble copper nanoparticles more difficult than noble metal

Received 20 May 2015; accepted 3 July 2015
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This work was supported by the Beijing Natural Science Foundation of China (No. 21176101).

DOI: 10.1016/S1872-2040(15)60882-9

nanoclusters. Researches showed that the stabilizer was an important factor affecting the formation of CuNCs and its optical property. In the preparation of water-soluble CuNCs, commonly used stabilizers included polyethylene imine[8], histidine[9], *L*-cysteine (*L*-cys)[10], penicillium amine[11], glutathione[12], DNA[13,14], bovine serum albumin (BSA)[15,16], lysozyme[17], trypsin[18] and transferrin[19], among which BSA was mostly used[20]. BSA contains 583 amino acid residues, 17 disulfide bonds composed of 35 *L*-cys and one free thiol group. The existence of free thiol group renders BSA certain reduction ability, so BSA can act as the stabilizer, chelating agent and reducing agent in the synthesis of CuNCs. Because the standard potential of copper is lower than that of gold and silver, the reduction of Cu^{2+} into Cu^0 by BSA is a very slow process, leading to a long time for the synthesis of CuNCs. Moreover, the as-prepared CuNCs contain a relatively high content of Cu^{2+}, which limits its direct application in biology, medicine and other fields. Recently, researchers attempted to use strong reducing agent such as hydrazine hydrate for improving the synthesis of water-soluble CuNCs, but the actual result was unsatisfactory[21].

In this study, the kinetics process of synthesizing water-soluble CuNCs with BSA as the stabilizer was investigated. The experimental results showed that hydrogen peroxide (H_2O_2) had a significant catalytic activity towards the formation of CuNCs. Based on fluorescence "on-off" response of the BSA-Cu system towards H_2O_2 and *L*-cys, a fluorescent method for quantitative detection of H_2O_2 and *L*-cys was established. The method had the advantages of high sensitivity, low cost and environment-friendliness, and was successfully applied to the detection of H_2O_2 and *L*-cys in drug samples.

2 Experimental

2.1 Main reagents and instruments

Bovine serum albumin (BSA), copper sulphate ($CuSO_4$), potassium sodium tartrate, sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and *L*-cys were purchased from Shanghai Chemical Company (Shanghai, China). The BSA solution was prepared by dissolving 1.5 g of BSA in 100 mL of water. The Cu(II) solution was prepared by dissolving 0.22 g of $CuSO_4$ and 0.42 g of potassium sodium tartrate in 100 mL of water, followed by adjusting pH to 12 with 1.0 M NaOH solution. The H_2O_2 solution was prepared by diluting 1.0 mL of 30% H_2O_2 with water to a final volume of 250 mL,and the exact concentration was determined by the $KMnO_4$ titration method. The *L*-cys solution was prepared by dissolving 0.121 g of *L*-cys in 100 mL of water. Phosphate-buffered saline (PBS, pH 7.4, Na_2HPO_4-NaH_2PO_4, 0.05 M) was used in the experiment. Other reagents were of analytical regent grade and purchased from Shanghai Chemical Company (Shanghai, China). Ultrapure water (18.2 MΩ cm) purified from Milli-Q system was used.

Fluorescence spectra were recorded on Cary Esclipse Fluorescence Spectrophotometer (Varian, USA). Absorption spectra were measured on TU-1901 Double Beams UV-vis Spectrophotometer (PERSEE, China)). Transmission electron microscope (TEM) image was accquired on JEOL 2010 FEG microscope (JEOL, Japan). Circular dichroism (CD) measurements were performed on MOS-450 Circular Dichroism Spectrometer (Bio-Logic, France). Zeta potential was measured on ZETASIZER 2000 Zeta Potential Analyzer (Brookhaven, USA). Lab dancer vortex oscillator was used for mixing of the solution (IKA, Germany). Fluorescent image of the solution was measured on ZF-1 type ultraviolet analyzer (Shanghai Jihui, China).

2.2 Procedure for detection of H_2O_2

1.0 mL of the BSA solution was transferred into a 5-mL centrifuge tube, and then 0.5 mL of Cu(II) stock solution was added. After oscillated for 5 s, different volume of H_2O_2 solution or real sample solution was added. Finally, water was added to a total volume of 2.0 mL. The mixed solution was oscillated for 5 s and then incubated in water bath at 45 °C for 5 min. The fluorescence intensity or spectrum of the solution was measured on fluorescence spectrophotometer at excitation wavelength of 320 nm and emission wavelength of 420 nm.

2.3 Procedure for detection of *L*-cys

0.25 mL of CuNCs solution was added to a 5-mL centrifuge tube containing 0.4 mL of PBS. After oscillated for 5 seconds, different volumes of *L*-cys solution were added into the mixture, and then diluted with water to 1.0 mL. The solution was oscillated for 5 seconds and left at room temperature for 30 min. The fluorescence intensity or spectrum of the solution was measured with excitation wavelength of 320 nm and emission wavelength of 420 nm.

2.4 Conversion of Cu^0 to Cu^{2+}

The wastewater containing Cu^0 produced after detection of *L*-cys was collected, and heated to dryness. The resulting residue was transferred into a platinum crucible and heated to 650 °C with a heating rate of 5 °C min^{-1} in a muffle furnace until the black carbon completely disappeared. The crucible was removed and cooled to room temperature. The residue was dissolved in 2 M H_2SO_4 to obtain a $CuSO_4$ solution.

3 Results and discussion

3.1 Formation of BSA-Cu complex

The reaction of $CuSO_4$ with a strong base will produce