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A label-free G-quadruplex DNA-based fluorescence method for highly sensitive, direct detection of cisplatin

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ABSTRACT

Cisplatin is a widely used anticancer chemotherapeutic, and fast, convenient detection methods for it are highly desirable. Based on the interaction of cisplatin with G-quadruplex DNA, we developed a simple label-free fluorescence method for rapid cisplatin detection. The titration experiment showed that the cisplatin concentration and the fluorescence signal change ratios (F_0/F) exhibited a consistent linear correlation within the 1 to 10 μM range with a limit of detection of 720 nM, which was even lower than the common concentration of cisplatin in chemotherapy patients' urine (54.3 to 321 μM). The equilibrium dissociation constant K_D value for cisplatin binding was determined to be 1.19×10^{-5} M. This result demonstrated that our method had relatively high affinity toward cisplatin and could bind micromolar concentrations of cisplatin in solution. Our method also shows obvious selectivity among tested drugs, even between cisplatin and oxaliplatin/carboplatin. We demonstrated the high sensitivity of this methodology in the direct detection of cisplatin in urine samples and the fluorescence imaging of cisplatin in living cells.

1. Introduction

Since it was approved by the Food and Drug Administration (FDA) in 1978, cisplatin [*cis*-diamminedichloroplatinum(II)] has been widely used for treating solid tumors, such as germ cell tumors, carcinomas of the head and neck and other tumor types [1–4]. However, cisplatin administration has frequently shown toxic side effects, including nephrotoxicity, neurotoxicity and the induction of nausea and vomiting [5–7]. Furthermore, a low concentration (8 μM) of cisplatin led to cell apoptosis, while a high concentration (800 μM) resulted in necrotic cell death [8]. Thus, the dosage control of cisplatin is a key factor in the successful execution of cisplatin-based chemotherapy [8,9]. Previous reports suggested that the concentration of cisplatin varied from 54.3 to 321 μM in the urine of patients when administered at 50 mg/m^2 by slow injection [10,11], while 90% of excreted cisplatin remained unchanged chemically [9]. Therefore, methods to monitor either extracellular or intracellular cisplatin concentrations are highly desired to ensure effective chemotherapy.

Currently, a series of analytical methods have been developed for measuring cisplatin [12]. The traditional methods utilising optical detection of cisplatin in biological samples required a complicated derivatization processes [9,10]. Recently, Petrlova et al. [13] and Mascini et al. [14] designed several powerful electrochemical biosensors for detecting cisplatin in solution. However, these biosensors encountered complications with cisplatin enrichment on the electrode surface [15]. Techniques such as atomic absorption spectrometry (AAS) [16] and inductively coupled plasma-mass spectrometry (ICP-MS) [17] could be used in analyzing the platinum content from cisplatin following complicated sample preparation processes. Thus, a fast, convenient and low-cost method with high cisplatin detection sensitivity in biological samples will be highly advantageous.

Herein, we report a simple method to detect cisplatin by utilising the interaction between cisplatin and G-quadruplex DNA. G-quadruplex DNA, a certain type of G-rich nucleic acid sequence

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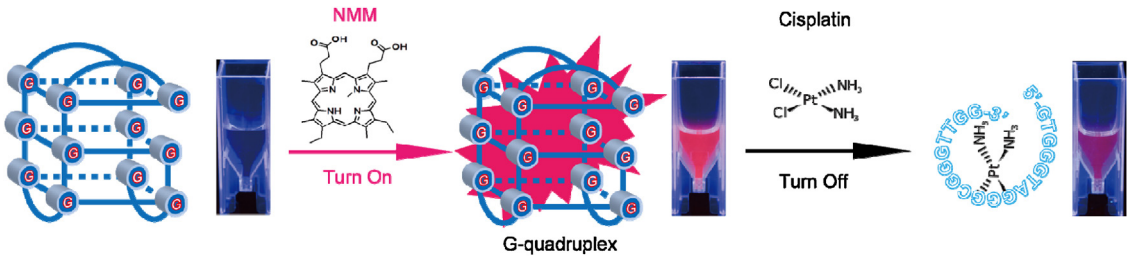

Scheme 1. Schematic illustration of the label-free fluorescence DNA probe based on the cisplatin-induced allosteric G-quadruplex for the detection of cisplatin.

