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DNA G-quadruplex-templated formation of the fluorescent silver nanocluster and its application to bioimaging

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ABSTRACT

Herein, a novel kind of silver nanocluster is synthesized simply by mixing G-quadruplex template with silver ions and reduction reagent ($NaBH_4$, here). AS1411 (a G-quadruplex that can bind nucleolin over-expressed in cancer cells) is used as the main model template to prove the synthesis protocol and its potential application. We used fluorescence assay, CD, MALDI TOF MS, and TEM to characterize the silver nanocluster. It is found that after formation of the silver nanocluster, AS1411 still keeps its structure and is able to bind with nucleolin in cancer cell. Meanwhile, this binding behavior can greatly enhance the fluorescence intensity of the silver nanocluster. This property can be directly employed into bioimaging HeLa cells. The cell toxicity (3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide, MTT) assay demonstrated that the silver nanocluster has only little affect on the cytotoxicity to the cells, which further proves the applicability of the method in tumor cell imaging. At last, the universality of the synthesis protocol is verified by using a series of other G-quadruplex sequences as templates. For a lot of functional nucleic acids, such as human telomeres and certain aptamers, are with G-rich sequences and can fold into G-quadruplexes in functioning conditions, our method displays a promising application space in future researches.

1. Introduction

Noble metal nanoclusters, consisting of a few metal atoms, have gained great attention due to their unique fluorescence properties and potential applications in the field of biological imaging. In particular, silver nanoclusters synthesized using polycytosines as matrices have attracted more attention in the field of medicine, pharmacology and biology. Silver nanoclusters encapsulated by the single-stranded oligonucleotide dC12 arguably consist of the most promising system for their very high photoemission rates and their excellent photostability [1–4]. It is generally known that silver ion and C base are easily incorporated. The high affinity of Ag^+ for deoxyribonucleic acid (DNA) bases has enabled creation of short oligonucleotide-encapsulated silver nanoclusters without formation of large nanoparticles. Time-dependent formation of nanocluster sizes ranging from Ag_1 to Ag_4 oligonucleotide was observed with strong characteristic electronic transitions between 400 and 600 nm [5]. The reduction of silver cations bound to the oligonucleotide dC12 was used to form silver nanoclusters. Mass spectra show that the oligonucleotides have 2–7 silver atoms that form multiple species, as evidenced from the number of transitions in the fluorescence and absorption spectra [6,7]. These silver nanoclusters made of few-atom offer great potential in pushing in vitro, and possibly in vivo, single-molecule study faster and also in longer time scales [1–4]. DNA-encapsulated silver nanoclusters are readily conjugated to proteins and serve as alternatives to organic dyes and semiconductor quantum dots. DNA/silver nanoclusters of guanine-rich DNA ("guanine" is abbreviated to "G") sequences could emit red fluorescence and enhanced 500-fold when placed in proximity to cytosine-rich DNA ("cytosine" is abbreviated to "C") [8]. The fluorescence of silver nanocluster is easily observed when nucleolin of the live cell surface is connected with that templated by AS1411. These nanomaterials offer new approaches for bulk and single molecule biolabeling [9]. Aptamers can bind with a wide array of targets with high affinity and specificity. Based on the phenomenon, we can search aptamer which is related to tumor and applied biomedical system. In this work, G-quadruplex formed by G-rich oligonucleotide was selected as matrices to synthesize silver nanocluster. No paper about G-quadruplex templated silver nanocluster has been published so far.

In this paper, AS1411 is selected to investigate as typical G-quadruplex. AS1411 (also known as AGRO100) is based on a 26-base G-rich oligonucleotide which recently entered in phase II human clinical trials. AS1411 represents the first nucleic

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acid-based aptamer to be tested in patient for the treatment of cancer [10]. AS1411 is easily formed a dimeric G-quadruplex structure in the presence of K^+ and targeted nucleolin with high affinity and specificity. At the same time, AS1411 is multifunctional aptamers in bioanalysis and has been applied in the chemiluminescence (CL) detection of the protein marker nucleolin expressed at the surface of HeLa cells [11]. The G-quadruplexes have also been shown to have antiviral activity and AS1411 is a multifunctional anticancer aptamer with the ability to combine with two ligands simultaneously. For example, AS1411 is incorporated with nucleolin in cancer cell and hemin. Furthermore, AS1411 is also able to bind another ligand protoporphyrin IX (PPIX). Herein, we report a novel approach to DNA G-quadruplex-templated formation of fluorescent silver nanocluster and its application to bioimaging. We demonstrate the method of generating G-quadruplex-encapsulated silver nanoclusters in aqueous solution. The G-quadruplex-encapsulated silver nanocluster chiefly made of 2–4 silver atoms was demonstrated by matrix assisted laser desorption ionization/time of flight mass spectrum (MALDI TOF MS).

