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PAPER

Human telomeric G-quadruplex formation and highly selective fluorescence detection of toxic strontium ions†

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Strontium ions play important roles in biological systems. The inhalation of strontium can cause severe respiratory difficulties, anaphylactic reaction and extreme tachycardia. Strontium can replace calcium in organisms, inhibit normal calcium absorption and induce strontium "rickets" in childhood. Thus, the development of sensitive and selective methods for the determination of trace amounts of Sr^{2+} in aqueous media is of considerable importance for environmental and human health protection. A number of methodologies, such as X-ray energy dispersive spectrometry, inductively coupled argon plasma atomic emission spectroscopy (ICP-AES), atomic absorption spectrometry (AAS) and instrumental thermal neutron activation analysis, have been reported. However, these methods are somewhat complex, costly, time consuming and, especially, need special instruments. Thus, the design of convenient and inexpensive approaches for the sensitive and selective detection of Sr^{2+} with rapid, easy manipulation is in ever-increasing demand. To the best of our knowledge, using DNA conformational change to detect Sr^{2+} has not yet been reported. Herein we utilized thiazole orange (TO) as a signal reporter to devise a simple Sr^{2+} detection assay based on Sr^{2+} induced human telomeric DNA conformational change in the presence of SWNTs. The limit of detection is 10 nM Sr^{2+} (0.87 μg L^{-1}), far below 4 mg L^{-1}, the U.S. Federal threshold in drinking water defined by the U.S. EPA.

Introduction

Human telomeric DNA has received great attention in recent years.[1–3] The G-rich telomeric sequence can fold into a G-quadruplex consisting of stacked G-tetrad planes. The formation and stabilization of these G-quadruplex structures in the human telomeric sequence have been shown to inhibit the activity of telomerase.[1–10] Thus the telomeric DNA G-quadruplex has been considered to be an attractive target for cancer therapeutic intervention,[6–10] and the in-depth investigation of G-quadruplex has great practical significance. G-quadruplex formation is known to be promoted by the presence of metal cations, such as Na^+, K^+, and Sr^{2+}. Intriguingly, Sr^{2+} can induce more stable G-quadruplex structure than K^+ and Na^+,[11,12] and facilitate the formation of distinct human telomeric G-quadruplex structures from K^+ and Na^+.[13]

Strontium ions play important roles in biological systems. The inhalation of strontium can cause severe respiratory difficulties, anaphylactic reaction and extreme tachycardia. Strontium can replace calcium in organisms, inhibit normal calcium absorption and induce strontium "rickets" in childhood. The United States Environmental Protection Agency (EPA) has mandated an upper limit of 4 mg L^{-1} for Sr^{2+} in drinking water. Thus, the development of sensitive and selective methods for the determination of trace amounts of Sr^{2+} in aqueous media is of considerable importance for the environmental and human health protection. A number of methodologies, such as X-ray energy dispersive spectrometry, inductively coupled argon plasma atomic emission spectroscopy (ICP-AES), atomic absorption spectrometry (AAS) and instrumental thermal neutron activation analysis, have been reported.[14] Though these methods have high selectivity and sensitivity, they are somewhat complex, costly, time consuming and, especially, need special instruments. Thus, the design of convenient and inexpensive approaches for the sensitive and selective detection of Sr^{2+} with rapid, easy manipulation is in ever-increasing demand.

As the leading nanodevice candidate, SWNTs have shown great potential applications ranging from molecular electronics to ultrasensitive biosensors.[15] Single-stranded DNA (ssDNA) can interact noncovalently with SWNTs, and form stable complexes with individual SWNTs by wrapping around them through π–π stacking between nucleotide bases and SWNT sidewalls.[16] However, double-stranded DNA (dsDNA) does not possess this feature.[17–22] Herein we utilized thiazole orange (TO) as a signal

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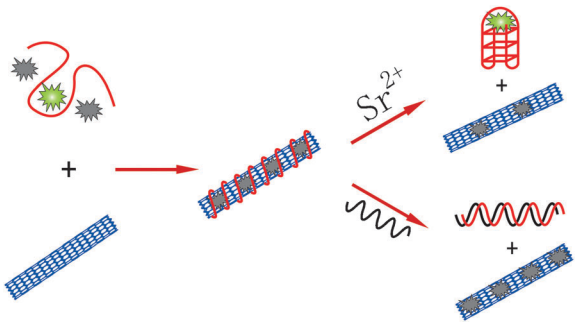

Scheme 1 Schematic representation of label-free fluorescent recognition of human telomeric G-quadruplex DNA and detection of Sr^{2+}. TO, $ssAG_3$ and SWNTs are mixed to quench TO fluorescence. Then, the formation of G-quadruplex induced by Sr^{2+} results in the restoration of TO fluorescence; however, the duplex formed by addition of the complementary strand cannot restore TO fluorescence.

