Talanta 188 (2018) 218–224

Contents lists available at ScienceDirect

Talanta

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

Highly selective detection of spermine in human urine *via* a nanometal surface energy transfer platform

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ARTICLE INFO

Keywords:
Gold nanorods
Spermine
Nanometal surface energy transfer
Porphyrin

ABSTRACT

As an important biomarker of malignant tumors, spermine is closely related with some diseases. In this work, a nanometal surface energy transfer (NSET) strategy *via* the positively charged gold nanorods (AuNRs) and the negatively charged tetrakis (4-sulfonatophenyl) porphyrin ($TPPS_4$) was developed to detect spermine in human urine. Under acidic condition, spermine possessed multi-cationic property and a strong affinity towards the anionic phosphate backbone of calf thymus DNA (ctDNA) by electrostatic attraction as well as the groove binding, which enabled to regulate the process of NSET between AuNRs and $TPPS_4$, leading to the fluorescence quenching of $TPPS_4$. Moreover, the quenched fluorescence was proportional to the concentration of spermine, which was applicable to monitor the level of spermine in human urine in the concentration range of 0.5–7.5 μM. The NSET platform for spemine is simple, selective and time-saving, which has great significance in early cancer diagnosis.

1. Introduction

Spermine, as a small-sized cationic polyamine, exists in bacteria, plants, animal tissues and body fluids [1]. Nowadays, many investigations have proven that the increase of polyamine in body fluids indicates a rapid growth of tissue [2,3], such as malignant tumors [4,5]. Therefore, the determination of spermine in blood or urine is a clinical index for the diagnosis of malignant tumors [6]. The traditional analytical techniques for the detection of spermine include immunoassays, high performance liquid chromatography (HPLC) [7], ion exchange liquid chromatography [8], and spectrometric methods [9–11]. However, some of them suffer from the complex, tedious and time-consuming pretreatment, such as derivation. Herein, developing the simple, efficient and selective assays is still highly desirable. In this work, we developed a simple and novel platform for sensing spermine *via* spermine and ctDNA to control the energy transfer between gold nanorods (AuNRs) and a near-infrared fluorophore, tetrakis (4-sulfonatophenyl) porphyrin ($TPPS_4$).

AuNRs, as an anisotropic nanomaterial, have achieved considerable attention in virtue of their advantages including easy preparation and unique optical properties [12]. They are normally passivated by positively charged surfactants, which are able to adsorb a variety of negatively charged biomacromolecules or small molecules through electrostatic attractions, such as DNA, peptide, and fluorescent dye [13]. Therefore, they have been widely applied in the field of chemical and biochemical sensing including metal ions [14], DNA [15], amino acids [16], antibodies [17]. More importantly, the tunable absorption of AuNRs enables them to function as the efficient acceptor to transfer energy from the near-infrared fluorophore [18], such as 5′-carboxytetramethylrhodamine (TAMRA) and porphyrin [12].

In general, energy transfer between dyes and nanomaterials involves Förster resonance energy transfer (FRET) and nanometal surface energy transfer (NSET), *etc*. Although FRET is a very effective and convenient technique, it has serious limitations regarding the distance (100 Å) and orientation between the donor and acceptor. Compared to FRET, NSET is a dipole-surface type energy transfer from a molecular dipole to a nanometal surface, which presents a more high quenching efficiency over much longer distance (> 400 Å) [19]. NSET can occur without resonant electronic transition, which has enabled its exploitation as an expedient optical nanoruler over FRET. Ray and co-workers have constructed a ultrasensitive gold-nanoparticle-based NSET for the screening of the hepatitis C virus (HCV) RNA [20].

In this work, we construct a NSET platform with $TPPS_4$ as donor and AuNRs as acceptor through the electrostatic interaction and coordination

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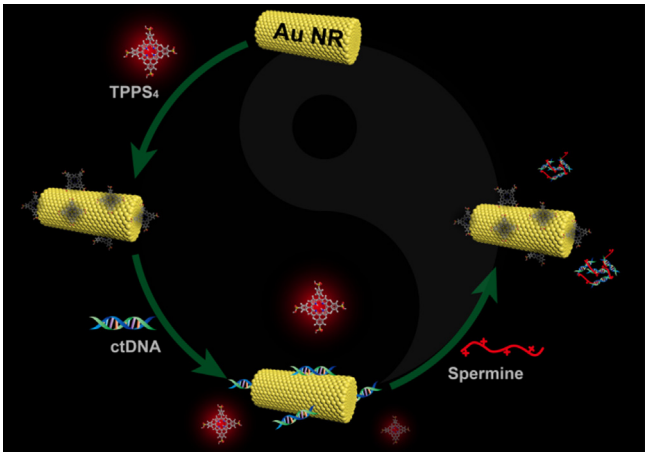
https://doi.org/10.1016/j.talanta.2018.05.086
Received 28 February 2018; Received in revised form 16 May 2018; Accepted 24 May 2018
Available online 25 May 2018



Scheme 1. The specific detection of spermine *via* NSET between AuNRs and $TPPS_4$.

interaction [21], leading to the fluorescence quenching of $TPPS_4$ [21,22]. When spermine was absent, ctDNA with the double-stranded structure presented stronger electrostatic interaction toward AuNRs [23], thus drove $TPPS_4$ away from the surface of AuNRs to result in the recovery of fluorescence. Under acidic condition, spermine showed multi-cationic property and a strong affinity towards the anionic phosphate backbone of ctDNA through electrostatic attraction as well as the groove binding to form condensation and aggregation [9] and release from AuNRs surface, thus $TPPS_4$ was adsorbed onto AuNRs surface to quench the fluorescence again, which was proportional to the concentration of spermine (Scheme 1). Furthermore, the energy transfer strategy was successfully used for the trace detection of biogenic amine spermine in urine specimens with high selectivity and sensitivity.

