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Griess reaction-based paper strip for colorimetric/fluorescent/SERS triple sensing of nitrite

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ABSTRACT

This study demonstrates a novel strategy for colorimetric/fluorescent/surface enhanced Raman scattering (SERS) triple-mode sensing of nitrite based on Griess reaction modulated gold nanorods (GNRs)-Azo-gold nanoparticles (GNPs) (GNRs-Azo-GNPs) assembly. The *p*-aminothiophenol (PATP)-capped GNRs (GNRs@PATP) and 1,8-diaminonaphthalene (DAP)-modified GNPs (GNPs@DAP) are first synthesized through Au–S and Au–N bonds, respectively. Upon being excited at 365 nm, the dispersion of GNRs-GNPs (GNRs@PATP and GNPs@DAP) emits cyan fluorescence. Next, the addition of nitrite into the GNRs-GNPs induces the formation of GNRs-Azo-GNPs assembly, resulting in the enhancement of color and decrease of fluorescence. Therefore, the GNRs-Azo-GNPs assembly can not only be used as a naked-eye indicator of nitrite changed from orange-yellow to purple, but also as a highly selective "fluorescence quenching" probe due to the fluorescence resonance energy transfer (FRET) between azo-moiety and DAP. The limit of detection (LOD) for nitrite is 0.05 μM by colorimetry and 0.01 μM by fluorescence. Meanwhile, the GNRs-Azo-GNPs assembly possesses controllable core–satellites nanostructures and enables on-field SERS detection of nitrite with the LOD of 0.8 nM. More importantly, the GNRs-GNPs sensing system can not only be used as a paper-based test strip for on-site fast screening of nitrite with a high sensitivity and selectivity, but also as a SERS substrate for reliable quantitative analysis of nitrite. This study offers a new method for on-site visual detection of nitrite in human urine and meat products, as well as provides a strategy for designing multi-mode sensing platform for various applications.

1. Introduction

Nitrite ion (NO_2^-) is widely present in food systems as food preservative for meat or meat products and has proven to be of a great threat to human health (Cross et al., 2010; D'Ischia et al., 2011). Moreover, the carcinogenic N-nitrosamines (N-nitroso compounds) could be generated in vivo from ingested NO_2^-, giving rise to severe human diseases including cancer, diabetes, and neurodegenerative diseases (Maia and Moura, 2014; Zhou et al., 2014). Excessive consumption of NO_2^- can lead to a number of medical issues such as esophageal cancer, infant methemoglobinemia (blue baby syndrome), spontaneous abortion, and birth defects in the central nervous system (Mishra et al., 2013; Dai and Mitch, 2013). According to the World Health Organization, the fatal dose of NO_2^- is in the range of 8.7–28.3 μM (Metters et al., 2013; Gopalan et al., 2010). Moreover, the Legislation of the European Union suggests maximum allowable level of NO_2^- in meat products should be 50 mg/kg (expressed as $NaNO_2$) (Turdean and Szabo, 2015). Considering the extraction and dilution process prior to analysis, the actual concentration of NO_2^- is at approximately micro-molar levels in solution. Therefore, analytical methods capable of detecting ultra-trace amounts of NO_2^- in complex matrices are highly desired. Traditional methods for NO_2^- determination include ion chromatography (IC) (López-Moreno et al., 2016; Iammarino et al., 2013; López-López et al., 2011), capillary electrophoresis (CE) (Merusi et al., 2010), gas chromatography-mass spectrometry (GC-MS) (Tsikas et al., 2010), chemiluminescence (CL) and electrochemical techniques (EC) (Lin et al., 2011; Dağcı and Alanyalıoğlu, 2016). Most of the above-cited laboratory methods have several disadvantages including high cost, complicated and time-consuming sample preparation procedures and are therefore not readily amenable to use for high-throughput screening of target analytes in complex samples. On-site detection methods are becoming increasingly more popular because of their easy test protocols and instantaneous results. Moreover, a rapid, cost-effective method for on-site detection of a target analyte in body fluid and food products would be of great value for law enforcement in field tests (Peters et al., 2015).