reporter to devise a simple Sr^{2+} detection assay based on Sr^{2+} induced human telomeric DNA conformational change in the presence of SWNTs. As demonstrated in Scheme 1, SWNTs possess exceptional quenching capability towards TO and the complex formed by TO and human telomeric DNA, $ssAG_3$, wrapped on SWNTs. However, in the presence of Sr^{2+}, G-quadruplex induced by Sr^{2+} would dissociate from the surface of SWNTs and enable TO to bind it, which disturbs the interaction of TO and SWNTs, resulting in the partial restoration of TO fluorescence. This strategy realized label-free, selective and sensitive detection of Sr^{2+}. Moreover, in the presence of $ssAG_3$ complementary strand, formation of dsDNA cannot restore TO fluorescence, this demonstrates that in the presence of SWNTs, TO-based fluorescence detection of Sr^{2+} is specific to G-quadruplex DNA. To the best of our knowledge, using DNA conformational change to detect Sr^{2+} has not yet been reported.

Results and discussion

TO, as an asymmetric cyanine dye, is a very interesting DNA-probe since it is highly fluorescent upon complexation with DNA, whereas totally non-fluorescent when free in solution.[23,24] The chemical structure of TO was shown in Fig. S1, ESI.† We first investigated the fluorescence quenching effect of SWNTs on TO binding to ssDNA. As shown in Fig. S3 (ESI†), in the absence of DNA and SWNTs, TO behaved totally non-fluorescent in 10 mM Tris-HCl buffer (pH = 7.0). Addition of $ssAG_3$ caused TO fluorescence dramatic enhancement because the nonspecific binding between TO and $ssAG_3$ restricted the rotation around the bond between the aromatic systems of TO, which closes a channel for nonradiative decay. Upon titration of SWNTs to the solution, the pronounced fluorescence quenching could be observed, and more than 97% quenching efficiency was observed at SWNTs concentrations up to 6 μg mL^{-1}. Electrostatic interaction and π–π stacking between the TO–$ssAG_3$ DNA complex and SWNTs resulted in fluorescence quenching because the $ssAG_3$ held random coil structure under salt-deficient conditions, and could wrap onto the surface of SWNTs, this further enhanced the quenching efficiency. The optimum concentrations for the system are 1 μM TO, 0.5 μM AG_3 and 6 μg mL^{-1} SWNT.

Next, we studied fluorescence change of TO/AG_3 by addition of different concentrations of metal ion Sr^{2+} in the absence or

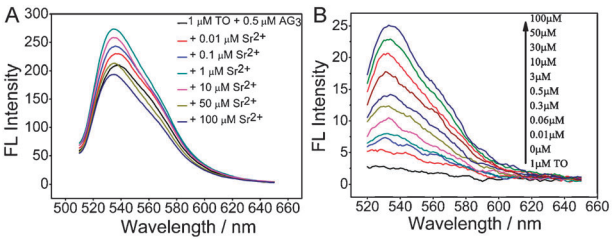

Fig. 1 Fluorescence emission spectra of $TO/ssAG_3$ upon addition of different concentrations of metal ion Sr^{2+} in the absence (A) and presence (B) of SWNTs.

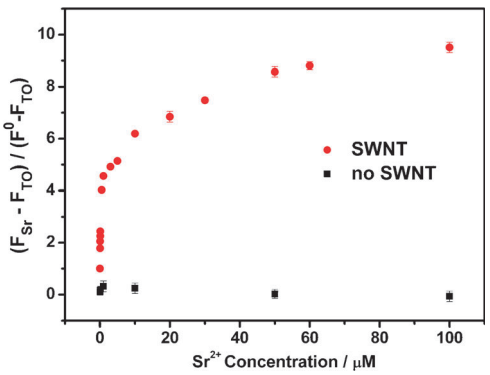

Fig. 2 $(F_{Sr} - F_{TO})/(F^0 - F_{TO})$ as S/B signal of the $TO/ssAG_3$ system plotted as a function of the concentration of Sr^{2+} in the absence (black squares) and the presence (red spheres) of SWNTs.

