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Rapid detection of heparin by gold nanorods and near-infrared fluorophore ensemble based platform *via* nanometal surface energy transfer

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ABSTRACT

As a mucopolysaccharide sulfate anticoagulant drug, heparin plays important roles in clinic. In this work, heparin was analyzed based on the nanometal surface energy transfer (NSET) between gold nanorods (AuNRs) and tetrakis (4-sulfophenyl) porphyrin ($TPPS_4$) fluorescent molecules. The results showed that AuNRs could bind $TPPS_4$ by electrostatic interaction and coordination to quench its fluorescence. While heparin was able to bind AuNRs with a stronger electrostatic interaction to compel $TPPS_4$ to leave AuNRs surface, leading to the fluorescence recovery of $TPPS_4$, which was proportional to the concentration of heparin in a range of 0.05 ~ 2 μg/mL with the detection limit as low as 6.7 ng/mL (3σ). Based on this, a NSET platform for the analysis of trace heparin sodium was proposed with fast response, high sensitivity and excellent specificity, which was successfully applied to the detection of heparin in injection with the satisfactory result. Importantly, the NSET mechanism was fully discussed.

1. Introduction

As a mucopolysaccharide sulfate anticoagulant drug, heparin sodium has attracted growing attention [1]. In clinic, heparin sodium is generally used to prevent the aggregation and destruction of platelet, and inhibit the activity of thrombin, as well as to promote fibrinolysis [2], which could act as an effective anticoagulant drug during cardiopulmonary surgery and under emergency deep venous thrombosis conditions. Other researches prove that heparin sodium also plays important role in hypolipidemic [3]. However, the overdose of heparin often induces potentially fatal bleeding neopathy, including hemorrhages and thrombocytopenia [4]. Therefore, it is of great significance to closely monitor the level of heparin in clinic.

By now, many efforts have been devoted to detecting the content of heparin. Numerous of methods, such as spectrophotometry [5,6], electrochemical analysis [7], fluorimetry [8–10] and high performance liquid chromatography (HPLC) [11], have been developed for heparin analysis. For instance, Cui's group developed an interesting HPLC approach by combining specific enrichment and highly efficient solid-phase tagging technique for the sensitive detection of heparin [11]. However, a few methods suffer from some problems such as the poor specificity and the time-consuming operation. Thus, it is necessary to develop new approaches to achieve the selective and fast analysis of heparin.

As an anisotropic nanoparticle, gold nanorods (AuNRs) present the transverse and longitudinal surface plasmon resonance, corresponding to the electron oscillation along the short and long axes of AuNRs, respectively [12]. In particular, the longitudinal surface plasmon resonance (LSPR) band can be tuned from visible to near-infrared region of the electromagnetic spectrum by adjusting the aspect ratio of AuNRs. By now, the synthesis of AuNRs has been very controllable [13,14] to adjust the LSPR features. Moreover, the surface plasmon resonance band of AuNRs endow with the higher absorption cross sections than those of spherical gold nanoparticles, which have found broad applications in colorimetric detection [5,12], fluorimetric determination [15] and thermal therapy [16,17]. What's interesting, AuNRs are suitable as a quencher in the energy transfer (ET) system when fluorescent dyes are located in their vicinity, which can increase the quenching of donors and further improve the quenching efficiency [16,17]. It has been proven that AuNRs present optical advantages in nanometal surface energy transfer (NSET), including the enhancement of the quenching efficiency that is related to the spectral overlapping between the adjustable absorption of AuNRs and the emission spectra of chromophores. The enhanced effect of NSET provides an outstanding low signal-to-noise and flexible distance between quencher and fluorophore [18], which breaks the barrier of the traditional Förster resonance energy transfer (FRET) for the sensitive and broader sensing [19]. For instance, Yang's group fabricated a near-infrared fluorogenic

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nanosensor based on NSET effect, *via* the conjugation of fluorescent proteolytic enzyme-specific cleavable peptides with AuNRs, which was capable of sensitive analysis of membrane-anchored membrane type 1-matrix metalloproteinases activity, providing the essential information in the clinical setting [20].

Previous investigations have proven that hexadecyltrimethylammonium bromide (CTAB) caps on the (111) facet of AuNRs [21], which enables the side of AuNRs with positive charge, leaving the ends of AuNRs are less positively charged. The special distribution of CTAB endows AuNRs with unique chemical and physical properties. On the one hand, the side of AuNRs can attract the negatively charged biomolecules, such as dsDNA [22] and heparin sodium [23], which could induce the side-by-side assembly of AuNRs. On the other hand, active biomolecules, including cysteine [24] and thiol modified DNA [25], may preferentially bind the ends of AuNRs, resulting the end-to-end assembly of AuNRs. The anisotropic chemical activity supplies ingenious binding mode for biomolecules, which is applicable to a wide range of applications.

In this work, we designed a NSET assay for heparin with AuNRs as the energy acceptor. To obtain a high efficiency, the near-infrared fluorophore porphyrin was employed as the energy donor, which spectrally overlapped with AuNRs, and thus was quenched effectively by AuNRs *via* electrostatic interaction between positively charged AuNRs and negatively charged porphyrin as well as coordination interaction between gold and pyrrole nitrogen atoms of porphyrin [26]. Due to the stronger negative charge of heparin, it was able to drive porphyrin away from AuNRs to restore the fluorescence intensity, which could be developed for heparin analysis.

