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Fluorescent labeling of cellular targets and multicolor imaging with gold nanorods

Shuguang Yao [a], Huai-Hong Cai [a,1], Mei Liu [a], Pei-Hui Yang [a,b,*]

[a] Department of Chemistry, Jinan University, Guangzhou 510632, China
[b] Key Laboratory of Optoelectronic Information and Sensing Technologies of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, China

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ABSTRACT

Gold nanorods (AuNRs) are among the promising emerging fluorescent labels for cellular imaging. However, it is unclear whether AuNRs can specifically label molecular target and fluorescent imaging at a subcellular level. Here we have used AuNRs linked to cystine and folic acid (FA) to label live cancer cells, and to detect the distributions of FA receptors on cell membrane. The labeling signals are specific for intended targets and more photostable than comparable organic dyes, rhodamine B. Using AuNRs with different excitation wavelength, we simultaneously detect one cellular target with different labeling colors. Furthermore, AuNRs-labeled red and AuNRs-labeled green came from the same cellular targets are observed with different filter sets. This result indicates that AuNRs-based fluorescent nanoprobes can be very effective in cellular imaging and offer substantial advantage over organic dyes in multicolor target detection.

1. Introduction

The development of new class fluorescent nanomaterials, which overcomes the intrinsic limitations of organic dyes or fluorescent proteins, is of considerable interest in many areas of research [1–3]. Gold nanorods (AuNRs), a kind of rod-shaped nanomaterial, have recently received great attentions as novel nanoprobes because of their unique optical properties [4,5]. Unlike gold nanospheres, AuNRs have two surface Plasmon absorption bands. A strong long-wavelength band is due to longitudinal oscillation of their electrons, while a weak short wavelength band around 520 nm is due to the transverse electronic oscillation [6]. The longitudinal absorption band is very sensitive to the size of the nanorods [7]. By increasing the aspect ratio (length divided by width) of AuNRs, the longitudinal absorption maximum of band has red shift with an increase in the absorption intensity. These properties of AuNRs open new possibilities for many biological and biomedical applications, including dark-field imaging, photothermal therapy, ultrasensitive bioassay and surface enhanced Raman scattering detection [8–11]. For instance, AuNRs have been applied as nanoprobes to localize blood vessels, tissue and cells by using two-photon luminescence (TPL) imaging [12,13]. AuNRs have been used to ultrasensitive detection of DNA and protein basing on fluorescence-based FRET assay [14,15]. Although studies have been reported on fluorescent characteristics of AuNRs [16], to our knowledge, the tunable wavelength of AuNRs has not been used in multicolor fluorescent labeling for living cells. El-Sayed and Eustis reported that the enhanced fluorescence intensity of AuNRs was related to their aspect ratio [17], but the fluorescent characteristics of AuNRs under different excited wavelength were unknown. Zhang et al. investigated GNRs as fluorescent labels in kidney cells by fluorescence lifetime imaging microscopy, which provided a better contrast ratio and more detailed features [18], but no multicolor labeling on cells was observed.

To demonstrate the labeling effectiveness of AuNRs on cells, folic acid (FA), a widely used targeting agent containing primary amines and carboxylic acid groups native to the structure, was chosen as our model system. FA is a low molecular targeting agent whose corresponding FA receptor, which is over-expressed on many types of cancer cells. In this work, we synthesized immunofluorescent nanoprobes of FA-conjugated AuNRs by linking AuNRs to FA molecules through cystine conjugation, and conducted investigations

* Corresponding author. Department of Chemistry, Jinan University, Guangzhou 510632, China. Tel./fax: +86 20 85223569.
E-mail address: typh@jnu.edu.cn (P.-H. Yang).
[1] Co-first author.

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into the multicolor labeling efficiency of these probes at the subcellular level (Scheme 1). We characterized luminescent properties, biomolecules conjugations, biocompatibility, and photostability of AuNRs or FA-conjugated AuNRs. Additionally, we detected FA receptors in the same cells simultaneously with different colors of AuNRs by changing excitation wavelength or filter sets. These results demonstrated the practicality of AuNRs as an attractive class of multicolor fluorescence labels for cellular imaging.

2. Experimental

2.1. Materials

All chemicals were of analytical grade and used without further purification. Hexadecyltrimethylammoniumbromide (CTAB, 98%), sodium borohydride (99%), chloroauric acid, silver nitrate, L-ascorbic acid, folic acid (FA), cystamine, thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma. RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco BRL (Gaithersburg, MD). Milli-Q water was used in all experimental processes.