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Therefore, it is desirable to develop an operationally simple, cost-effective, highly-selective and highly-sensitive technique for on-site detection of NO_2^- in complex matrix samples.

For several decades, the Griess-based colorimetric assay has been the mainstay of on-site analysis of NO_2^- and has the advantages of simplicity, visuality and low-cost (Ruiz et al., 2014; Daniel et al., 2009; Zurcher et al., 2014; Jayawardane et al., 2014). The classical Griess-based assay, which relies on the color of the azo-compounds, is prone to interference caused by colored pigments that may exist in the solution. Moreover, the Griess method is not sensitive enough for detection NO_2^- at sub-micromolar concentrations (Silanikove et al., 2009). The serious interferences or lack of sensitivity limited the applications of the colorimetric methods for quantitation of NO_2^- in complex matrix samples (Chatterjee et al., 2015). The development of new methods to detect NO_2^- with high sensitivity and selectivity are highly desired. To address these concerns, efforts are focused on designing of novel sensor technologies for the NO_2^- (Saha et al., 2012; Unnikrishnan et al., 2014). Among the various detection technologies, fluorescence sensing has gained considerable attention for NO_2^- detection because of its high sensitivity and ease of fabrication (Kumar et al., 2012; Chatterjee et al., 2015; Menon et al., 2016). However, the fluorescent compounds may suffer from low selectivity toward NO_2^- because of the interference from coexisting cationic and anionic ions, and are therefore not suitable for on-site detection of NO_2^- (Tabares et al., 2011). Fortunately, surface-enhanced Raman scattering (SERS) can provide "fingerprint" signatures of analytes, thus allowing for on-site monitoring of additives and pollutants directly (Anker et al., 2008). More importantly, SERS can boost the weak Raman signal as high as 10^{15} and could be used for label-free detection of small organics (Gong et al., 2015), metal ions (Song et al., 2017) and inorganic anions (Kim et al., 2012; Correa-Duarte et al., 2015) as well. However, only the target analytes that have a strong affinity toward the SERS substrates can yield strong Raman signals. Most inorganic anions possess low SERS cross-section and weak affinity toward metallic surfaces, limiting the application of SERS detection of inorganic anions (Kim et al., 2012). Correa-Duarte et al. (2015) proposed silver nanoparticle aggregates-induced hot spots for direct detection of NO_2^- and NO_3^- with sub-picomolar detection limits. Moreover, NO_2^-- triggered conversion of PATP to DMAB on GNPs and aggregation of GNPs, thus permitting the SERS detection of NO_2^- (Liu et al., 2015). The results show that SERS is very sensitive for the quantitative detection of ultra-trace amounts of NO_2^- due to the excellent SERS activity of nanostructures. The SERS substrates combining sensitivity with controllable nanostructures are still a challenge for the practical SERS applications. The unsuitability of SERS for quantitative analysis is mostly attributed to the instability, non-uniformity and low reproducibility of SERS substrates (Li et al., 2010). Moreover, these methods may suffer from the potential interference in the complicated matrix (Chatterjee et al., 2015; Tabares et al., 2011). Considering the unique advantages and disadvantages of these analytical techniques, there is an increasing demand for building up a complementary multi-mode sensing methods within one system because they can offer more than one kind of output signal simultaneously, thus making the detection results more convincing.