2. Material and methods

2.1. Instruments

Fluorescence and absorption spectra were scanned with an F-2700 fluorescence spectrophotometer (Hitachi, Japan) and a UV-2600 UV–vis spectrophotometer (Shimadzu Corporation, Japan), respectively. FL-TCSPC fluorescence spectrometer (Horiba Jobin Yvon Inc., France) tested the fluorescence lifetime of porphyrin. Dynamic light scattering particle nanosizer (ZEN3600, Malvern, UK) measured the potential and size of AuNRs. The electron microscopic images of AuNRs were recorded with S-4800 scanning electron microscope (SEM, Hitachi, Japan), a Tecnai G2 F20 S-TWIN transmission electron microscope (TEM, FEI Company, USA) respectively. The dark-filed light scattering images was obtaiined by light BX-51 dark field microscope (Olympus, Japan) coupled with a monochromator (SR303i-B, Andor), which were analyzed with Image-Pro Plus 6.0 (IPP) software (Media Cybernetics, USA). A H1650 W high-speed centrifuge (Hunan Xiangyi Company) was used to purify AuNRs and HCJ-6D water bath digital magnetic stirrer (Changzhou Longyue Company) was for controlling temperature during the synthesis of AuNRs.

2.2. Reagents

Heparin sodium was offered by Aladdin Reagent Co., Ltd. (Shanghai). Tetrakis (*p*-sulfobenzene) porphyrin ($TPPS_4$) was supplied by Sigma Company (Shanghai). CTAB (> 99%) and chlorauric acid ($HAuCl_4{\cdot}4H_2O$) were commercially obtained from China Pharmaceutical Co., Ltd. (Shanghai, China). All other reagents were of analytical grade. Ultra-pure water was used throughout this work. Britton-Robinson (BR) buffer and sodium chloride were employed to adjust the acidity and ionic strength of solutions, respectively.

2.3. Preparation of AuNRs

AuNRs were prepared according to a previous report [14]. Firstly, gold seeds were synthesized by mixing 5.000 mL of 0.20 M CTAB, 1.000 mL of 0.01 M ice-cold $NaBH_4$, and 0.103 mL of 1% $HAuCl_4$, which followed by vigorously stirring for 2 min.

Secondly, the growth solution was prepared by following the procedure: 1.8000 g of CTAB and 0.3086 g of sodium oleate were dissolved in 50 mL of water at 50 °C to completely dissolve, which was then cooled to 30 °C. After introducing 3.600 mL of 4.00 mM $AgNO_3$, the above mixture stood at 30 °C for 15 min. Then, 50 mL of 1.00 mM $HAuCl_4$ was placed into the mixture, which was stirred at 700 rpm for 90 min. After that, 0.300 mL of 37% HCl was mixed and stirred at 400 rpm for 15 min. Another vigorous blending for 30 s was performed after the injection of 0.250 mL of 0.064 M L-ascorbic acid.

Finally, 0.040 mL of gold seeds were mixed with the growth solution, which was allowed to stand overnight at 30 °C. The obtained AuNRs presented a uniform size in TEM images (Fig. S1).

2.4. Fluorescence assay for heparin

In a 1.50 mL tube, 100 μL of 0.05 M NaCl and 100 μL of BR buffer (pH 5.72) were mixed with 140 μL of 0.10 μg/mL of $TPPS_4$ and 50 μL of AuNRs. Then, an appropriate amount of heparin sodium solution was added into above mixture, which was then diluted to 1.00 mL with water. After fully mixing for 1 min, the solution was transferred to F-2700 fluorescence spectrophotometer to scan spectra with the slit at 5.0 nm and voltage at 700 V.

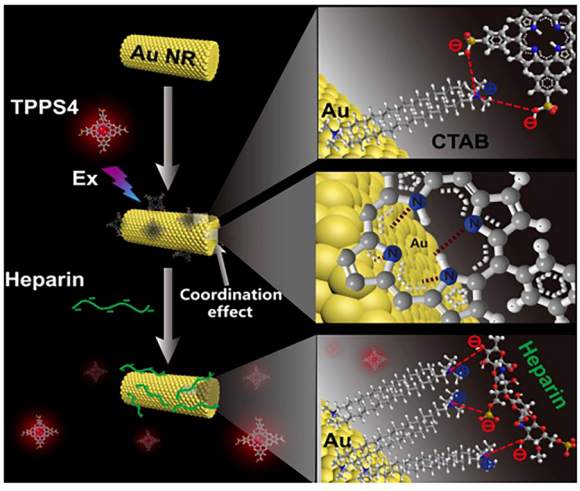
2.5. Detection of heparin sodium in injection

The injection samples were firstly diluted 5000-fold with water, which were then detected according to the above procedure as described in 2.4. Finally, the corresponding concentrations were calculated by the standard curve and compared with the labelled concentration on the trademark.

3. Results and discussion

3.1. Schematic diagram

The NSET platform for heparin detection is shown in Scheme 1. As mentioned above, the cationic surfactant (CTAB) coats on the (111) facet of AuNRs and less caps on the (100) crystal facet [21], thus AuNRs are able to bind $TPPS_4$ by electrostatic interaction as well as coordination interaction [26]. In brief, on the one hand, $TPPS_4$ is negatively charged because of the presence of four sulfonate groups, which is capable of binding CTAB on the side of AuNRs by electrostatic interaction, reducing the distance between $TPPS_4$ and AuNRs to quench



Scheme 1. Schematic diagram of the reaction mechanism.