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Highly selective and sensitive detection of coralyne based on the binding chemistry of aptamer and graphene oxide

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ABSTRACT

In this contribution, an organic small molecule (OSM)-participating interaction between its aptamer and graphene oxide (GO) is investigated by taking coralyne as an example. Based on their interactions, a simple, rapid, highly sensitive and selective fluorometric method for the detection of coralyne is developed. GO can effectively quench the fluorescence of dye-labeled aptamer, while stronger binding of the aptamer and its target can make the fluorescence be recovered, which have been well demonstrated by the studies of the fluorescence spectra, fluorescence anisotropy, and circular dichroism spectra. In this case, the coralyne can be quantificationally detected by the variation of the fluorescence intensity, where GO acts as an efficient signal-to-background enhancer. With the increase of the coralyne, the fluorescence intensity increases gradually and linearly proportional to the concentration of the coralyne in the range of 10–700 nmol L^{-1}. This method is reliable, and has been successfully applied for the detection of coralyne in complicated matrixes.

1. Introduction

Over the past decade, developing analytical methods with the advantages of *Simple*, *Speedy*, *Sensitive*, and *Selective* features, combined with *Automatic* operation, *Accurate*, and *Efficient* outputs (so-called 4S+2A+E) has attracted increasing attention. The established methods with these advantages have played important roles in a variety of different areas related to environmental monitoring, food analysis, clinic diagnosis, and drug quality control [1]. Although a large number of methods have been successfully demonstrated for the detection of a specific target, however, most of them suffer from the limitations such as time-consuming, cumbersome, low sensitivity and selectivity, and poor performance in complex matrixes. Taking organic small molecules (OSMs) as an example, there are several challenges during their detection by the optical methods: (i) most of the OSMs do not have any optical signals in UV or visible region, and thus they can hardly be monitored by their own signals; (ii) the strategies for the assay which depend on the intermolecular forces (*e.g.*, electrostatic interaction or Van der Waals force) between the probes and OSMs are usually with low-selectivity and high-background signals [2–4]; (iii) design and synthesis of specific molecular probes for the recognition of OSMs via coordination or covalent bonds can achieve high selectivity and sensitivity, however, these probes cannot be widely extended to other systems. Therefore, developing simple, rapid, highly sensitive and selective, and general platforms for the detection of OSMs is still a grand challenge.

Recently, graphene has attracted increasing attention due to its unique structure and properties. Owing to its high quenching efficiency to the fluorescence, graphene has been developed as nanoplatforms in optical detections, especially for fluorescence resonance energy transfer [5–7]. Based on the change of optical signals, these graphene-involved systems have been used for DNA and protein analysis, metal ions detection, single bacterium detection, drug delivery, and biological imaging [8–14]. Therefore, it is easy to develop high-performance sensing platforms consisted of graphene with high quenching efficiency and a specific recognition system with high affinity to the target. To this end, aptamer is used as a recognition element to achieve the high affinity and selectivity toward the target in the present work. Aptamer is selected from a large number of random sequences of oligonucleotides or peptides, and can specifically bind to its target with high binding constant [15]. Aptasensors for the recognition of small molecules, proteins and cancer cells also have been developed in recent years due to the specific binding affinities to their targets [16].

Herein, coralyne, a kind of alkaloid, is used as a model OSM to investigate the interaction among graphene oxide (GO), OSM and its aptamer. It has been demonstrated that coralyne can restrict the leukemia growth in cells and exhibit a noticeable antitumor activity, and thus a promising new medicine for the cancer therapy [17,18].

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As such, quality control and quantitive detection of coralyne *in vivo* and *in vitro* is of great importance. To date, there have been only a few reports on the detection of coralyne. One example was the colorimetric detection of coralyne with gold/silver nanoparticles as probes [19,20]. In these cases, aggregation of metal nanoparticles might occur and result in high background signals and low sensitivity, and these methods can also hard to be used in complicated matrixes. In this contribution, GO was employed as an efficient signal-to-background enhancer in order to achieve the high sensitivity. Meanwhile, the aptamer of coralyne, poly adenosine (poly A) [19–22], was used as a selective recognition element for strong binding of the coralyne in this system. Base on the emulative bindings of GO and coralyne to the aptamer, the coralyne could be quantificationally detected by the variation of the fluorescence intensity. This method is highly sensitive and selective, and has been applied to practical analysis in complicated matrixes.

