Sensors and Actuators B 247 (2017) 188–196

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Fabrication of thermoresponsive near-infrared fluorescent gold nanocomposites and their thermal manipulation

Nobuo Uehara*, Yoshikuni Numanami

Department of Material and Environmental Chemistry, Graduate School of Engineering, Utsunomiya University, 7-1-2 Yoto, Utsunomiya, Tochigi, 321-8585, Japan

ARTICLE INFO

Article history:
Received 22 July 2016
Received in revised form 25 February 2017
Accepted 2 March 2017
Available online 2 March 2017

Keywords:
Fluorescent gold nanoparticles
Thermoresponsive polymers
Near infra-red emission

ABSTRACT

Thermoresponsive gold nanocomposites emitting near-infrared fluorescence were fabricated and used for fluorometric staining of HeLa cells. The gold nanocomposites were composed of two types of gold nanoparticles conjugated with a thermoresponsive polymer bearing triethylenetetramine groups. One type of gold nanoparticle was fluorescent and had a particle size of 2 nm, and the other type was non-fluorescent, had a particle size of 6 nm, and exhibited an absorbance band at 310 nm. Conjugation with the thermoresponsive polymers brought the two types of gold nanoparticle together, forming gold nanocomposites that fluoresced at 830 nm. Changing the solution temperature induced the phase transition of the thermoresponsive polymers in the gold nanocomposites, altering the fluorescence of the gold nanocomposites. The gold nanocomposites accumulated in HeLa cells after 24 h incubation and were detected fluorometrically.

1. Introduction

Gold nanoparticles have attracted great interest as functional materials with unique optical properties and high chemical stability [1–5]. The optical properties of the gold nanoparticles are size-dependent. Gold nanoparticles larger than 6 nm show a plasmonic band in the visible region [6–10], whereas those smaller than the Fermi wavelength (2 nm) exhibit molecule-like characteristics such as luminescence arising from the electron transition of gold atoms in gold nanoclusters [11–13]. Another type of luminescent gold nanoparticles has been fabricated by the reaction of gold nanoparticles with thiol compounds [14–30]. Luminescence of the gold nanoparticles modified with thiol compounds comes from ligand-to-metal charge transfer of bonds of gold surface to thiols [31,32]. Luminescent gold nanoparticles have been used in bioimaging as inorganic fluorescent dyes because they are biocompatible and non-bleaching [14–19]. In particular, gold nanoparticles emitting near-infrared (NIR) fluorescence are promising for bioimaging because water and biological fluids are transparent to NIR.

Several methods for preparing NIR fluorescent gold nanoparticles have been reported [20–27]. The methods can be divided into two categories. The first is chemical reduction of tetrachloroaurate ions ($AuCl_4^-$). Chen at al. [22] fabricated NIR fluorescent nanogels by reducing $AuCl_4^-$ in a nanogel with cysteamine and sodium tetrahydroborate. The NIR fluorescent nanogels were used as fluorometric stains for tumor tissues. During the chemical reduction of $AuCl_4^-$ with bovine serum albumin, the protein acted as a reductant and a stabilizer of the gold nanoparticles, improving biocompatibility [21,26]. The gold nanoparticles were nanoclusters of 25 gold atoms and they exhibited fluorescence with a maximum wavelength around 700 nm. Similar gold nanoclusters were prepared by chemical reduction with dihydrolipoic acid [27]. The second type of preparation is chemical etching of larger gold nanoparticles with thiols [25,28–30]. Muhammed and coworkers etched 4–5 nm gold nanoparticles with glutathione to prepare NIR luminescent gold nanoparticles, which were nanoclusters of 25 gold atoms protected with glutathione [25].

