

Nano-graphite as New Biosensing Material for Highly Sensitive and Selective Fluorescent Detection of Silver(I) Ion in Aqueous Solution

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Abstract: This paper describes a novel approach utilizing nano-graphite as an effective fluorescent sensing platform for highly sensitive and selective detection of Ag^+ in aqueous solution for the first time. Nano-graphite can effectively quench the fluorescence of dye-labeled cytosine-rich single-stranded DNA due to their strong π - π stacking interactions; however, Ag^+ is able to specifically bind to the cytosine-cytosine (C-C) base pair in a DNA duplex, which does not adsorb on nano-graphite and thus retains the dye fluorescence. This sensing platform exhibits high selectivity and sensitivity toward Ag^+ versus other metal ions with a limit of detection of 500 pm.

1. Introduction

Silver ions are assigned to the highest toxicity class of heavy metal pollution. It is very important to monitor its content level in aquicolous ecosystems because a large amount of silver(I) ions is released to the environment annually from industrial wastes and emissions, especially to the sludge waste and even to surface waters [1-3]. A number of methodologies, such as electrothermal atomic absorption spectrometry (ETAAS) [4], voltammetry [5,6], inductively coupled plasma atomic emission spectrometry (ICP-AES) [7], inductively coupled plasma mass spectroscopy (ICP-MS) [8,9], potentiometry [10-12], and the fluorescence method have been reported in the past few years. However, these techniques suffer either from extensive, time consuming procedures or the use of sophisticated instrumentation.

On the other hand, the interactions between metal ions and nucleic acids have been paid considerable attention and have laid the foundation for oligonucleotide-based metal ions detection assay. It is well-known that Ag^+ can bind to two cytosine (C) residues of DNA to form the C- Ag^+ -C complex. The oligonucleotide (OND) is rich in cytosine and readily forms a hairpin structure in the C- Ag^+ -C configuration in the presence of target Ag^+ ion. Various Ag^+ ion detection assays based on this property of C- Ag^+ -C coordination chemistry have been developed in recent years.

In this paper, we demonstrate the first use of nano-graphite as a cheap, effective fluorescent sensing platform for Ag^+ detection, which is based on the noncovalent assembly of nano-graphite and dye-labeled cytosine -rich single-stranded DNA. This fluorescent sensing platform achieves a detection limit as low as 500 pM, which is much lower than that of carbon nanotubes and grapheneoxide system and exhibits excellent selectivity.

2. Experiment

The chemically synthesized oligonucleotide was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Oligonucleotide sequence is listed as follows: 5'-ROX-CCT CCC TCC TTT TCC ACC CAC C-3'. DNA concentration was estimated by measuring the absorbance at 260 nm. The nano-graphite (10-15 nm) was purchased from Nanjing XFNANO Materials Tech Co. Ltd. (Nanjing, China). It is important to mention that although nano-graphite tends to sink in the mixing solution of water and ethanol, they can be well-dispersed by shaking and kept stable during our measurements. All the other chemicals were purchased from Beijing Solarbio Co.Ltd. (Beijing, China) and used as received without further purification. The

water used throughout all experiments was purified through a Millipore system. The total volume of each sample for fluorescence measurement was 500 μL in 10 mM 3-(N-morpholino) propanesulfonic acid (MOPS) buffer containing 50 mM NaNO_3 (pH:7.5). The volume of nano-graphite used for each measurement was 40 μL , and the fluorescence quenching measurement was done after 20 min incubation in buffer. Transmission electron microscopy (TEM) measurements were made on a FEI Tecnai G2 F20 S-TWIN instrument (FEI, USA). Fluorescent emission spectra were recorded on a PerkinElmer LS55 Luminescence Spectrometer (PerkinElmer, UK).

