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A label-free fluorescent biosensor for determination of bovine serum albumin and calf thymus DNA based on gold nanorods coated with acridine orange-loaded mesoporous silica

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ABSTRACT

A simple fluorescent assay for sensing bovine serum albumin (BSA) and calf thymus DNA (ctDNA) was developed based on the gold nanorods-based probe. Gold nanorods coated with mesoporous silica coated ($Au@SiO_2$) which loading acridine orange (AO) was prepared by one-pot synthesis method. Interestingly, BSA is detected based on the quenching fluorescence signal while ctDNA is determined based on the enhanced fluorescence signal by the proposed method. Under the optimal conditions, the declined values of the fluorescent intensity of the biosensor are proportional to the BSA concentration in the range of 0.75–33.86 μmol/L. The limit of detection is 0.25 μmol/L. The enhanced values of the fluorescent intensity of the biosensor are linear with the ctDNA concentration in the range of 0.5–10 μg/mL, and the detection limit is 0.1667 μg/mL. The proposed method shows a good selectivity for BSA and ctDNA and can be applied to the determination of the analytes in real samples. The strategy of Au NRs@SiO_2-AO complex as a novel fluorescent probe paves a new way to design fluorescent sensor.

1. Introduction

Fluorescence-based techniques have emerged as the powerful tools in analytical chemistry [1–3]. Various materials were used to develop the new fluorescent probe. So far, the most popular fluorescent probes reported include the organic dyes, quantum dots (QDs), carbon dots, and metal nanoclusters [4–7]. The common feature of the probes mentioned above is the fact that all materials themselves possess the fluorescent signal. However, it is few reported that the non-fluorescent material is selected for constructing fluorescent probe.

Gold nanorods (Au NRs) have emerged as an anisotropic one-dimensional nanomaterials for a wide range applications in biology and biomedicine areas due to the exceptional radiative and nonradiative properties [8–10]. As for possessing high specific surface area, gold nanorods are usually selected as the nanocarrier for loading various objects to construct multifunctional nanoprobes [11,12]. However, the developed biosensors are mainly based on the surface plasmon resonance property of gold nanorods [13,14]. Therefore, it is a great important to explore the new application of gold nanorods.

In this work, a simple label-free fluorescence assay for sensing BSA and ctDNA using gold nanorods coated with mesoporous silica which loading acridine orange (AO) (Au NRs@SiO_2-AO) as the probe was proposed. The mesoporous silica with large specific surface area was chosen to decorate on the gold nanorods, which guarantees a high AO payload [15], and the complex was applied as a new fluorescent sensing platform. In this experiment, AO dye molecules were absorbed into mesoporous silica with the electrostatic interaction. As AO interacts with both bovine serum albumin (BSA) and calf thymus DNA (ctDNA) [4,16], it would induce the change of fluorescent signal of the biosensor. Interestingly, it appears the decreased fluorescent signal after the addition of the BSA solutions while it turns up the increased fluorescent signal after the introduction of certain concentration of ctDNA. It is due to the different binding mode for BSA and ctDNA with AO. The results suggested that AO molecule interacts with BSA by electrostatic interaction while AO molecule binds with ctDNA by intercalation binding mode. To the best of our knowledge, this is the first report in which Au NRs@SiO_2-AO have been employed as the fluorescent probe for the rapid and sensitive detection of BSA and ctDNA.

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2. Experimental

2.1. Materials

Ascorbic acid, sodium borohydride and hexadecyltrimethylammonium bromide (CTAB, 99%) were obtained from Aladdin Industrial Corporation (China). Calf thymus DNA (ctDNA) was purchased from Sigma–Aldrich (China). Silver nitrate was obtained from Shanghai Fine Chemical Materials Research Institute (China). Ethyl orthosilicate was obtained from Tianjin Institute of Fine Chemicals Retrocession (China). Methanol and hydrochloric acid were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. (China). Acridine orange, sodium oleate, albumin from bovine serum (BSA), tetrachloroauric (III) acid hydrate and other reagents were all purchased from Sinopharm Chemical Reagent Co. Ltd. (China). All reagents were of analytical grade and used without further purification. The ultrapure water with 18.2 MΩ cm^{-1} was used throughout the whole experiments.

2.2. Instruments and characterizations

UV–vis absorption spectra were carried out on a Shimadzu 245 UV–vis spectrophotometer (Shimadzu, Japan). The fluorescence spectra were recorded with a model Jasco FP-6500 fluorescence spectrometer (Jasco, Japan). The ξ-potential was measured by ZEN3500 Zetasizer Nano particle analyser (Malvern, UK). Transmission electron microscopy (TEM) images were recorded on a field emission transmission electron microscope at an accelerating voltage of 200 kV (JEM-2100F, JEOL, Ltd., Japan). FT-IR spectra were obtained by using a Nicolet-6700 FT-IR spectrometer (Thermo Ltd., USA) within a range of 400–4000 cm^{-1}.