2. Experimental

2.1. Chemicals and materials

GO sheets were synthesized according to a modified Hummers method [23,24]. Coralyne chloride, 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), sybr green1(SG 1), and acridine orange (AO) were commercially available from Sigma (St. Louis, USA). Berberine chloride and jatrorrhizine chloride were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All of the oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the DNA used in this work were listed as follows: 5′–TAMRA-AAA AAA AAA AAA AAA A-3′ (the aptamer of coralyne, TAMRA-aptamer), 5′–TAMRA-CTT ACG GTG GGG CAA TT-3′ (DNA 2), 5′–TAMRA-AGC TTC TTT CTA ATA CGG CTT ACC-3′ (DNA 3), 5′–GCG AGT GTT AAA AGA GAC CAT CAA TGA GCT CGC-TAMRA-3′ (DNA 4), 5′–FAM-ACT CCT GGG GGA GTA TAT AAT-3′ (DNA 5). Phosphate buffer (PB, 0.2 mol L^{-1}) was used to control the acidity of the solution. Mili-Q purified water (18.2 MΩ cm) was used throughout the experiment.

2.2. Experimental instrumentation

Fluorescence spectra and fluorescence anisotropy were measured with an F-2500 fluorescence spectrophotometer (Hitachi, Japan). The Jacso J-810 circular dichroism (CD) spectropolarimeter (Japan) was employed to confirm the conformation of the aptamer. The fluorescence images were recorded with an IX81 microscope with a 40 × objective (Olympus, Japan). A QL-901 vortex mixer (Haimen, China) was employed to mix the solution. A high-speed TGL-16 M centrifuge (Hunan, China) was used during the purification of GO and uric samples.

2.3. Synthesis of graphene oxide

The details for the synthesis of GO sheets were identical with our previous works [6,25], which is also shown in the Supporting Information.

2.4. Experimental measurements

A certain concentration of coralyne was added to the mixture of 10 nmol L^{-1} TAMRA-aptamer, 20 mmol L^{-1} PB buffer, and 8 μg mL^{-1} GO at pH 7.4 (total volume, 500 μL). After 20 min at room temperature, the mixture was measured on the F-2500 fluorescence spectrophotometer with an excitation wavelength of 550 nm.

For the practical detection of uric samples, a series of uric samples were firstly centrifuged at 10000 rpm for 20 min to remove the possible aggregates and then diluted for 50 times. The testing samples were prepared by mixing uric samples with standard coralyne solution. The samples (50 μL) were then added to the mixture of GO and TAMRA-aptamer (total volume, 500 μL) containing 10 nmol L^{-1} TAMRA-aptamer, 20 mmol L^{-1} PB (pH 7.4), and 8 μg mL^{-1} GO.

For the experiments of cell imaging, human bone marrow neuroblastoma (SK-N-SH) cells were firstly incubated in medium supplemented with 10% fetal bovine serum at 37 °C under 5% CO_2. Then, the cells were cleaved by trypsin and replaced onto 18 mm glass coverslips in a 24-well tissue culture plate and allowed them to grow for 24 h. Then, coralyne, GO, and TAMRA-aptamer (or their complex) were added into the medium for incubation. After 3 h, the cells were washed thrice in PBS buffer, fixed with 4% p-formaldehyde for 30 min, and mounted on microscope slides for the imaging.

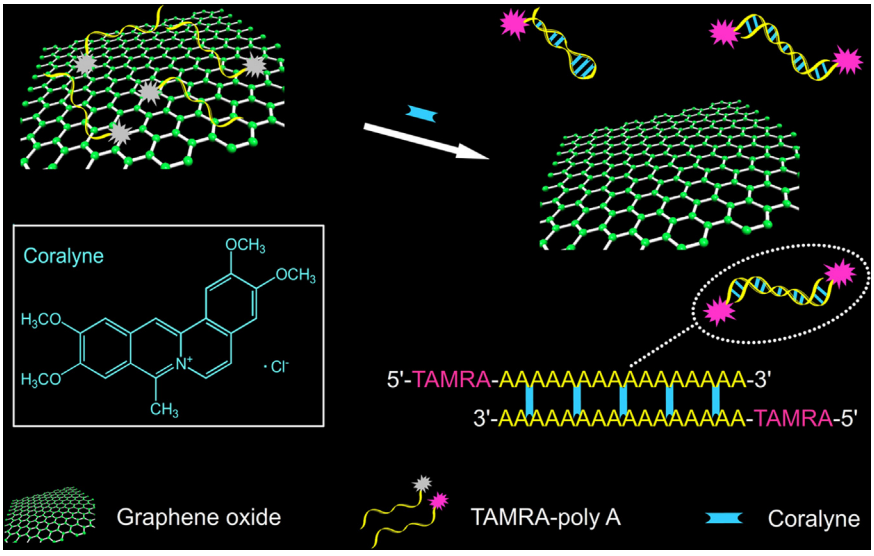

Fig. 1. Schematic illustration showing the OSMs-participating interaction between aptamer and GO, in which coralyne is a typical example of OSMs. Based on this strategy, coralyne can be detected by the turn-on fluorescence after the recognition of its aptamer.