3. Results and Discussion

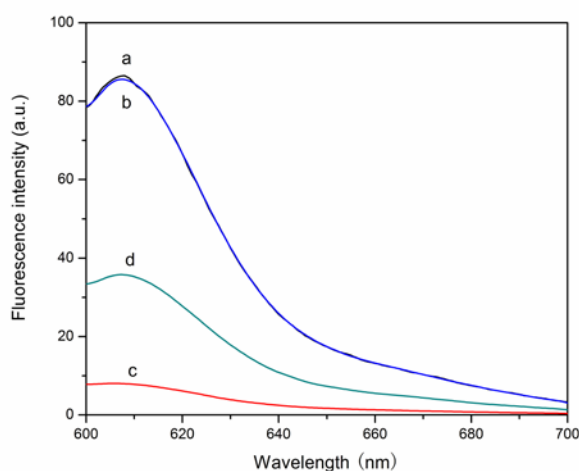


Figure 1. Fluorescence emission spectra of: (a) ssDNA; (b) ssDNA + Ag^+ ; (c) ssDNA + nano-graphite; (d) ssDNA + nano-graphite + Ag^+ . Excitation was at 580 nm and the emission intensity was monitored at 606 nm. All experiments were carried out in MOPS buffer (pH: 7.5) containing 50 mM of NaNO_3 and 100 nM of ssDNA. ($[\text{Ag}^+]$: 200 nM.)

Figure 1 shows the fluorescence emission spectra of ROX-labeled ssDNA probe under different conditions. Upon the presence of the fluorescein-based dye, ssDNA shows strong fluorescence emission at 606 nm (curve a). The fluorescence of ssDNA was slightly influenced by Ag^+ (curve b). However, the presence of nano-graphite results in about 87% fluorescence quenching after 20 min incubation in MOPS solution (curve c), indicating that nano-graphite can efficiently quench the fluorescence of ROX, and the observed quenching phenomenon may be largely originated from the electron or energy transfer between the fluorophore and nano-graphite. In the presence of 200 nM Ag^+ , the fluorescence intensity is about 4-fold higher than that without Ag^+ (curve d). The fluorescence enhancement is the result of the formation of dsDNA from ssDNA by Ag^+ , which detaches from nano-graphite and hampers the energy transfer between ROX and nano-graphite. We thus expect the fluorescence intensity changes of ssDNA/nano-graphite to provide a quantitative readout for Ag^+ .

To evaluate the sensitivity of this detection system, we collected emission spectra of ssDNA/nano-graphite in the presence of different concentrations of Ag^+ ranging from 0 to 400 nM, as shown in Fig 2. It is obvious that the fluorescence intensity of the mixture increases with the increase of Ag^+ concentration. The inset in Figure 2 shows the value of $F/F_0 - 1$ plotted against the concentration of Ag^+ , where F and F_0 are the fluorescence intensity of ssDNA/nano-graphite with and without Ag^+ , respectively. Even at concentration as low as 5 nM, the fluorescence intensity is distinct from that without Ag^+ . The detection limit is estimated to be 500 pM (three times the standard deviation of the blank solution), which is lower than the toxicity level of Ag^+ in drinking water (4.6×10^{-7} M) defined by U.S. Environmental Protection Agency (EPA) [1]. The result indicates this fluorescent sensor holds a promising potential for practical applications.

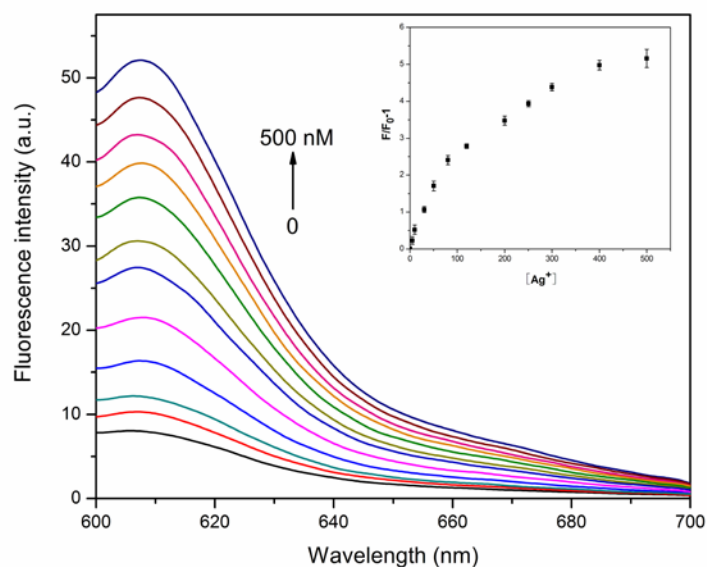


Figure 2. Fluorescence spectra of ssDNA/nano-graphite in the presence of different Ag^+ concentrations (from bottom to top: 0, 5, 10, 30, 50, 80, 120, 200, 250, 300, 400, 500 nM). Inset: Inset: $F/F_0 - 1$ value plotted against the concentration of Ag^+ with error bar (standard deviation from the mean, $n=3$), where F and F_0 are the fluorescence intensity of ssDNA/nano-graphite with and without Ag^+ , respectively.

