Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 122 (2014) 238–245

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Fluorescence enhancement of quercetin complexes by silver nanoparticles and its analytical application

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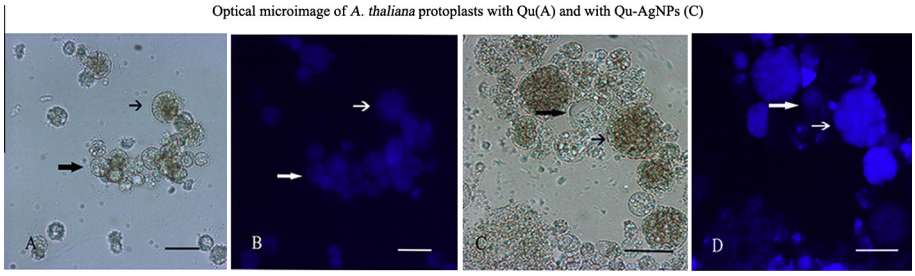
HIGHLIGHTS

- The system with AgNPs exhibited stronger luminescence and higher photostability.
- The Qu–AgNPs complex was used as a fluorescence probe for nucleic acids detection.
- The Qu–AgNPs system was applied in fluorescence image analysis of protoplasts.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form 1 November 2013
Accepted 10 November 2013
Available online 20 November 2013

Keywords:
Fluorescence enhancement
Quercetin
Silver nanoparticles
Nucleic acids

ABSTRACT

It is found that the plasmon effect of silver nanoparticles (AgNPs) helps to enhance the fluorescence intensity of the quercetin (Qu) and nucleic acids system. Qu exhibited strong fluorescence enhancement when it bound to nucleic acids in the presence of AgNPs. Based on this, a sensitive method for the determination of nucleic acids was developed. The detection limits for the nucleic acids ($S/N = 3$) were reduced to the ng mL^{-1} level. The interaction mechanism of the AgNPs-fish sperm DNA (fsDNA)–Qu system was also investigated in this paper. This complex system of Qu and AgNPs was also successfully used for the detection of nucleic acids in agarose gel electrophoresis analysis. Preliminary results indicated that AgNPs also helped to improve sensitivity in the fluorescence image analysis of Qu combined with cellular contents in *Arabidopsis thaliana* protoplasts.

Introduction

Silver nanoparticles (AgNPs) have been shown to possess high binding affinity for nucleotides in nucleic acids [1,2]. They have also served as the enhancement reagent in the study of the surface enhanced resonance Raman scattering (SERRS), resonance light scattering (RLS) [3–5] and surface enhanced fluorescence (SEF) [6,7]. The SEF on metal nanoparticles is related to the shape and size of the particle, and the distance between dye molecules and the metal nanoparticles surface. Due to their unique properties and potential applications, many studies have been done on complex probes of AgNPs and dyes [3,8]. Farcău and Astilean [9] demonstrated a 28-fold emission enhancement of Rose Bengal fluorophore when it was placed about 1 nm above silver half-shells. Xu's lab [10] reported the use of fluorescence probes hybridized by Aptamer/Oligomer-A/Cy3-modified AgNPs and Aptamer/Oligomer-B/Cy3-modified AgNPs for the ultrasensitive detection of immunoglobulin E (IgE). They also exhibited a new fluorescence aptasensor based on the SEF effect of silver nanoparticles for the detection of adenosine [11]. In our previous study in which AgNPs–Curcumin (CU)-Cetyltrimethylammonium Bromide (CTAB) was used for the detection of nucleic acids. It showed that AgNPs

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http://dx.doi.org/10.1016/j.saa.2013.11.055

could serve as a fluorescence enhancement reagent for the CU–CTAB nucleic acids system [12].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone, Qu) is a ubiquitous plant flavonoid found in many herbs and fruits (Fig. 1). The interactions between Qu and biomacromolecule [13,14] are commonly studied through fluorescence spectra of Qu complexes. Combining with metal ions [15] or biomacromolecule, the Qu complexes could exhibit higher fluorescence intensity than Qu alone. Our previous study found that DNA could enhance the fluorescence intensity of Qu [16]. And a sensitive determination method has been established. Here, we describe the effective enhancement effect of AgNPs on the fluorescence of the Qu-nucleic acid system. The complex system containing AgNPs coupled with Qu was used in the determination of nucleic acids in solution and in an agarose gel. We also used the complex system to probe and observe the fluorescence microimage of *Arabidopsis thaliana* (*A. thaliana*) protoplasts. In contrast to the system without AgNPs, the fluorescence of the AgNPs–fsDNA–Qu system showed an obvious synergistic enhancement effect. In addition, the interaction mechanism of the system of AgNPs–fsDNA–Qu was studied using multiple techniques, including fluorescence spectrometry, fluorescence polarization (FP), UV–vis spectrometry, circular dichroism (CD) and transmission electronic microscopy (TEM), etc.

Experimental

Apparatus

Fluorescence and fluorescence polarization measurements were performed with a LS-55 spectrofluorimeter (Perkin Elmer, USA) in a 1 cm quartz cuvette. All the absorption spectra were measured using a U-4100 spectrophotometer (Hitachi, Japan). The TEM images were taken on a JEOL JEM-1400 transmission electron microscope. All circular dichroism spectra were performed on a J-810S circular dichroism spectrometer (JASCO, Japan). All pH measurements were made with a Delta 320-S acidity meter (Mettler Toledo, Shanghai). Agarose gel electrophoresis was performed in a horizontal gel electrophoresis apparatus (DYY-6C, Beijing Liuyi instrument factory, China). The λDNA separation pattern was analyzed with a Syngene fluorescence gel imaging system (Syngene, USA). The fluorescence images were obtained using a fluorescence microscope with a CCD camera (Nikon ECLIPSE TE2000-U).

