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Analytical Methods

A G-quadruplex based fluorescent oligonucleotide turn-on probe towards iodides detection in real samples

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ABSTRACT

A basket-type G-quadruplex (GQ) fluorescent oligonucleotide (OND) probe is designed to detect iodides dependent on thymine-Hg(II)-thymine (T-Hg(II)-T) base pairs and the intrinsic fluorescence quenching capacity of GQ. In the presence of Hg(II) ions (Hg^{2+}), the two hexachloro-fluorescein-labeled ONDs form a hairpin structure and the fluorophores are dragged close to the GQ, leading to fluorescence quenching of the probe due to photoinduced electron transfer. Upon addition of iodide anions, Hg^{2+} are extracted from T-Hg(II)-T complexes which attributes to the stronger binding with iodide anions, resulting in the fluorescence recovery. Through performing the fluorescence quenching and recovery processes, this probe developed a fluorescence turn-on sensor for iodide anions determination over a linear range of 20–200 nmol/L with a limit of detection of 5 nmol/L. The practical use of the turn-on technology was demonstrated by its application in determination of iodides in water, food, pharmaceutical products and biological samples.

1. Introduction

Iodide is essential for normal growth and development of human and plays a crucial role in neurological activity, thyroid gland, pituitary gland functions and many other biological processes (Taurog, 2002). Both iodide excessive intake and deficiency could lead to thyroid diseases. Iodine excess can lead to high iodine goiter, hyperthyroidism, hypothyroidism, autoimmune thyroid diseases and thyroid cancer, even to disorders of mental development (Suzuki, 1980). While iodine deficiency can harm the baby's brain development, adult's brain function and cause iodine deficiency disorders, such as endemic goiter and cretinism etc (Haldimann, Zimmerli, Als, & Gerber, 1998). The elemental iodine has also been frequently used in many areas of food and chemistry applications, such as synthesizing organic chemicals, manufacturing dyes and drugs and preparing iodized table salt (Lyday, 2000; Singh & Mehtab, 2008). Due to the significant role of iodide ions in maintaining the health of human body, more sensitive and selective method is necessary for iodide ion detection in real samples.

Currently available techniques for detecting iodide ions (I^-) include gas chromatography–mass spectrometry (GC–MS) (Bichsel & von Gunten, 1999), ion chromatography (Villagran, Deetlefs, Pitner, & Hardacre, 2004), capillary electrophoresis (Ito et al., 2003; Yokota, Fukushi, Takeda, & Wakida, 2004), atomic absorption spectrometry (Bermejo-Barrera et al., 2001), ion selective electrode (Malon et al., 2003), inductively coupled plasma mass spectrometry (ICP-MS) (Schone, Zimmermann, Quanz, Richter, & Leiterer, 2006) and so on. Due to high sensitivity and selectivity, fluorescence spectra based methods have been developed for a plenty of analytes detection (Peng, Stolwijk, Sun, Wegener, & Wolfbeis, 2010; Kim, Guo, Zhu, Yoon, & Tian, 2011). In literatures, a lot of fluorescence turn-off probes based on fluorescence quenching response have been utilized for I^- detection (Singh & Jang, 2007; Lee, Singh, Kim, & Jang, 2011; Li, Han, & Zhang, 2008). And with the improvement of fluorescence method, more sensitive turn-on probes based on fluorescence recovery response (Lin, Yuan, Cao, Chen, & Feng, 2009; Ma, Zeng, Zheng, & Wu, 2011; Mahapatra, Roy, Sahoo, Mukhopadhyay, & Chattopadhyay, 2012; Zhu et al., 2012) have already been reported for iodide ion determination. The iodide ions detection limits of these probes were reported to be within the range of 30–450 nmol/L. In general, fluorescence enhancement is advantageous

Abbreviations: GC–MS, Gas chromatography–mass spectrometry; GQ, G-quadruplex; HEX, Hexachloro-fluorescein; Hg^{2+}, Hg(II) ions; I, Iodide ions; ICP-MS, Inductively coupled plasma mass spectrometry; K^+, Potassium ions; MOPS, 3-(*N*-morpholino)propanesulfonic acid; OND, Oligonucleotide; RSD, Relative standard deviation; T-Hg(II)-T, Thymine-Hg(II)-thymine; T-T, Thymine-thymine.