Herein, we develop a triple-mode colorimetric, fluorescent, and SERS sensor for detection of NO_2^- based on hybrid gold nanorods (GNRs)-azo-gold nanoparticles (GNPs) (GNRs-Azo-GNPs) assembly (Scheme 1). Upon addition of NO_2^-, azo-compounds immediately appear based on the specific Griess reaction between *p*-aminothiophenol (PATP) and 1, 8-diaminonaphthalene (DAP), resulting in a quantitatively colorimetric response to the concentration of NO_2^-, whereas fluorescence intensities decrease upon the addition of NO_2^- because of the fluorescence resonance energy transfer (FRET) from DAP to azo-compounds. Moreover, the hybrid GNRs-Azo-GNPs assembly enables the SERS detection of NO_2^-. Different from the existing multi-mode sensing systems, the proposed strategy needs not any spacer layers and possesses a compact core–satellites nanostructure. The application of GNRs-Azo-GNPs assembly based triple-mode assay has several advantages: (1) The formation of GNRs-Azo-GNPs assembly after the addition of NO_2^- results in the enhancement of color and decrease of fluorescence intensity, which enables the colorimetric and fluorescent dual-mode sensing of NO_2^-. (2) The GNRs-GNPs based test strip enables on-site fast screening of NO_2^- with a high sensitivity and selectivity. The distinct fluorescent intensity can be obtained in the low-concentration region (< 0.5 μM), whereas the colorimetric assay is more suitable for sensing NO_2^- at the higher concentrations (≥ 0.5 μM). (3) The GNRs-Azo-GNPs core–satellites nanostructures combined with a handheld Raman spectrometer facilitates the reliable quantitative SERS detection of NO_2^- with different concentrations ranging from 0 to 100 μM. (4) The colorimetric, fluorescent, and SERS triple-mode sensing strategy can be used individually or in combination with another to achieve the optimal sensing performance for NO_2^- in complex matrix samples. This versatility is not available with the conventional optical sensors. More importantly, by acquiring data from multiple-channel sensing system, the risk of false positive and false negative detection can be mitigated (Schmittel and Lin, 2007), which ensures the reliability and accuracy of sensing system and is more suitable for on-site fast screening and point-of-care diagnosis without any sample pretreatment.

2. Experimental section

2.1. Materials

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$, 99.9%), hexadecyltrimethylammonium bromide (CTAB, ≥ 99%), tetraoctylammonium bromide (TOAB, 98%), sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$, 99.9%), sodium borohydride ($NaBH_4$, 99%), silver nitrite ($AgNO_3$, ≥ 99.9%), hydrochloric acid (HCl, 37 wt%), 1-dodecanethiol (DT, ≥ 98%) and were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,8-diaminonaphthalene (DAP, 97%), *p*-aminothiophenol (PATP, 98%) and sodium nitrite ($NaNO_2$, ≥99%) were obtained from Aladdin-Reagent Co., Ltd. (Shanghai, China). Other reagents were of analytical grade and used without further purification. Ultrapure water with a conductivity of 18 MΩ cm^{-1} was used in all experiments.

2.2. Synthesis of GNRs-Azo-GNPs assembly

First, PATP-capped GNRs (GNRs@PATP) were synthesized according to the modified seeded growth method (Dong et al., 2015). Then, synthesis of the DAP-modified GNPs (GNPs@DAP) was performed as reported previously (Hostetler et al., 1998). The experimental details on their synthesis and purification were provided in the Supporting information.

The GNRs-Azo-GNPs assembly was synthesized by self-assembly processes. Initially, GNRs-GNPs (GNRs@ PATP + GNPs@DAP) was prepared by mixing GNPs@DAP (100 μL, 0.45 μM) with a dispersion of GNRs@PATP (200 μL, 1.8 nM). The pH of the mixture was adjusted to 3.0 with 0.1 M HCl. Then, 10 μL of NO_2^- with final concentrations ranging from 0 to 100 μM was added into the resulting GNRs-GNPs. Within 5 min, the color of solution was changed from orange-yellow to purple. The resultant GNRs-Azo-GNPs assembly was then centrifuged at 8000 rpm for 5 min and washed with ultrapure water several times.

2.3. Characterization

UV–vis spectra were obtained on a UV-2100 spectrophotometer (Tokyo, Japan). Fluorescent spectra were recorded on a RF-5301PC FL Spectrophotometer (Tokyo, Japan) equipped with a Xe lamp and a plotter unit and a 1 cm quartz cell. The scanning electron microscopy (SEM) were carried out on an FEI-Sirion 200 field-emission scanning electron microscope (FEI Co. with 20 kV operating voltage).