Chemicals

Stock solutions of nucleic acids (1.0×10^{-4} g mL^{-1}) were prepared by dissolving commercial fish sperm DNA (fsDNA) (Sigma), salmon serum DNA (smDNA) (Chemical Co. USA) or yeast RNA (yRNA) (Beijing Baitai Co., China) in 0.05 mol L^{-1} sodium chloride solution, respectively. A stock solution of Qu (1.0×10^{-3} mol L^{-1}) was made by dissolving 0.0302 g of Qu in absolute ethyl alcohol and diluting to 100 mL with anhydrous ethanol. A stock solution of silver nanoparticles (5.0×10^{-3} mol L^{-1} calculated by the concentration of the silver ion added) was synthesized by an electrochemical method as described in reference [17]. 1.0 g PVP k30 was dissolved in mixed solution of 1.0×10^{-2} mol L^{-1} of $AgNO_3$ (25.0 mL) and 1 mol L^{-1} of KNO_3 (5.0 mL), then diluting it to 50 mL with 0.22 μm-filtered ultra pure water (18.25 MΩ cm). The mixed solution was electrolyzed with stirring at 800 r/min for 12 min. The solution color changed gradually from colorless to yellow. The plasma resonance absorption peak of AgNPs is at 402 nm. All the solutions used were stored in a refrigerator at 0–4 °C. A formic–NaOH buffer solution was prepared with 0.2 mol L^{-1} formic acid solution adjusted to pH 4.40 with 0.2 mol L^{-1} NaOH solution.

Unless otherwise noted, all reagents and solvents were analytical grade and ultra pure water was used throughout.

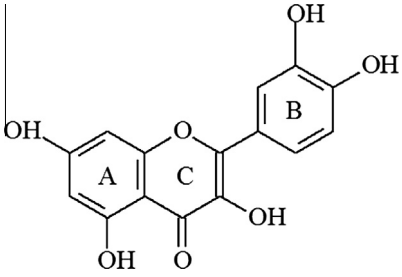

Fig. 1. The chemical structure of quercetin.

Procedure

Fluorescence spectra

To a 10-mL colorimetric tube, solutions were added in the following order: 0.3 mL of 0.2 mol L^{-1} formic–NaOH buffer solution (pH = 4.40), an appropriate amount of nucleic acids, 0.3 mL of 5.0×10^{-5} mol L^{-1} AgNPs and 0.15 mL of 1.0×10^{-4} mol L^{-1} Qu. The mixture was diluted to 5 mL with water and allowed to stand for 50 min. The excitation and emission slits were both 10 nm and the scan speed was 500 nm min^{-1}. The fluorescence intensity at 490 nm with an excitation wavelength of 440 nm was recorded. The enhanced fluorescence intensity of the AgNPs–fsDNA–Qu system was calculated as $\Delta I_f = I_f - I_f^0$ where I_f and I_f^0 represent the intensity of the system with and without nucleic acids.

Fluorescence polarization

Fluorescence polarization were recorded on a Perkin–Elmer LS-55 spectrofluorometer, both excitation and emission slits set at 10 nm. Solution used in the measurements of fluorescence polarization was prepared in the same way as described in fluorescence spectra measurements. The solutions were excited at 440 nm and the fluorescence was monitored at 490 nm through a pair of polariser filters. The Fluorescence polarization parameter P is defined as:

$$P = \frac{I_{//} - I_{\perp}}{I_{//} + I_{\perp}}$$

where $I_{//}$ and $I_{\perp}$, are the fluorescence components parallel to and orthogonal to the polarization of the exciting light.

Agarose gel electrophoresis protocol

Prior to gel casting, 0.6 g dried low temperature agarose gel was dissolved in 60 mL TAE buffer (1 × pH 8.3 tris-acetate-EDTA buffer) by being heated in a microwave for 2–4 min and was then poured into a 8 cm × 8 cm glass mold, which was fitted with a well-forming comb, and the processing procedures were performed according to Sambrook and Russel [18]. The conventional λDNA/*Hind*III + *EcoR* I digest molecular weight markers (λDNA marker) were used as DNA samples in the experiment. A total of 20 μL of DNA mixture containing 6 μL λDNA marker samples (1 μL, 0.5 μg λDNA marker + 1 μL of 6 × DNA Loading Dye + 4 μL of deionized water) and 14 μL Qu or Qu–AgNPs solution (Qu: 7.5×10^{-5} mol L^{-1}; AgNPs: 7.5×10^{-5} mol L^{-1}; formic–NaOH: 3.6×10^{-2} mol L^{-1}, pH = 4.40) were loaded in each sample well. Then 1 × TAE electrophoresis buffer was added into a horizontal electrophoresis apparatus until the buffer just covered the agarose gel. Electrophoresis was performed at 70 V for 30–60 min at room temperature, depending on the desired separation. After electrophoresis, the gel was cut into two pieces, one was immersed into