[18,19], plays an important role in genomic functions, including transcription, recombination and replication [20–23]. Interestingly, unlike triplex, duplex or single-stranded forms of DNA, G-quadruplex DNA can be selectively recognized by N-methyl mesoporphyrin IX (NMM) [24,25]. The fluorescence intensity of NMM exhibits a dramatic enhancement upon binding to G-quadruplex DNA [26,27]. Recent research progress has shown that this interaction can be utilized as a signal reporter to detect heavy metal ions [28], NAD^+ [29] and RNA [30,31]. Furthermore, it was reported that cisplatin hydrolysed to produce cationic species after passing through the blood into the cells. The platinum atoms of the cationic species would subsequently covalently bond to the nitrogen atoms of guanine on the basic backbone of the DNA, forming intra- and inter-strand crosslink [32] that result in structural destruction of the target DNA [33]. Inspired by these reports, we proposed that an easy, highly sensitive method of cisplatin detection could be developed by adding cisplatin to the combination of G-quadruplex DNA and NMM. As shown in Scheme 1, the self-folding of G-quadruplex DNA without cisplatin would form a G-quadruplex DNA structure that binds NMM and results in a remarkable hyperchromic effect. When cisplatin was added, it would bind to G-quadruplex DNA and disintegrate the DNA structure of the G-quadruplex, which would result in an obvious hypochromic effect.

2. Experimental

2.1. Chemicals and materials

The synthetic G-rich oligonucleotides DNA purified by PAGE were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Stock solution of G-rich oligonucleotides (100 μM) was prepared by DNase- and RNase-free water. Before used, the oligonucleotides solutions were diluted to required concentration with the Tris–HCl buffer (10 mM, pH 7.6). Cisplatin and NMM were purchased from J&K Scientific Ltd. (Beijing, China). Other anti-cancer drugs were purchased from Sigma-Aldrich. All chemicals were analytical reagent. The water used was purified by Millipore Milli-Q (18 MΩ/cm). The stock solution of NMM (6 mM) was prepared in DMSO [dimethyl sulfoxide], stored in darkness at −20 °C. Before being used, NMM was diluted to required concentration with the Tris–HCl buffer (10 mM, pH 7.6).

2.2. Fluorometric analysis

All fluorescence measurements were performed on an F-7000 spectrometer (Hitachi, Japan). The instrument settings were as follows: Excitation wavelength λ_{EX} = 399 nm (bandpass 10 nm), Emision wavelength λ_{EM} from 550 nm to 700 nm (bandpass 10 nm) and the photomultiplier tube (PMT) detector voltage = 500 V. The cisplatin titration was performed by adding 1 μM to 100 μM cisplatin into 125 nM PS2.M oligonucleotide DNA in Tris–HCl buffer (10 mM, pH 7.6) containing 2 mM KCl and 1.5 μM NMM. All samples were incubated at 37 °C for 80 min to ensure completed reaction and signal stabilization. The fluorescence signal change ratios were calculated with the formula $Y = F_0/F$, where F_0 and F are the fluorescence intensities at 610 nm (maximum emission wavelength) in the absence and presence of cisplatin, respectively.

2.3. Circular dichroism (CD) spectra measurement

The G-quadruplex oligonucleotides (30 μM) were dissolved in 10 mM potassium acetate. The CD spectra were measured using a JASCO J-810 CD spectropolarimeter (Jasco, Japan). The data were recorded for the 220 to 320 nm range at room temperature in a quartz cuvette with a 1 mm optical path length. The data reported herein were averaged from at least 5 scans to improve the signal-to-noise ratio so that the contribution from the buffer was diminished.

2.4. Rat urine experiments

SD rats (8 weeks old) were purchased from Model Animal Research Center of Nanjing University. The rats were provided with a standard pelleted food and water and were placed in metabolism cages. Rats treated with 10 mg/kg cisplatin by intraperitoneal injection and treated with the same volume of normal saline as a control. The urine was collected for 24 h and stored frozen until analysed. Before measured, the urine samples were centrifuged and filtered by a 0.22 μm filter. The animal study proposal was approved by the Institutional Animal Care and Use Committee (IACUC) of the Nanjing University. All rat experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China. An experiment of normal urine samples with extra added cisplatin was also conducted by the same method.

2.5. Cellular imaging experiments

Human breast adenocarcinoma (MCF-7) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were allowed to grow to 80% confluency before being transfected. At that time, oligonucleotide transfections were performed using 500 nM G-quadruplex DNA, 6 μM NMM, 2 mM K^+ and Lipofectamine 2000 reagent (Invitrogen). One hour after transfection, the media was removed and fresh media was added to remove any material left in solution and to optimize the background signal. For the cisplatin treatment, the cells were incubated with 10 μM cisplatin for 12 h. The Hoechst 33342 was added 20 min before the imaging experiments. All imaging experiments were performed using a confocal microscope (Olympus FV 1000). Both NMM and Hoechst 33342 used the DAPI filter and their excitation wavelengths were both set at 387 nm; however, their emission wavelengths were 610 nm and 440 nm, respectively.