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A “turn-off” fluorescent biosensor for the detection of mercury (II) based on graphite carbon nitride

Jingshuai Li, Huan Wang, Zhankui Guo, Yaoguang Wang, Hongmin Ma, Xiang Ren, Bin Du, Qin Wei*

Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China

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ABSTRACT

A novel and simple fluorescent biosensor has been realized through the fluorescence quenching of graphite carbon nitride (g-C_3N_4) for mercuric ion (Hg^{2+}) detection. In this assay, the g-C_3N_4 sheets which were functionalized with single-stranded DNA (ssDNA) aptamer showed strong fluorescence emission at 440 nm under the excitation of 380 nm in the absence of Hg^{2+}. When added to the assay solution, Hg^{2+} was embedded in the hairpin-shaped double-stranded DNA (dsDNA) due to the formation of the thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) complex, which made the Hg^{2+} close to the surface of g-C_3N_4 sheet. Therefore, the fluorescence of g-C_3N_4 was quenched. This sensor has good selectivity with a limit of detection as low as 0.17 nM under optimal conditions. The present work demonstrates that the g-C_3N_4-based fluorescent sensor has a promising application for detection of metal ions in real samples.

1. Introduction

As is known to all, heavy metal mercury exists in nature widely and plenty of evidences prove that it is harmful to human health. With the rapid development of industrialization, the pollution of heavy metal mercury to the environment, which mainly comes from industrial waste, is becoming more and more serious [1]. The Hg^{2+} ions, severe environmental pollutants, have serious medical effects because the heavy metal pollution can be accumulated through the food chain in the environment [2]. Moreover, it is hard to be removed but transferred. Thus the presence of mercury threatens human health seriously [3]. The maximum allowable Hg^{2+} levels regulated by the U.S. Environmental Protection Agency (EPA) are 2 ppb [4]. Therefore, it is necessary to develop and establish a method that can analyze trace levels of heavy metal ions. Currently, conventional methods for Hg^{2+} detection mainly include stripping voltammetry [5,6], mass-spectrography, plasma induced spectrum [7,8], atomic fluorescence spectrometry [9] and ultraviolet-visible spectrometry [10], etc. Although these methods have their own advantages, they also have some defects, such as sophisticated pretreatment of samples, expensive instruments operated by professionals and time-consuming. To overcome these shortcomings, several attempts have been made to establish better sensors for rapid and facile detection of Hg^{2+}, including electrochemical, colorimetric and fluorescent sensors [11–14].

Most of all, fluorescent approaches have been used for detection of trace Hg^{2+} with high selectivity and sensitivity due to the research progress of DNA probes [15]. So a new approach for Hg^{2+} detection was put forward. In this method, thymine-thymine (T-T) mispairs are introduced in g-C_3N_4-based fluorescent sensor [16]. T-T mispairs can specifically capture Hg^{2+} to form T-Hg^{2+}-T structure in DNA duplexes [17,18]. According to this approach, interference factors can be effectively eliminated.

Nowadays, g-C_3N_4, which is a chemically metal-free stable semiconductor, has been widely used as a new fluorophore because of its high mechanical strength, long-persistent luminescence [19] and good water solubility [20]. Besides, it also has been found that g-C_3N_4 can bind ssDNA via hydrophobic and π-π stacking interactions [21]. Therefore, g-C_3N_4 can serve as the superior sensing material.

In our study, a simple, effective, highly sensitive and selective g-C_3N_4-based fluorescent “on-off” sensor has been developed for Hg^{2+} detection without complicated operating steps and skills. Herein, g-C_3N_4 acts as a fluorophore and ssDNA are considered as the molecular recognition probes that can specifically combine with Hg^{2+} ions [22]. This newly-developed research method not only has a wide linear range but also exhibits a low detection limit for the determination of Hg^{2+}.

* Corresponding author.
E-mail address: sdjndxwq@163.com (Q. Wei).

