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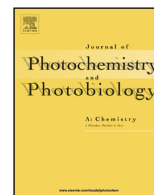

Competitive binding assay for G-quadruplex DNA and sanguinarine based on room temperature phosphorescence of Mn-doped ZnS quantum dots

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ABSTRACT

G-quadruplex DNA is an effective target for anticancer drug, and investigation of the G-quadruplex DNA with natural compounds plays a key role in the development of anticancer drugs.

In this article, room temperature phosphorescence sensing system based on L-cysteine-capped Mn-doped ZnS QDs has been established. Sanguinarine can adsorb on the surface of Mn-doped QDs and quenched the phosphorescence emission of the sensor. When the G-quadruplex sequences combined with the sanguinarine of the QDs-sanguinarine system, the phosphorescence intensities of the QDs sensors would be restored, and the binding abilities of the G-quadruplex DNA with the sanguinarine can be evaluated by the phosphorescence recoveries of the sensor system. Based on the developed approach, the interaction of sanguinarine and six G-quadruplex DNA with various sequences (tel24-1, tel24-2, tel22, k-ras, HIF-1α and c-kit1) has been investigated. The results showed that the binding abilities of the G-quadruplex DNA with sanguinarine were related to their number of bases and conformations. The conformational changes of the six G-quadruplex DNA, after binding with sanguinarine, have also been probed by circular dichroism. Combining the changes of the phosphorescence lifetime, the binding mechanism of the G-quadruplex DNA and sanguinarine has been discussed. The developed method was simple, rapid and specific, and can avoid fluorescence interferences of the system, and it would be prospective to evaluate the interaction of the drug with different G-quadruplexes.

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1. Introduction

G-quadruplex DNA, formed by folding of guanine-rich sequences, has been recognized to be promising target for genetic research and pharmacotherapeutics due to its structural particularity and important physiological functions [1]. In human body, G-quadruplex structure in terminal human telomere DNA can restrain the telomere maintenance induced by overexpression of telomerase in cancer cells, so it is an effective antitumour method to inhibit the activity of telomerase or stabilize G-quadruplex structures of telomeric DNA [2]. G-quadruplexes in promoters and introns of some genomes related to cancer or other diseases also play a key role in some physiological events, such as genetic transcription, protein expression and the regulation of ion channels [3–5]. As one of the important members for ras gene family, k-ras gene encodes the P21 protein with GTP enzyme activity [3,6]; the proto-oncogene c-kit can encode the tyrosine kinase receptor with the molecular weight of 145–160 kDa [7,8]; hypoxia-inducible factor-1 (HIF-1) is a kind of heterogeneity dimer transcription factor of human and mammalian under hypoxic condition [5,9]. The overexpression or mutation of these genes may induce various tumor diseases [10].

Sanguinarine (13-methyl-[1,3]benzodioxolo[5,6-c]-1,3-dioxolo [4,5-I] phen-anthrdinim) is a kind of benzo-phenanthridine alkaloid, existing mainly in the roots of *Sanguinaria canadensis* L. and the poppy-fumaria species, as well as the seeds of *Argemone mexicana* L. [11]. Sanguinarine possessed antibacterial, antiplatelet, antifungal, antischistosomal, anti-inflammatory, antitumor activity and had the potential to eliminate cancer cells [12]. It has been found that the pharmacological actions of sanguinarine related to its binding with the G-quadruplex DNA and regulating to its structure [13]. So, investigation on the interaction of G-quadruplex DNA and sanguinarine can provide rich information for understanding their therapeutic effects in molecular level, and it also would be beneficial to anticancer drug development.

In recent years, much attention has been paid to the interaction of DNA and the active components of natural products. Kumar's group has investigated the interactions of sanguinarine with double-strand DNA and human telomeric G-quadruplex by NMR, CD spectra, fluorescence and calorimetric approaches

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[13,14]. However, there is no fluorescence for these DNA molecules, and generally organic dyes, as a fluorescence probe, should be introduced to the interaction system, which would be participated the interaction process and finally influence on the experimental results. To overcome this inconvenience, some novel techniques have been tried to the investigation. Teulade' group developed a high-throughput G4-FID assay for screening and evaluation of small molecules binding with quadruplex DNA [15–17]. Dash et al. applied the FRET assay for ligand selective of G-quadruplexes [18]. Mass spectrometry [19], NMR spectroscopy [20], circular dichroism (CD) [3], UV-melting [21], surface plasmon resonance (SPR) [18] and equilibrium dialysis [22] have also applied to the investigation of biological macromolecules interacting with small molecular ligands.

The modificatory fluorescence quantum dots (QDs) have become the supplementary and replacer of organic dyes in basic research due to their superior performance, such as wide excitation, narrow and size-tunable emission, high luminous stability, good solubility in water and so on [23]. Because of these advantages, fluorescence QDs are applied in various kinds of biological sensing systems, including nucleic acid hybridization, gene delivery, enzyme-activity detection, and drug screening [24–28]. Raymo et al. constructed a new method to investigate receptor–substrate interactions based on QDs sensor [29], and the interaction of anticancer drug and DNA has also been investigated based on fluorescent probe composed of QDs and ruthenium complexes [30,31].