2.2. Preparations of AuNRs

The AuNRs were synthesized according to the seed-mediated growth method [6]. The preparation of the seed solution used the following typical protocol: CTAB solution (5 mL, 0.20 M) were mixed with 5.0 mL of 0.00050 M $HAuCl_4$. To the stirred solution, 0.60 mL of ice-cold 0.010 M $NaBH_4$ was added, which resulted in the formation of a brownish yellow solution. Vigorous stirring of the seed solution was continued for 2 min. After the solution was stirred, it was kept at 25 °C. Then, CTAB (5 mL, 0.20 M) was added to (0.050, 0.10, 0.15, 0.20, 0.25 mL) of 0.0040 M $AgNO_3$ solution at 25 °C, respectively. Next, 5.0 mL of 0.0010 M $HAuCl_4$ was added to this solution, and then 70 μL of 0.0788 M ascorbic acid was added under gentle mixing. Ascorbic acid as a mild reducing agent changed the growth solution from dark yellow to colorless. It is worth noting that different aspect ratios of AuNRs above are identical processexcept for their silver ion content. The final step was the addition of 12 μL of the seed solution to the growth solution at 27–30 °C. The color of the solution gradually changed within 10–20 min. The temperature of the growth medium was kept constant at 27–30 °C in all the experiments.

2.3. Synthesis of FA-conjugated AuNRs

FA-conjugated AuNRs were synthesized through the modification of FA molecules on cystamine-functionalized AuNR surfaces. Firstly, the prepared AuNRs were reacted with cystamine at a AuNRs/cystamine molar ratio fo 1:50 at pH 7 for 3 h (25 °C), generating amine-linked AuNRs. The final functional AuNRs were purified by centrifugation at 100,000 *g* for 30 min. Secondly, FA molecules-conjugated AuNRs were prepared by using cross-linking reagent (1 mM EDC) and under the same experimental conditions (1:15 AuNRs/folic acid molar ratio, pH 7, 25 °C and 2 h) as for AuNR-biomolecule conjugates. The product was purified by centrifugation at 5000 *g* for 10 min to remove the unmodified FA molecules. After resuspension in PBS buffer (pH 7), aggregated FA–AuNRs were redispersed by centrifugation at 6000 g for 5 min.

2.4. GNRs characterizations

To visualize the nanostructure of AuNRs, a drop of the stock for each sample was placed on a TEM grid and viewed using a Hitachi (H-7650) transmission electron microscope (TEM) operated at an accelerating voltage at 80 kV. Fourier transform infrared spectroscopy (FT-IR) spectra of the samples were recorded on Equinox 55 IR spectrometer in the range of 4000–400 cm^{-1} using the KBr-disk method. The zeta potential of different AuNRs was measured by a Nano-ZS instrument (Malvern Instruments Limited).

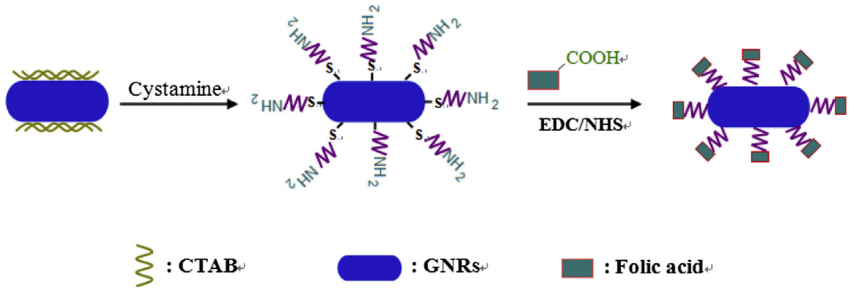
2.5. Cell culture and MTT assay

HepG2 hepatocellular carcinoma cells were purchased from the type culture collection of the Chinese Academy of Sciences (Shanghai, China). Cell lines were maintained in RPMI-1640 media supplemented with penicillin (100 units/mL), fetal bovine serum (10%), and streptomycin (50 units/mL) at 37 °C in a humidified incubator with 5% CO_2 atmosphere.

Cell viability was determined by measuring the ability of cells to transform MTT to a purple formazandye. Cells were seeded in 96-well tissue culture plates at 2.5×10^3 cells/well for 24 h. The cells were then incubated with FA–AuNRs at different concentrations for different periods of time. After treatment, 20 μL/well of MTT solution (5 mg/mL PBS) was added to the well and incubated for another 5 h. To dissolve the formazan salt, the medium was aspirated and replaced with 150 μL/well DMSO. The cell growth condition was reflected by the color intensity of the formazan solution. Absorbance at 570 nm was taken by a 96-well microplate reader (MD VERSA max).

2.6. Flow cytometric analysis of cells

The fluorescence-labeling cells were monitored by flow cytometric analysis. Briefly, the cells treated with or without FA–AuNRs were harvested and washed with PBS. After being fixed with 70% ethanol at −20 °C overnight, the fluorescence-labeled cells were washed twice with PBS and then subjected to flow cytometric analysis. Fluorescent signals of cells were analyzed using Multi-Cycle software (Phoenix Flow Systems). For each experiment, 10,000 events per sample were recorded.



Scheme 1. Schematic illustration of FA-conjugated AuNRs for fluorescence-labeling cells.