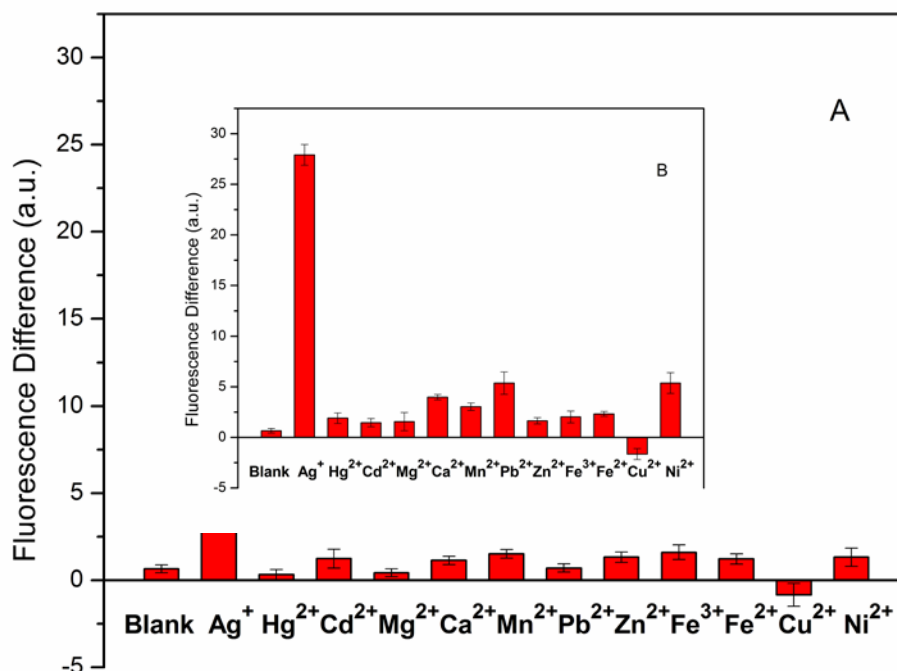


Figure 3. Fluorescence difference with error bars of ssDNA/nano-graphite between the blank and solutions containing different ions. Fluorescence difference = FL Intensity (ssDNA/nano-graphite + Metal ions) - FL Intensity(ssDNA/ nano-graphite). (A: [other metal ions]: 2 uM; B: [other metal ions]: 20 uM).

The selectivity of the present detection system for Ag^+ was also investigated. The enhanced fluorescence intensities were plotted against various environmentally relevant metal ions, including Hg^{2+} , Cd^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Pb^{2+} , Zn^{2+} , Fe^{3+} , Fe^{2+} , Cu^{2+} , Ni^{2+} were examined. Fig 3 A shows the fluorescence difference between the blank and solutions containing Ag^+ (200 nM) and other metal ions (2 uM). It can be seen that all the other metal ions presented negligible effects on the fluorescence of the ssDNA/ nano-graphite detection system. Furthermore, other metal ions at the concentration of 20 uM were also investigated (Fig. 4 B). Under such conditions, although the fluorescent sensing platform produces slightly bigger fluorescence differences in most cases than those observed at 2 uM, the use of Ag^+ still gives the best result. In all, these results clearly demonstrate that the ssDNA/nano-graphite system is highly selective toward Ag^+ over the other metal ions.

4. Conclusions

In conclusion, for the first time, we have demonstrated the successful use of nano-graphite as a cheap, effective fluorescent sensing platform for Ag^+ detection with high selectivity and sensitivity. This assay is based on the interaction between the target-induced conformational change of the ROX-ssDNA probe and nano-graphite quenching effects. We suggest that this assay offers several advantages. First, nano-graphite is cheaper and easier to produce than carbon nanotubes and grapheneoxide and nano-graphite can be easily synthesized with large quantities; Second, compared to SWCNT and GO sensing platforms, it exhibits a lower detection limit of 500 pM. We believe that this sensing platform will find great application in real environmental analysis.

Acknowledgments

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