presence of SWNTs in Fig. 1. In the absence of SWNTs, addition of Sr^{2+} to the TO/AG_3 solution caused TO fluorescence enhancement. However, addition of more Sr^{2+} resulted in TO fluorescence decrease. The reason for the decrease can be that excessive Sr^{2+} would compete with TO binding to G-quadruplex DNA. In the presence of SWNTs, gradual fluorescence enhancement of TO was observed following addition of Sr^{2+}, and the fluorescence reached a platform with increasing Sr^{2+} concentration. We found that there was a rather large difference in the signal-to-background ratio (S/B) in the absence or presence of SWNTs due to different background signals. Therefore, we evaluated the S/B which was defined as $S/B = (F_{Sr} - F_{TO})/(F^0 - F_{TO})$, where F_{Sr}, F^0, and F_{TO} are the measuring signals of TO/AG_3 with different concentrations of Sr^{2+}, TO/AG_3, and TO alone, respectively. As shown in Fig. 2, in the absence of SWNTs, the maximum S/B is 0.31 when 1 μM of Sr^{2+} was added because of a strong background signal, then a decreased S/B signal was observed with continuing addition of Sr^{2+}. On the other hand, in the presence of SWNTs, a gradual signal enhancement of TO fluorescence was observed in a Sr^{2+} concentration range of 0.01–100 μM and a maximum S/B reached as high as 9.5 by addition of 100 μM Sr^{2+}. The limit of detection we directly observed was 10 nM Sr^{2+} (0.87 μg L^{-1}), comparable to that obtained by the conventional methods (ICP, AAS *et al.*), and far below 4 mg L^{-1}, the U.S. Federal threshold in drinking water defined by the U.S. EPA. These results indicate that TO/AG_3/SWNTs can be used as a sensitive fluorescent approach for Sr^{2+} detection. The high sensitivity demonstrates that SWNTs greatly reduce the background signal, and improve S/B ratios.[15]

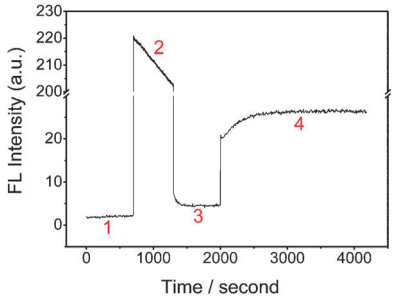

Fig. 3 The kinetics of TO fluorescence response toward different targets. First, the cell was filled with (1) 1 μM TO in buffer; then (2) 0.5 μM AG_3, (3) 6 μg mL^{-1} SWNTs, and (4) 50 μM Sr^{2+} was added sequentially. $\lambda_{ex}/\lambda_{em}$ = 480 nm/535 nm.

The response kinetics of Sr^{2+} detection was tested by real-time monitoring of TO fluorescence variation in Fig. 3. TO alone has a negligible fluorescence in buffer. The response of TO with addition of AG_3 is prompted and has strong fluorescence enhancement. However, the fluorescence decreases quickly upon the light irradiation; it reflects the notable photobleaching effect of the dye, which severely limits the application of the dye in sensors. By addition of SWNTs, pronounced fluorescence quenching was caused and reached equilibrium immediately within 3 min. Upon addition of 50 μM Sr^{2+}, TO fluorescence has partially restored and reached equilibrium within 10 min. Strong adsorption of $ssAG_3$ and TO to the nanotube surface may account for the slow kinetics of G-quadruplex formation and relatively long restoration time of TO fluorescence.

To determine the assay selectivity, we studied the fluorescence response of TO/AG_3/SWNTs to other typical G-quadruplex-related metal ions, including K^+, Na^+, Ca^{2+}, Mg^{2+}, Li^+, and NH_4^+ at a concentration of 50 μM under the same conditions as used in Sr^{2+} experiments (Fig. 4A). Remarkably, no apparent fluorescence signal changes were observed with these metal ions; only Sr^{2+} resulted in significant increases of the fluorescence signal. The metal ions including Ca^{2+}, Mg^{2+}, Li^+, and NH_4^+ are known to have negligible influences towards the structure of single-stranded AG_3. Sr^{2+} can induce more stable G-quadruplex structure than K^+ and Na^+,[11,12] and facilitate the formation of distinct human telomeric G-quadruplex structures from K^+ and Na^+.[13] DNA UV melting studies (Fig. S2, ESI†) show that the human telomeric G-quadruplex structures have T_m values of about 63 °C and 73 °C at 50 μM and 100 μM Sr^{2+}, respectively, comparable to the conditions of 100 mM Na^+ (61 °C) and 100 mM K^+ (69 °C). This can be the reason for the significant fluorescence enhancement induced by Sr^{2+}.

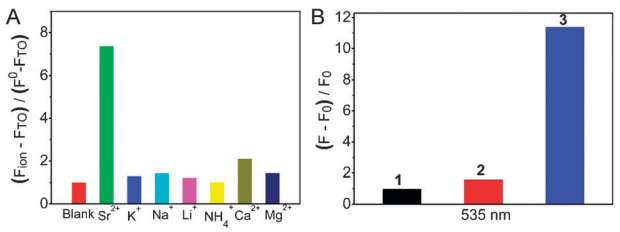

Fig. 4 (A) Selectivity of the TO/AG_3/SWNTs to different metal ions (K^+, Na^+, Li^+, NH_4^+, Ca^{2+} and Mg^{2+}). All metal ions were tested at 50 μM. (B) Selectivity of TO to different AG_3 structures (1: $ssAG_3$, 2: duplex, 3: G-quadruplex) in the presence of SWNTs.

