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Carbon dots-silver nanoparticles fluorescence resonance energy transfer system as a novel turn-on fluorescent probe for selective determination of cysteine

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ABSTRACT

In this paper, we investigated the interaction of carbon dots (C-dots) prepared from orange juice with silver nanoparticles (AgNPs) using fluorescence spectroscopy. It was found that AgNPs efficiently quench the fluorescence of C-dots as a result of fluorescence resonance energy transfer (FRET). Thus, a novel FRET system between C-dots (as the donor) and AgNPs (as the acceptor) was introduced. Moreover, it was found that cysteine even at nanomolar levels could recover the fluorescence of C-dots due to the competitive adsorption of this compound onto AgNPs. This was exploited to design a simple and selective method for the determination of cysteine in the concentration range from 6.0 to 300 nM, with a detection limit of 4.0 nM. Cysteine was determined by this method in human plasma and urine samples with satisfactory results.

1. Introduction

Fluorescence resonance energy transfer (FRET) is a non-radiative process whereby an excited state donor (usually a fluorophore) transfers its energy to a proximal ground state acceptor through long-range dipole–dipole interactions [1]. In conventional FRET systems, fluorescent dyes are usually employed as both the energy donor and the acceptor. However, such FRET systems suffer from disadvantages such as photobleaching and interferences. In recent years, various nanomaterial-based FRET systems have been developed. In these systems, luminescent nanomaterials such as quantum dots (QDs), dye-doped silica nanoparticles, rare-earth upconversion nanophosphors and dye labeled microspheres could be applied as alternative energy donors for fluorescent dyes. These nanomaterials have several unique optical properties such as brighter fluorescence, wider selections of excitation and emission wavelengths and higher photostability. Moreover, noble metal nanoparticles like gold (AuNPs) and silver nanoparticles (AgNPs) could be applied as efficient acceptors for most fluorophores due to their high extinction coefficient and the broad absorption band [2]. Some nanomaterials, such as semiconductor QDs which are usually applied as energy donors in FRET systems, suffer from the complicated preparation process and high toxicity. Therefore, it is necessary to find a safer and simpler alternative to QDs with comparable fluorescent properties.

Carbon dots (C-dots) are a fascinating class of luminescent nanomaterials which were discovered in 2004 during the purification of single-walled carbon nanotubes. They are quasi-spherical carbon nanoparticles with sizes below 10 nm which have attracted tremendous attention in various fields due to their alluring properties such as size- and excitation wavelength-dependent luminescence emission, excellent photostability, favorable biocompatibility, simplicity of synthesis and good water solubility [3,4]. In addition, C-dots appear to be promising alternative to semiconductor QDs duo to low toxicity, high chemical stability and low environmental hazard [5,6]. Therefore, they have been applied in various fields such as bioimaging [7,8], catalysis [9,10], chemiluminescence [11,12] and fluorescence sensing [13–20]. A few C-dot-based FRET systems have been reported [21–24], in which C-dots serve as energy donors and AuNPs as acceptors. These systems have been applied for the determination of melamine [21], polybrominated biphenyl [22] and glutathione [24] in real samples. To the best of our knowledge, the FRET process between C-dots and AgNPs has not yet been studied.

Cysteine (Cys) is a sulfur-containing amino acid that plays an important role as a critical substrate for protein synthesis. The disulfide bonds, which could be formed between two Cys residues, play essential structural roles in many proteins [25]. Cys could be

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also employed as a biomarker for various medical conditions. For example, elevated levels of plasma Cys have been observed in a number of pathological conditions such as motor neuron, Parkinson and Alzheimer disease [26,27]. Therefore, determination of Cys levels in biological samples has appeared of great importance in health monitoring. Various optical methods including spectrophotometry [28–30] and fluorimetry [31–39] have been reported for this purpose. Recently C-dot-based optical probes have been developed for the assay of biothiols such as Cys homocysteine and glutathione [36,40].

In the present work, the FRET process between C-dots and AgNPs has been studied. Furthermore, it was shown that in presence of Cys the FRET efficiency of C-dots-AgNPs system reduced, and the fluorescence intensity of C-dots was restored. On the basis this finding, a fluorescent "turn-on" method was developed for selective determination of nanomolar levels of Cys in human plasma and urine samples.

2. Experimental

2.1. Apparatus

The fluorescence spectra were recorded using a Shimadzu RF-540 fluorescence spectrophotometer (Kyoto, Japan) equipped with a 1.0 cm quartz cell. UV–vis absorption spectra were obtained by a Cary-100 spectrophotometer (Varian, Sydney, Australia). Transmission electron microscopy (TEM) images of the C-dots and AgNPs were obtained using a Leo 906 transmission electron microscope (Zeiss, Oberkochen, Germany).