In this paper, we describe a method for preparing NIR luminescent gold nanocomposites that does not belong to either of the two categories mentioned above. Conjugation of two types of gold nanoparticle with thermoresponsive polymers bearing triethylenetetramine groups brings the two types of gold nanoparticle into contact, producing a fluorescence band around 830 nm. Although the direct contact between the types of gold nanoparticle produced fluorescence, the fluorescence spectra decayed rapidly. The thermoresponsive polymers between the nanoparticles induced indirect contact, increasing the fluorescence of

* Corresponding author.
E-mail address: ueharan@cc.utsunomiya-u.ac.jp (N. Uehara).

http://dx.doi.org/10.1016/j.snb.2017.03.004
0925-4005/

the nanocomposites. Thermoresponsive fluorescent nanocomposites composed of gold nanoparticles and fluorophores have been reported [33–38]; however, thermoresponsive fluorescent gold nanocomposites consisting of fluorescent gold nanoparticles and thermoresponsive polymers have not. The preparation conditions, such as the type of thermoresponsive polymer, polymer concentration, temperature, and number of heating cycles, were examined. The resulting gold nanocomposites were used to stain HeLa cells.

2. Experimental

2.1. Reagents

Hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4$), sodium tetrahydroborate, glutathione and *N*-isopropylacrylamide, were obtained from Kanto Chemical (Tokyo, Japan). Triethylenetetramine and a 30% solution of polyethyleneimine (PEI) were purchased from TCI Fine Chemicals (Tokyo, Japan). *N*-Isopropylacrylamide was recrystallized with hexane before use. Reagent grade methanol was distilled before use. Ultrapure water (<18 MΩ) was used by PURELAB Ultra, Organo ELGA (Tokyo, Japan). All other reagents and solvents were obtained from commercial sources and were used as received.

2.2. Apparatus

UV–vis absorption spectra and fluorescence spectra of solutions were measured with a spectrophotometer (V-650, JASCO [Tokyo, Japan]) and a spectrofluorometer (F-6300, JASCO), respectively. Hydrodynamic diameters of gold nanoparticles were measured with a dynamic light scattering (DLS) system (Zetasizer Nano ZS, Malvern Instruments [Malvern, UK]). The gold nanoparticles were observed by field emission transmission electron microscopy (TEM; JEM-2010, JEOL[Tokyo, Japan]) operating at 200 kV.

2.3. Preparation of gold nanoparticles and thermoresponsive polymer

Fluorescent gold nanoparticles ($AuNC_{@GSH}$) were prepared by reducing $HAuCl_4$ with glutathione according to a previously described protocol [39]. In brief, a mixture of hydrogen tetrachloroaurate (2.5×10^{-3} mol/L, 1 mL) and glutathione (2.5 m mol/L, 1 mL) was stirred at 32 °C under ambient light for 36 h. The resulting solution was centrifuged at 13,000 rpm for 30 min at 4 °C to immerse and remove larger gold particles. The supernatant was taken and transferred to an Amicon Ultra-4 filter cartridge (Merck Millipore [Darmstadt, Germany]). The cartridge was centrifuged at 4500 rpm for 4 h to reduce the solution volume to *ca.* 0.5 mL for removal of starting materials dissolved in the solution. The remaining nanocluster solution in the filter cartridge was diluted with deionized water (3 mL) and then centrifuged at 4500 rpm for 4 h. The rinse steps were repeated three times.

A solution of non-fluorescent gold nanoparticles ($AuNP_{310}$) 6 nm in size was prepared by the chemical reduction of $HAuCl_4$ with sodium tetrahydroborate according to a previous method [40]. A hydrogen tetrachloroaurate solution (2.5×10^{-3} mol/L, 10 mL) was mixed with tetraethyleneglycol (5.15×10^{-2} mol/L, 50 mL), and the pH of the mixture was adjusted to 2.5 with hydrochloric acid. The volume of the mixture was adjusted to 100 mL by adding water. Subsequently, sodium tetrahydroborate solution (0.1 mol/L, 1.0 mL) was added and the mixture was stirred for 6 h at 25 °C. The mixture was passed through a silica gel column to remove the gold nanoparticle aggregates.