2. Experimental section

2.1. Materials

$TPPS_4$ was offered by Tokyo Chemical Industry. The solution of ctDNA (Sigma, Steinheim, Germany) was stored at 4 °C. Polyamines, such as spermine (Spm), putrescine (Put), spermidine (Spd), cadaverine (Cad), L-Arginine were obtained from Sigma (Steinheim, Germany). Cetyltrimethyl ammonium bromide (CTAB) was supplied by Aladdin Reagent Co. (Shanghai, China). All other reagents were of analytical grade and used without further purification. Britton-Robinson (BR) solution was used as a buffer to control the samples acidity. Ultrapure water (18.2 MΩ cm) was utilized throughout this work.

2.2. Instruments and characterizations

The absorption and fluorescence spectral features were obtained with a Hitachi U-3010 spectrophotometer (Tokyo, Japan) and a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan), respectively. Zeta potential was carried out with a Malvern ZEN 3600 Nano ZS Zetasizer (Malvern Instruments, England). An S-4800 scanning electron microscope (SEM, Tokyo, Japan) was used to scan SEM images of AuNRs. The fluorescence lifetime was measured with an FL-TCSPC fluorescence spectrophotometer (Horiba Jobin Yvon Inc., France). Our proposed method was validated by a D-2000 Elite high performance liquid chromatography (HPLC) system.

2.3. Synthesis of AuNRs

AuNRs were prepared by following a seed-mediated method as previously reported [24]. In brief, 5.000 mL of 0.20 M CTAB solution and 0.103 mL of 1% (*w/w*) $HAuCl_4$ were mixed in a 10 mL round-bottomed flask. And then, 1.000 mL of fresh 0.01 M $NaBH_4$ prepared by ice-cold water was quickly injected to the mixture solution under vigorous stirring (1200 rpm) for 2 min, which changed from yellow to brown. The above seed solution was aged at room temperature for 2–3 h.

The growth solution was prepared as follows: 0.3090 g of sodium oleate (NaOL) and 1.8000 g of CTAB were dissolved in 50 mL of water at 50 °C, and then was allowed to cool down to 30 °C. 2.400 mL of $AgNO_3$ (4.00 mM) was added into the above mixture, which was kept undisturbed at 30 °C for 15 min. Then, 50 mL of 1.00 mM $HAuCl_4$ solution was introduced. After of stirring at 700 rpm for 90 min, 0.300 mL of HCl (12.1 M) was employed to adjust the acidity. After standing for 15 min, 0.250 mL of 0.064 M ascorbic acid was added under vigorous stirring for 2 min. Finally, 0.040 mL of seed solution was injected into the growth solution under stirring for 30 s and the mixture was then left undisturbed overnight.

2.4. Removal of CTAB

It has been reported that many water-soluble anionic porphyrins interact with surfactant molecules, such as SDS and CTAB, resulting in a stable structure of porphyrin-surfactant complexes or aggregates, such as J- and H- aggregates [25], which can lead to the fluorescence quenching of porphyrin. In order to remove excess CTAB and prevent $TPPS_4$ aggregation, AuNRs were centrifuged at 7000 rpm for 10 min and the precipitate was resuspended in water. The overall procedure was repeated twice, and then the concentration of AuNRs (0.47 nM) was calculated by UV–Vis absorption spectrum of AuNRs based on an extinction coefficient of $3.2 \times 10^9\ M^{-1}\ cm^{-1}$ at $\lambda = 662$ nm [26].

2.5. Spermine standards preparation

20 μL of ctDNA (93.4 μg/mL) were mixed with 40 μL of BR buffer (pH 3.78) in 1.50 mL tube. Next, 80 μL of spermine solution at different concentrations (0, 2.5, 5, 7.5, 15, 25, 37.5 μM) was added into the above mixture. After stirring, the mixture was incubated at 28 °C for 10 min, then 35 μL of AuNRs (0.47 nM) was mixed and incubated for another 5 min. Finally, 80 μL of $TPPS_4$ (20 μM) was added to the above mixture and diluted to 0.4 mL with doubly distilled water (18.2 MΩ cm).

2.6. Urine sample preparation

Urine samples of healthy people were obtained from the Southwest University, which were pretreated by following procedure. In order to remove the tiny solid particles and protein, 2 mL of urine sample was mixed with 2 mL of 5% perchloric acid and vortexed for 30 s. Subsequently, the mixture was separated by centrifugation at 12,000 rpm for 10 min and then placed at room temperature for 20 min. Then, the supernatant fluid was filtered with 0.22 μm syringe filters and adjusted to neutral with sodium hydroxide. Finally, the concentration of spermine in urine sample was detected according to the procedure described in Section 2.5.

2.7. Determination of spermine with HPLC

HPLC was employed to verify the accuracy of our method by following the previous report [27]. First of all, spermine was derived with benzoyl chloride according to the following procedure. 2 mL of spermine solutions (0.01, 0.05, 0.1, 0.5, 1, 5, 10 mM) or human urine were mixed with 2 mL of NaOH (2 M) and 20 μL of benzoyl chloride in a 15 mL tube, which was vortexed for 20 s and followed by incubating at 37 °C for 20 min. Afterwards, 4 mL of saturated NaCl and 4 mL of ether were mixed with above solution and centrifuged at 10,000 rpm for 5 min. Then, 2 mL solution in ether phase was concentrated and dried at 37 °C. After that, the above product was dissolved in 1 mL of methanol