2.2. Reagents

All reagents were of analytical reagent grade. Doubly distilled deionized water (obtained from Ghazi Serum Co., Tabriz, Iran) was used throughout the experiment. Cysteine, silver nitrate ($AgNO_3$), triphenylphosphine (PPh_3), sodium borohydride ($NaBH_4$) and trisodium citrate were purchased from Merck (Darmstadt, Germany).

2.3. Synthesis of AgNPs

2.0 mL of 1% sodium citrate aqueous solution were added to 50 mL aqueous solution of 2.0×10^{-4} mol L^{-1} $AgNO_3$, with vigorous stirring. After 10 min, 1.0 μL of 10 mmol L^{-1} freshly prepared ice-cold $NaBH_4$ solution was added to the mixture. The colloid was stirred for 30 min and aged for 2 days at room temperature before being used.

The concentration NP solution was estimated by UV–vis absorption spectroscopy using the molar extinction coefficients at the wavelength of the maximum absorption of AgNPs, as obtained by Mie theory ($\varepsilon_{(15\,nm)} = 2.84 \times 10^9\ M^{-1}\,cm^{-1}$ [41]).

2.4. Synthesis of C-dots

C-dots were synthesized solvothermally according to the literature with some modifications [42]. Briefly, 20 mL of pulp-free orange juice (prepared from oranges grown in Gilan province, Iran) was mixed with 15 mL ethanol. The mixture was sealed into a 100-mL Teflon equipped stainless steel autoclave and heated at 120 °C for 5 h in a muffle furnace. The solution was cooled down to ambient temperature naturally. Afterward, in order to remove the unreacted organic moieties, 3 mL of the resulted solution was washed with 3.0 mL of dichloromethane. The mixture was centrifuged at 900 × *g* for 15 min to separate the less-fluorescent deposit. Then 4 mL of acetone was added to the upper brown solution and centrifuged at speed of 1600 × *g* for 15 min. The supernatant solution which contains the highly fluorescent C-dots was diluted to 50 mL with water and used for experiments.

2.5. General procedure for determination of cysteine

Typically, 1.0 mL of C-dot solution, 625 μL of 0.12 M Britton–Robinson buffer solution (pH 6.0) and 2.0 mL of AgNPs were added to a 5.0 mL volumetric flask. Then, an appropriate amount of Cys standard or sample solution was added and final volume of the mixture was adjusted to 5.0 mL with deionized water. After incubation for 7 min at room temperature, the fluorescence intensity was recorded at 455 nm with an excitation wavelength of 380 nm.

2.6. Preparation procedure for real samples

Human plasma samples were obtained from Blood Transfusion Center (Tabriz, Iran). A 0.5-mL aliquot of plasma was placed into a centrifuge tube and 40 μL of 0.2 M HCl and 20 μL of 0.4 M PPh_3 were added to it and vigorously mixed. After incubating for 20 min, 0.5 mL of acetonitrile was added into the obtained hydrolyzed plasma in order to precipitate proteins. The solution was centrifuged for 15 min and the supernatant solution was transferred into a 10-mL volumetric flask and diluted to the mark with water. A 300-μL portion of this solution was analyzed according to the general procedure.

No pretreatment was necessary for urine samples. The samples were diluted 100-fold with deionized water and 500 μL of the diluted urine was taken for analysis according to the general procedure.

3. Results and discussion

3.1. Characterization of C-dots and AgNPs

In this study, C-dots were prepared by hydrothermal treatment of orange juice [42]. As revealed by the TEM image (Fig. 1a), the synthesized C-dots were monodispersed and uniform in size. The diameter of C-dots was distributed in the range of 8 ± 2 nm.

FT-IR spectrum was applied to identify the surface functional groups of the synthesized C-dots. As shown in Fig. 1b, the peaks attributed to C=C and C=O stretching vibrations can be observed at 1643 and 1710 cm^{-1}, respectively. The peaks at about 1407 and 2930 cm^{-1} are related to the stretching vibration of C—H. In addition, the peak at 1055 cm^{-1} indicates the existence of C—O bonding, and the broad intense peak corresponding to O—H stretching vibrations can be seen at 3397 cm^{-1}.

The optical properties of synthesized C-dots were also investigated. The UV–vis absorption spectrum of C-dots demonstrated a broad absorption at 285 nm (Fig. 2a). The fluorescence spectra of C-dots are shown in Fig. 2b. As can be observed, the maximum emission was obtained at about 455 nm with an excitation wavelength of 380 nm. Furthermore, the prepared C-dots exhibited an excitation-dependent fluorescence behavior, which revealed a distribution of the different surface energy traps of the C-dots. The fluorescence quantum yield of the prepared C-dots was found to be about 11% by using quinine sulfate as a standard.

The prepared AgNPs was characterized by TEM and UV–vis spectroscopy. According to the TEM image shown in Fig. S1a (Electronic Supplementary material, ESM), the prepared nanoparticles are almost spherical with average sizes of around 15 nm.