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compared to fluorescence quenching for the detection of analytes, since the latter usually leads to low signal-to-noise ratio and false positive results by other quenchers in actual samples (Johansson & Cook, 2003; Nolan & Lippard, 2008; Doose, Neuweiler, & Sauer, 2009).

It is reported that thymine-thymine (T-T) base pairs can selectively capture Hg(II) ions (Hg^{2+}) to form thymine-Hg(II)-thymine (T-Hg(II)-T) complexes in DNA duplexes (Tanaka et al., 2007; Torigoe, Ono, & Kozasa, 2010). It is known that Hg^{2+} are able to interact with I^- to form mercuric iodide precipitates (HgI_2 and HgI_4^{2-}) with the formation constants of 8.3×10^{23} and 5.6×10^{29}, respectively; while the formation constants of T-Hg(II)-T is nearly 10^6 (Hepler & Olofsson, 1975; Chiang, Huang, Liu, & Chang, 2008), indicating that Hg^{2+} have greater affinity for I^- than thymines. Hence, I^- can disrupt T-Hg(II)-T complex and give rise to the release of thymines. In addition, the G-quadruplex (GQ) structure, formed by a guanine (G)-rich single-stranded DNA, can be served as favorable electron donor and quencher for a variety of fluorophores. Therefore, a photoinduced electron transfer or resonant energy transfer may occur between the GQ and fluorophores (Wang, Tian, Li, Zhang, & Sun, 2011), resulting in the quenching of fluorophore in excited state (Seidel, Schulz, & Sauer, 1996).

In this work, we have developed a novel basket-type GQ fluorescent OND probe for convenient detection of Hg^{2+} ions based on T-Hg(II)-T base pairs and the intrinsic quenching capacity of G-quadruplex (Fig. 1). A GQ OND sequence (5′-GGAAGGAAAG GAAGG-3′) was designed to situate at the middle of the probe and two pieces of T-rich OND sequences (5′-ATTGTTTCCTTTCTT-3′ and 5′-TTCTTTCCTTTGTTA-3′) labeled with hexachlorofluorescein (HEX) to locate at the both ends of the probe. In the presence of potassium ions (K^+), the OND sequence folded into a GQ. When Hg^{2+} was added into the system, the two HEX-labeled ONDs formed a hairpin structure and the fluorophores were closed to the GQ, resulting in a clear fluorescence quenching of HEX. Upon addition of iodide anions, the strong fluorescence of the probe was recovered due to the extraction of Hg^{2+} from the T-Hg(II)-T complexes and the release of HEX-labeled ONDs. Under the optimal conditions, this method allows a good response to iodide ions and was successfully utilized for the detection of iodide ions in real samples.

2. Experimental

2.1. Reagents and instrument

The HEX-labeled basket-type GQ fluorescent OND probe (5′-HEX-ATTGTTTCCTTTCTT-GGAAGGAAAGGAAGG-TTCTTTCCTTTGTTA-HEX-3′) was provided by Takara Biotechnology Co. Ltd. (Dalian, China) and its purity was confirmed by analytical anion exchange and reversed-phase high performance liquid chromatography. Concentrations of DNA were determined at the wavelength of 260 nm. MOPS (3-(*N*-morpholino)propanesulfonic acid) sodium salt, potassium salts of the anions I^-, CO_3^{2-} and $C_2O_4^{2-}$, sodium salts of the anions F^-, Cl^-, Br^-, NO_3^-, CH_3COO^-, HCO_3^-, $H_2PO_4^-$, SO_4^{2-}, HPO_4^{2-} and PO_4^{3-}, nitrate salts of the cations Ag^+ and Pb^{2+}, and chloride salts of the cations K^+, Na^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, Al^{3+} and Cr^{3+} were obtained from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). The salts were analytical grade and used without further purification.