A thermoresponsive polymer, poly(*N*-isopropylacrylamide (90 mol%)-co-*N*-acryloyl- triethylenetetramine (10 mol%)) ($p(NIP_{0.9}\text{-}TETA_{0.1})$), was prepared according to a previous method [41]. In brief, prior to the radical copolymerization, a precursor, *N*-acryloyl triethylenetetramine, was synthesized from acryloyl chloride and triethylenetetramine. 25 mL of a 1,4-dioxane solution containing acryloyl chloride (0.01 mol, 0.91 g) was added to a cooled 1,4-dioxane solution (100 mL) containing 0.1 mol (14.6 g) of triethylenetetramine. The resulting white precipitate was filtered followed by suspension in 100 mL of methanol containing 0.01 mol (0.59 g) of potassium hydroxide. After filtration of the precipitated potassium chloride, the filtrate, which contained *N*-acryloyl triethylenetetramine, was subjected to the following copolymerization immediately without purification.

After the methanol solution of *N*-acryloyl triethylenetetramine was transferred into a 500 mL round-bottom separable flask equipped with a condenser, 0.09 mol (10.2 g) of *N*-isopropylacrylamide, 0.5 mL of 3-mercaptopropionic acid, and 0.82 g of azobisisobutyronitrile were added into the flask. The mixture was kept at 60 °C under nitrogen atmosphere. After cooling, the solution was poured into the same volume of cooled diethylether. The crude precipitation of the copolymer was dissolved into methanol and the solution was dialyzed with an ultrafiltration membrane (10 kDa).

2.4. Preparation of NIR-fluorescent gold nanocomposites and fluorometric imaging

NIR-fluorescent gold nanocomposites were prepared by mixing solutions of $AuNC_{@GSH}$, $AuNP_{310}$, and $p(NIP_{0.9}\text{-}TETA_{0.1})$. Solutions of $AuNC_{@GSH}$ (0.016 g/L, 1 mL), $p(NIP_{0.9}\text{-}TETA_{0.1})$ (0.2 wt%, 0.5 mL), and $AuNP_{310}$ (0.048 g/L, 0.5 mL) were added to a centrifuge tube. The mixture was heated at 90 °C for 30 min and cooled at 20 °C for 30 min. If necessary, the heating process was repeated. The NIR-fluorescent gold nanocomposites were characterized by spectrofluorometry, UV–vis photometry, TEM, and DLS.

Fluorometric imaging was conducted the following procedure. After culture of the HeLa cells in a Dulbecco's Modified Eagle Medium media (Sigma-Aldrich, St. Louis, US), the gold nanocomposite solution was added to each plate to make its concentration one-quarter. The cells were incubated for 24 h. The resulting cells were washed with a saline phosphate buffer (×1, pH 7.4) twice. The fluorescent gold nanocomposites were excited by exciting source with a 400–440 nm filter.

3. Results and discussion

3.1. Synthesis and characterization of gold nanoparticles and thermoresponsive polymer

Fluorescent gold nanoparticles, $AuNC_{@GSH}$ were synthesized by reducing $AuCl_4^-$ with glutathione according to reported methods [39,42–44]. Fluorescence of $AuNC_{@GSH}$ comes from ligand-to-metal charge transfer (LMCT) of glutathione bonded with gold surface [39,44]. Although the maximum fluorescence wavelength of the gold nanoparticles was reported as 650 nm [37], the gold nanoparticles we prepared had a shoulder peak at 830 nm in addition to the main peak at 650 nm (Fig. 1a) because LMCT is influenced by the environment of ligand [31,32]. The evolution of the shoulder peak suggests that the fluorescence band at 830 nm arose from the properties of the fluorescent gold nanoparticles [22], because 830 nm corresponds to 1.5 eV, which corresponds to the *sp-sp* transition in gold nanoclusters [45]. The TEM images in Fig. 1b show that the fluorescent gold nanoparticles were smaller than 2 nm, which is consistent with previous results [39]. MS spectra of $AuNC_{@GSH}$ depicted in Fig. S1 in SI showing periodic peaks with certain intervals of a gold atom and glutathione residues.