2.3. Procedures

2.3.1. Preparation of gold nanorods

Au NRs were prepared according to the improved seed-mediated growth method with little modification [17]. First, the seed solution for Au NRs growth was prepared as follows: 2 mL of 0.5 mM $HAuCl_4$ was mixed with 2 mL of 0.2 M CTAB solution in a 10 mL test tube. Then, 0.4 mL $NaBH_4$ (0.06 M, freshly prepared and ice-cold) was added in the tube under vigorous stirring for 2 min. The seed solution was kept at 25 °C before use.

Secondly, 0.56 g of CTAB and 0.0987 g of sodium oleate were added into a 50 mL round-bottomed flask and then 20 mL ultrapure water was added. The flask was kept in a water bath under 50 °C for about half an hour, then cool down to 30 °C. After that, 1.92 mL of 4 mM $AgNO_3$ solution was added. 20 mL of 1 mM $HAuCl_4$ solution was added after the mixture was kept at 30 °C for 15 min. It was kept stirring in intermediate speed for 90 min and then changed from dark yellow to colorless. 0.168 mL of HCl (37 wt.% in water, 12.1 M) was then added to adjust the pH of the mixture and then slow stirring for 15 min. 0.100 mL of 0.064 M ascorbic acid (AA, freshly prepared) was injected in and the solution was vigorously stirred for 30 s. The final step was the addition of 64 μL the seed solution. Then the growth medium was stirred for 30 s and left undisturbed at 30 °C over night. The concentration of prepared Au NRs was 0.6489 nM [18,19].

2.3.2. Preparation of gold nanorods decorated with mesoporous silica which loading acridine orange

The functional gold nanorods (Au NRs@SiO_2-AO) were carried out according to the reported literature with little modification [20]. Briefly, 12 mL of as-synthesized Au NRs were centrifuged at 7000 rpm for 20 min at a time to remove excess CTAB surfactant, and the precipitate was then redispersed in a 25 mL beaker with 8 mL of ultrapure water. And the beaker was kept in a water bath under 25 °C. Then under vigorous stirring, 20 μL of acridine orange aqueous solution (10 mg/mL) and 80 μL 0.1 M NaOH solutions were added in the beaker. Last, 16 μL 20% TEOS (in methanol) was injected into the beaker three times under gentle stirring at 30 min intervals. The reaction mixture should be kept the reaction for 24 h. The product was washed by ultrapure water for several times and was redispersed in water at the same volume for subsequent experiments.

2.3.3. Detection of BSA based on decreased fluorescence

The Au NRs@SiO_2-AO solution was diluted to 2 mL with Britton–Robinson buffer solution (BR buffer) and then mixed with different concentrations of BSA solution. The reaction was kept for certain minutes at room temperature. Then the fluorescent intensity of the reacted solution was measured by fluorescence spectrometer with slit width at 5.0 nm for the excitation and emission.

2.3.4. Detection of ctDNA based on enhanced fluorescence

The Au NRs@SiO_2-AO solution was diluted to 2 mL with BR buffer solution and then mixed with different ctDNA concentrations. The reaction was kept for certain minutes at room temperature. After that, the fluorescent intensity of the reacted solution was detected by fluorescence spectrometer with slit width at 3.0 nm and 5.0 nm for the excitation and emission, respectively.

3. Results and discussion

3.1. Characterization of the Au NRs and Au NRs@SiO_2-AO

Fig. 1a presents the TEM image of the Au NRs, which suggests that we have successfully synthesized gold nanorods. Besides, it is observed that the Au NRs possess a 100 ± 4 nm length and a

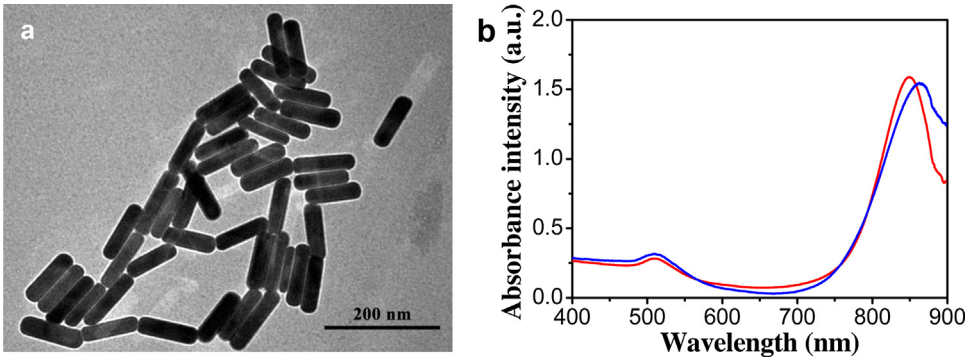

Fig. 1. (a) The image of Au NRs. (b) Surface plasmon resonance spectra of Au NRs before and after modification. Gold nanorods (red curve), Au NRs@SiO_2-AO (blue curve).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)