The phosphorescence properties of the doped QDs have potential applications for the interaction of DNA with drugs. The doped QDs with phosphorescence have longer lifetime, which can allow the suitable delay time to avoid interference of the autofluorescence and scattering light and improve the selectivity and specificity of the sensoring system [32]. Taking of this advantage, room-temperature phosphorescence (RTP) means for sensing DNA, enoxacin, folic acid [32–34], pentachlorophenol, ascorbic acid, heparin [35–37], glucose, proteins [38,39] have been developed based on Mn-doped ZnS QDs. Thus it can be seen, phosphorescence sensor also has a broad application prospect for determining the target analytes and investigating the interaction of biomacromolecules with ligands.

In this work, a comparative binding assay has been established for evaluating the binding capacity of sanguinarine with six G-quadruplex DNA (tel24-1, tel24-2, tel22, kras, c-kit1 and HIF-1α) with the room-temperature phosphorescence of the Mn-Doped ZnS QDs as a sensor. In this phosphorescence sensing system, as shown in Scheme 1, sanguinarine adsorbed on the surfaces of the QDs, and quench its phosphorescence intensity. When G-quadruplex DNA was added, the phosphorescence intensity of the sensing system could be partly restored due to the combination of the G-quadruplexes with sanguinarine in the system, and their binding ability could be judged by observing the signal changes of the sensing system. With the developed method, the binding abilities of sanguinarine and the G-quadruplex DNA with various sequences have been assessed and compared. Combining with CD spectra, the conformational alterations of the G-quadruplex DNA after binding with sanguinarine were probed and discussed.

Table 1
Sequences of DNA oligonucleotides.

Oligo name	Sequence
tel24-1	5′-TTAGGGTTAGGGTTAGGGTTAGGG-3′
tel24-2	5′-AGGGTTAGGGTTAGGGTTAGGGTT-3′
tel22	5′-AGGGTTAGGGTTAGGGTTAGGG-3′
k-ras	5′-AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG-3′
HIF-1α	5′-GGGGAGGGGAGAGGGGGCGGGA-3′
c-kit1	5′-TGAGGGTGGTAGGGTGGGTAA-3′

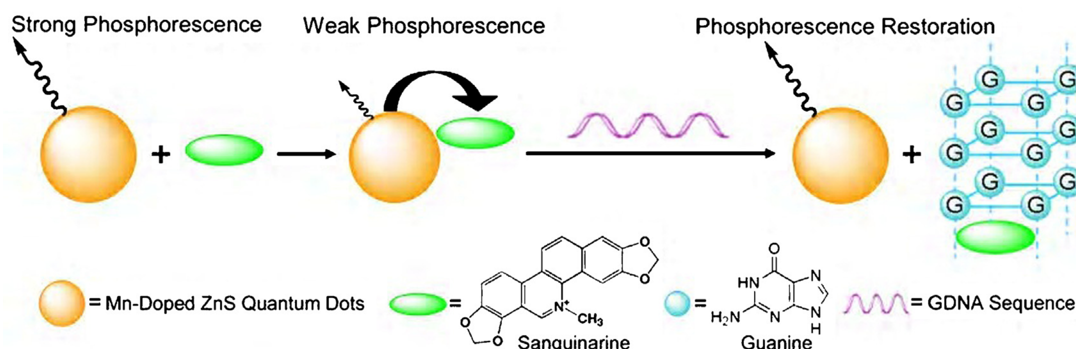
2. Materials and methods

2.1. Materials

All the reagents and starting materials of Mn-doped ZnS QDs synthesis were purchased from commercial suppliers and were used without further purification. Sanguinarine was obtained from Aladdin reagent Co. Ltd. (Shanghai, China). L-Cysteine was obtained from Acros, $ZnSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $Na_2S{\cdot}9H_2O$, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Tianjin Kermel Chemical Reagent Co. Ltd. (Tianjin, China). Six quadruplex-forming oligonucleotides were used in these experiments, as shown in Table 1. These oligomers were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and used without further purification. All experiments were prepared with doubly deionized water obtained from an Aquapro ultra pure water system (18.25 MΩ cm, Chongqing, China).

2.2. Instrumentation

The morphology and microstructure observation of the QDs were performed on the Philips Tecnai G2 F20 microscope (FEI, America) high resolution transmission electron microscopy (HRTEM). The X-ray diffraction (XRD) spectra were recorded on a X' Pert PRO MPD X-ray diffractometer (PANalytical, Poland) with Cu K R radiation. The FT-IR spectra (4000–400 cm^{-1}) were collected using a Nexus-670 spectrometer (Nicolet, Madison, WI) in KBr tabletting. The samples were dried for detecting by FT-IR. UV–visible spectra were measured on the SPECORD 200 UV-Visible spectrophotometer (Jena AG, Germany). Phosphorescence spectra and phosphorescence decay were performed on a FluoroMax-4 fluorescence spectrophotometer (HORIBA Jobin Yvon, France) equipped with a quartz cell in phosphorescence mode. CD spectra



Scheme 1. Signal transduction mechanism diagram for combination of sanguinarine and G-quadruplex mediated by phosphorescence QDs.

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