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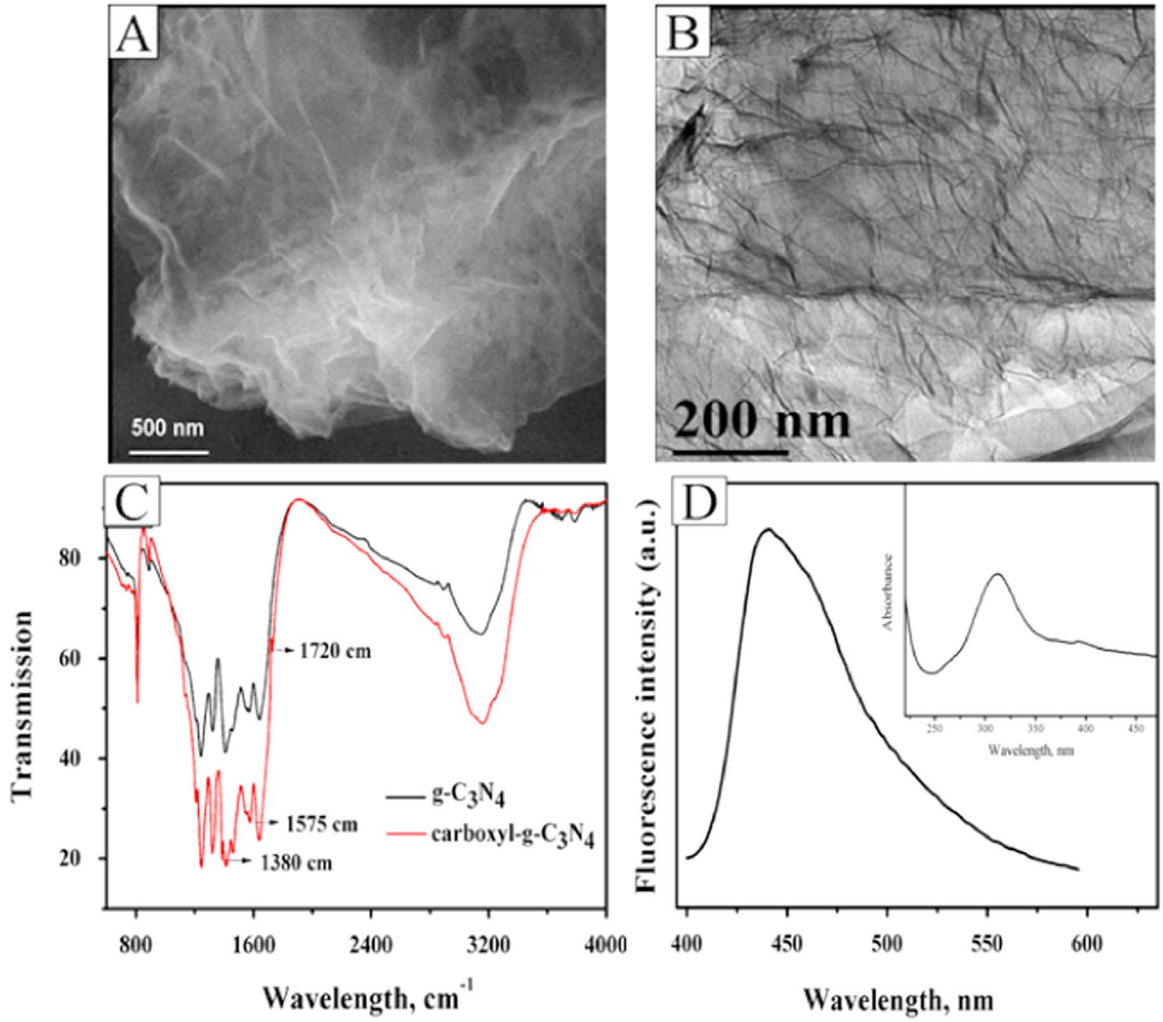

Fig. 1. SEM image of carboxylated g-C_3N_4 (A); TEM image of carboxylated g-C_3N_4 (B); FT-IR spectroscopy of carboxylated g-C_3N_4 (C) and fluorescence spectrum of the g-C_3N_4 (D) in the Tris solution (inset: UV–visible absorption spectrum). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Chemicals and materials

Melamine was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The Hg^{2+} specific-DNA aptamer (ssDNA) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China), and their base sequences in details were as follows: 5′-NH_2-TTCTTCCCCTTgTT-3′. $Fe(NO_3)_3{\cdot}9H_2O$, $Al(NO_3)_3{\cdot}9H_2O$ and $AgNO_3$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). $Mg(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $MnCl_2{\cdot}4H_2O$ and $BaCl_2{\cdot}2H_2O$ were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). $Cd(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were obtained from Sigma-Aldrich (St. Louis, MO). Diethanol amine (DEA) and Thioglycollic acid (TGA) were purchased from Aladin Ltd. (Shanghai, China). All the reagents and chemicals were of analytical grade without further purification. The reaction buffer solution (pH=7.6) containing 25 mM trihydroxymethyl aminomethane (Tris) and 150 mM NaCl was used throughout the experiment. The Hg^{2+} stock solution (20 mM) was prepared with ultrapure water. Deionized (D.I.) water produced by a Milli-Q Millipore system (Millipore Corp., Billerica, MA) was used for the preparation of all the solutions by standard methods.

2.2. Apparatus

Scanning electron microscope (SEM) images were obtained with a JSM-6700F microscope (JEOL, Japan). Transmission electron microscope (TEM) images were obtained from a Tecnai G220 TEM (FEI Company, USA). FT-IR spectra were collected using a FT-IR-410 infrared spectrometer (JASCO, Japan). Ultraviolet absorption spectra were obtained from a Lambda35 UV–Vis spectrophotometer (PerkinElmer, America). All the fluorescence measurements were performed on a LS-45/55 fluorescence/phosphorescence/light spectrophotometer (Perkin Elmer Company, America). The pH value was acquired on a PB-10 pH-meter (Beijing, China).

2.3. Preparation of carboxylated g-C_3N_4

The g-C_3N_4 nanosheet was synthesized according to the previous report with minor modifications [23]. In brief, g-C_3N_4 was prepared by heating 5.0 g melamine in alumina crucible to 500 °C and maintaining for 4 h in muffle. Then took out the crucible and let it cool to room temperature. The yellow powder was g-C_3N_4. In order to improve the solubility of g-C_3N_4 in water, the yellow product was treated with 5 M HNO_3 and refluxed for 24 h at 125 °C. The refluxed product was centrifuged and washed with ultrapure water. Finally, the ultimate product was dried in vacuum oven for 12 h at 35 °C.