Fluorescent OND probe (1.0 μmol/L) was dissolved in 10.0 mmol/L MOPS buffer with 400.0 mmol/L $NaNO_3$ and 20.0 mmol/L KNO_3 (pH 7.0). The ultrapure water (18.2 MΩ·cm) was prepared by a Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA). For iodide anion detection, stock solution of T-Hg(II)-T (0.5 μmol/L) and a variety of anions and cations (5.0 mmol/L) were prepared using MOPS buffer (10.0 mmol/L, pH 7.0) and ultrapure water, respectively. All the testing solutions were diluted to requisite amounts with MOPS buffer (10.0 mmol, pH 7.0). All the mixture solutions were stirred for 5 min before the fluorescence spectra were recorded.

Tap water sample was taken from a household water pipe. "Wa-Ha-Ha" mineral water, "Bi-Luo-Chun" green tea, "Rong-Chen" kelp, "Guang-Ming" milk and "Bing-Ling" iodized table salt were obtained from a supermarket. Amiodarone hydrochloride, diatrizoic acid and thyroid dispersible tablets were purchased from a drug market. Human serum and urine were from a healthy female.

Shimadzu UV-2450 (Kyoto, Japan) and Hitachi F-4500 (Tokyo, Japan) fluorescence spectrophotometers were utilized to detect the ultraviolet–visible absorbance spectra and fluorescence emission spectra of the samples.

2.2. Fluorescence emission spectra

In order to make the fluorescent OND probes displayed only in their primary structure, the probe solution (10 μL, 1.0 mmol/L) was diluted by 490 μL of MOPS buffer and incubated at 95 °C for 10 min. The probe solution mixture was then stored at 25 °C overnight, transferred into a quartz cell of 1.0 cm path length and the emission spectra from 530 to 600 nm were collected. The total volume of the testing solution was 500 μL containing 20.0 nmol/L of probe, 10.0 mmol/L of MOPS, 400.0 mmol/L of $NaNO_3$ and 20.0 mmol/L KNO_3 (pH 7.0). Two minutes later, different concentrations of Hg^{2+} solutions (5.0 μL) were separately added to the above mixture and the fluorescent emissions were recorded. Finally, under the optimized condition of Hg^{2+} concentrations, various concentrations of 5.0 μL iodide anion solutions were added and the fluorescent emissions were collected under the same conditions. The excitation and emission wavelengths were set at 520 and 554 nm with the excitation and emission slits of 10 nm, respectively.

2.3. Calibration curve and validation of the method

A stock solution of iodide ions (5 mmol/L) was prepared by dissolving 8.3 mg of potassium iodide in 10.0 ml of 10.0 mmol/L of MOPS, 400.0 mmol/L of $NaNO_3$ and 20.0 mmol/L KNO_3 (pH 7.0). The stock solution was diluted by the same MOPS buffer to yield working solutions containing 0.02, 0.05, 0.10, 0.125, 0.15, 0.175, 0.20, 0.5, 1.0, 2.0, 2.50 and 5.0 μmol/L of I^-. The concentrations of I^- were determined by the proposed fluorescence "turn-on" method. The accuracy and precision of the method were investigated at three concentration levels of standard solutions of I^- (20, 80 and 160 nmol/L).

2.4. Detection of iodide anions

For evaluating the applicability of this method, the iodide anions in real samples including two water samples (running water and mineral water), an iodized table salt sample, three food samples (tea, kelp and milk), three drug samples (amiodarone hydrochloride, diatrizoic acid and thyroid dispersible tablets), and two biological samples (human serum and urine) were determined.

All the samples were centrifuged at 15000 rpm for 10 min and filtered through a 0.22 μm membrane before use. The iodized table salt solution was prepared with ultrapure water. Water samples (100 μL) were separately mixed with 400 μL of incubated probe solution at 25 °C and the fluorescence responses were monitored.

Tea and kelp samples were cleaned with ultrapure water and dried in a 50 °C oven for 2 h. Then the samples were triturated to powder and stored in a thermostatic drier for further use. Two