2. Materials and methods

2.1. Chemicals and materials

Silver nitrate ($AgNO_3$, (Sigma-Aldrich, 99.9999%)), sodium borohydride (AF granules, 10–40 mesh, 98%, Sigma-Aldrich), and all chemicals of analytical grade were used as received without further purification. 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (USA). The oligodeoxynucleotide used synthesizing silver nanocluster synthesis for cell imaging was AS1411, an antiproliferative G-rich oligodeoxynucleotide (GRO) which sequence is 5′-d(GGTGGTGGTGGTTGTGG TGGTGGTGG)-3′. The other GROs also used in silver nanocluster synthesis were human telomeres, OXY, PW17, T30695, PS2M, 93del and their sequences were 5′-d (GGGTTAGGGTTAGGGTTAGGG)-3′, 5′-d(GGGGTTTTGGGGTTTTGGGGTTTTGGGG)-3′, 5′-d (GGGTAGGGCGGGTTGGG)-3′, 5′-d(GGGTGGGTGGGTGGGT)-3′, 5′-d(GTGGGTAGGGCGGGTTGG)-3′, 5′-d(GGGGTGGGAGGAGGGT)-3′, 5′-d(AATTCCCCCCCCCCCCCCCCAATT)-3′(called "C16″ for short) respectively. All the GROs were synthesized and HPLC purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Before using, these GRO powders were dissolved in the 100 mM KCl, 20 mM potassium phosphate buffer (pH 7.4). The concentrations of them were quantified by using UV/vis/near IR spectrophotometer and determined using the 260 nm UV absorbance and the corresponding extinction coefficient: $A = 15{,}400$, $C = 7400$, $G = 11{,}500$, $T = 8700$. Phosphate Buffered Saline (PBS) [10 mM phosphate (NaH_2PO_4 and Na_2HPO_4), pH 7.4] was also prepared. The PBS buffer is used to rinsing suspension of HeLa cells. All the solutions were prepared by using distilled water and stored at 4 °C before use. HeLa cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% standard Fetal bovine serum (Defined FBS) (HyClone Laboratories, UT) at 37 °C and in 5% CO_2. Glass chamber slides (14 mm bottom well) were purchased from Hangzhou Sanyou Biotechnology Co. Ltd. (Hangzhou, China).

2.2. Apparatus

UV/vis absorption spectra were carried out by a CARY 500 UV/vis/near-IR spectrophotometer (Varian). Fluorescence measurements were recorded at room temperature using a LS 55 luminescence spectrometer (Perkin-Elmer). CD signal was performed by JASCO-820 Circular Dichroism spectrometer (Tokyo, Japan). The sample for cell imaging was obtained by fixing the bound cells using silver nanocluster on a 35 mm tissue culture dish (World Precision Instruments) and acquired the fluorescence images using LEICA TCS SP2 laser scanning confocal microscope (Germany) with a 100× oil immersion objective. TEM images were obtained with a FEI TECNAI G^2 transmission electron microscope (Netherlands) operating voltage of 120 kV.

2.3. Synthesis of GRO_m:Ag_n solutions

The purified GROs were dissolved in a buffer containing 100 mM KCl, 20 mM potassium phosphate buffer (pH 7.4) at a concentration of 10 mg/mL and allowed to dissolve over several hours. Samples were then equilibrated for 10 min at 90 °C in a water bath, followed by spontaneous cooling to room temperature overnight (annealed samples). The DNA was also subjected to rapid cooling by heating a 50 μM sample in the same buffer to 90 °C for 10 min. The DNA samples were then refrigerated until use.

The GROs and $AgNO_3$ solutions (concentration ratio = 1:6) were mixed and cooled to 0 °C with ice. After 15 min, the solutions were reduced with $NaBH_4$ (concentration ratio of $AgNO_3$:$NaBH_4$ = 1:1) and shaken intensively to form silver nanoclusters (GRO_m:Ag_n). In practically, concentration ratio of $AgNO_3$:GRO:$NaBH_4$ was 6:1:6. The $NaBH_4$ was dissolved in deionized water and added within 30 s. The solvents for all solutions were deionized water unless otherwise mentioned.

CD spectra were recorded using a 1 mm optical path length-quartz cell and an instrument scanning speed of 100 nm/min with a response time of 2 s at room temperature. CD spectra were obtained by taking the average of three times scans made from 210 to 340 nm. All DNA samples at a final concentration of 10 μm were dissolved in 50 mM PBS buffer (pH 7.4).

2.4. Bioimaging

HeLa cells incubated with AS1411 encapsulated silver nanocluster. HeLa cells were plated onto 35 mm glass chamber slides. Stock solutions of AS1411 encapsulated silver nanocluster dissolved in TE buffer were prepared at concentrations of 10 μM. Diluted solutions in complete growth medium were then freshly prepared and placed over the cells for 2–3 h. All cells were washed with PBS buffer (3×) at room temperature. After that, cells were scanned by LEICA TCS SP2 laser scanning confocal microscope.

2.5. MTT assay

In order to evaluate the silver nanocluster dose on cellular toxicity, the complex treated cells were illuminated for a serial concentration. After treatment, cells were incubated in fresh medium for 24 h. The culture medium was replaced by 100 μL fresh medium containing 0.5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assays reagent (MTT assay), and then incubated at 37 °C and 5% CO_2 for 4 h. Then, the MTT containing medium was added with 100 μL of acid/isopropanol solution, in which the concentration of HCl was 0.04 M to dissolve the MTT product, formazan. Viability of non-silver nanocluster-treated control cells was arbitrarily defined as 100%. Finally, the absorption at 490 nm of each well was measured by an EL808 ultramicroplate reader (Bio-TEK Instrument, Inc., Winooski, VT, USA). The relative cell viability was recorded and shown (Fig. 4).

3. Results and discussion

3.1. General consideration of the bioimaging design

In this paper, we demonstrate the method of generating G-quadruplex-templated silver nanoclusters in aqueous solution.