We further evaluated whether $ssAG_3$ complementary strand DNA can influence the fluorescence of the TO/AG_3 system (Fig. S3–S7, ESI†). We observed that addition of excess $ssAG_3$ complementary strand DNA had little effect on the fluorescence of TO in the absence and presence of SWNTs, we assume that the duplex formed by DNA hybridization is dissociated from the surface of SWNTs, however the duplex cannot compete with TO binding to SWNTs due to strong adsorption of TO on SWNTs surfaces. Compared with TO fluorescence variations induced by $ssAG_3$, duplex and G-quadruplex induced by 50 μM Sr^{2+} in the absence or presence of SWNTs, we found that in the absence of SWNTs, TO produced almost identical fluorescence enhancement when interacted with three kinds of DNA, this is consistent with the previous report that TO has less sequence dependent affinity for different DNA structures.[24] In the presence of SWNTs, however, significant increases of TO fluorescence were observed by G-quadruplex other than $ssAG_3$ and duplex. It indicates that SWNTs have endowed TO with the excellent selectivity to recognize the G-quadruplex structure induced by Sr^{2+}, which TO itself does not possess. We defined $(F - F_0)/F_0$ to evaluate the selectivity ability of TO to $ssAG_3$, duplex and G-quadruplex induced by 50 μM Sr^{2+} in the presence of SWNTs, its value is 0.98, 1.58 and 11.38 for $ssAG_3$, duplex and G-quadruplex, respectively (Fig. 4B). Therefore, our designed chemosensor can realize exclusive detection of Sr^{2+}.

Conclusions

In summary, we have designed a non-covalent label-free fluorescent assay for detection of Sr^{2+}. SWNTs can dramatically reduce the background fluorescence of TO, which can significantly improve the detection sensitivity. The high selectivity is due to the stronger effect of Sr^{2+} on G-quadruplex than that of K^+, Na^+, Ca^{2+}, Mg^{2+}, Li^+, and NH_4^+ ions. Our design reported here will shed light on developing SWNTs-based DNA devices and their applications to environmental and human health protection.

Experimental section

Materials

SWNTs (Φ = 1.1 nm, purity > 90%) were purchased from Aldrich (St. Louis, MO), purified as described previously by sonicating SWNTs in a 3 : 1 v/v solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 10 h at 35–40 °C, and washed with water, thereby leaving an open hole in the tube side and functionalizing the open end of SWNTs with a carboxyl group to increase their solubility in aqueous solution. The stock solution of SWNTs (0.2 mg mL^{-1}) was obtained by sonicating the SWNTs for 8 h in pH 7.0 aqueous solution.

DNA oligomers were purchased from Sangon (Shanghai, China) and used without further purification. Concentrations of these oligomers were determined by measuring the absorbance at 260 nm. Extinction coefficients were estimated by the nearest-neighbor method by using mononucleotide and dinucleotide values. All experiments were carried out in aqueous Tris-HCl buffer (10 mm Tris, pH 7.0) unless stated otherwise.

DNA sequences are as follows: human telomeric G-quadruplex sequence (AG_3): 5′-A GGG TTA GGG TTA GGG TTA GGG-3′; human telomeric i-motif sequence (i-motif): 5′-CCC TAA CCC TAA CCC TAA CCC T-3′.

Sr^{2+} was prepared by dissolving some $SrCl_2$ in water and was diluted into a specific concentration when used. Other metal salts used in this work include NaCl, KCl, LiCl, NH_4Cl, $MgCl_2$, and $CaCl_2$.

Apparatus

Fluorescence measurements were carried out using a JASCO FP-6500 spectrofluorometer. An excitation wavelength of 480 nm was used, and the fluorescence emission was monitored from 510 to 650 nm with the slit width for the excitation and emission of 10 nm. UV experiments were carried out using a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory. Primary data were transferred to the graphics program Origin for plotting and analysis.

Fluorescence quenching assay of TO by SWNTs. 1 μM TO was mixed with 0.5 μM AG_3, then titrated with SWNTs aqueous solution and then fluorescence spectra were recorded.

Fluorescence assays for Sr^{2+}. 1 μM TO was mixed with 0.5 μM AG_3, then 6 μg mL^{-1} SWNTs was added. The resulting solution was mixed with different concentrations of Sr^{2+} ions. The mixture was allowed to react for 1 h and then fluorescence spectra were recorded. Control experiments in the absence of SWNTs were carried out under identical conditions. The selectivity for Sr^{2+} was confirmed by adding other metal-ion stock solutions or the AG_3 complementary strand (i-motif) instead of Sr^{2+} in a similar way. All experiments were performed at room temperature.

Acknowledgements

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