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A ratiometric nanosensor based on lanthanide-functionalized attapulgite nanoparticle for rapid and sensitive detection of bacterial spore biomarker

Jun Xu [a, b], Xiaoke Shen [a], Lei Jia [a, b, *], Chuanxiang Zhang [a, b], Tieliang Ma [c], Tao Zhou [a], Taofeng Zhu [c, **], Zhouqing Xu [a], Jianliang Cao [a], Baozhong Liu [a], Ning Bi [a], Lianqi Liu [a], Yizhe Li [a]

[a] College of Chemistry and Chemical Engineering, Henan Polytechnic University, No. 2001 Shiji Road, Jiaozuo, Henan 454000, China
[b] Collaborative Innovation Center of Coal Work Safety, Henan Province, No. 2001 Shiji Road, Jiaozuo, Henan 454000, China
[c] The Affiliated Yixing Hospital of Jiangsu University, No.75, Tongzhenguan Road, Yixing, Jiangsu 214200, China

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ABSTRACT

Since the 2001 anthrax attacks, researchers have focused on the development of a bacterial spore biomarker detector with rapid response time and high sensitivity and selectivity. Hence, a ratiometric fluorescent nanosensor based on lanthanide-functionalized attapulgite (Atta) was designed and prepared using dye-doped Atta as an internal reference. Atta, which is famous because of Maya Blue, is a hydrated magnesium aluminum silicate present in nature, with pronounced adsorption and a large specific surface area rich in silicon hydroxyl. The adsorption of dye molecules benefits the formation of an internal reference with orange fluorescence. Meanwhile, silicon hydroxyl would be available for terbium (Tb^{3+}) complex grafting, which favors the sensitization of the bacterial spore biomarker. With increasing dipicolinic acid (DPA) concentrations, energy transfer from DPA to Tb^{3+} gradually enhanced, thereby resulting in strong and predominant green fluorescence. The ratiometric fluorescence intensity is highly sensitive. It has a limit of detection of 9.8 nM, which is much lower than the infectious dosage of *Bacillus anthracis* spores (60 μM) for humans. The rapid response time (10s), high selectivity, and reliable and practical performances in actual applications make this ratiometric fluorescent nanosensor useful in the detection of biomarkers for various bacterial spores. Moreover, the reported construction of visual ratiometric detection system follows the sustainable development idea of “from nature, for nature, into the nature” and promotes the use of naturally renewable resources to produce promising and eco-friendly functional materials for various applications.

1. Introduction

Anthrax is an acute disease and a potential biological warfare agent that infects both animals and humans. Inhalation of *Bacillus anthracis* leads to high mortality rates within 36 h after the onset of respiratory distress [1]. One of the key aspects of an efficient response to this attack is the development of a technology for its detection in various matrices with high sensitivity and selectivity. Dipicolinic acid (DPA) is a major and unique component of these bacterial spores, accounting for 5%–15% of the dry mass of the spore [2–4]. Thus, attempts have been made to detect bacillus spores by targeting DPA [5]. However, traditional medical methods usually require lengthy cycles, complicated operations, expensive reagents, and professional analysis, making them unsuitable for on-line monitoring. Rapid, sensitive, and straightforward detection of bacillus spores is crucial for disease and bioterrorism prevention [4]. Optical techniques for dipicolinic acid detection have attracted the interest of many researchers because of their low cost, fast response, and easy portability. Lanthanide ion-based luminescence is one of the most promising methods for the detection of DPA because of its large Stokes shift, narrow emission bands, and long

* Corresponding author. Henan Polytechnic University, #2001 Shiji Street, Jiaozuo, Henan 454000, China.
** Corresponding author. The Affiliated Yixing Hospital of Jiangsu University, #75 Tongzhenguan Road, Yixing, Jiangsu 214200, China.
E-mail addresses: jlxj@hpu.edu.cn (L. Jia), Staff1639@yxph.com (T. Zhu).

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fluorescence lifetime [6,7]. In previous reports, lanthanide ions (Ln^{3+}) were effective for DPA detection [8,9], but nonselective for interferents. Lanthanide-based nanocomposites had shown improved selectivity [4]. However, lanthanide-based "off-on" fluorescence nanosensors determined DPA concentrations through emission intensity variation, which was greatly influenced by excitation, environmental, and instrumental factors [10]. Sensing reliability can be improved by using a ratiometric fluorescent sensor, which measures the ratio between analyte and reference signals [11,12].

Therefore, a fluorescent reference with good thermal and chemical stability should be used. Maya Blue, a vividly colored organic–inorganic composite that was in use presumably more than 2000 years ago in the ancient Mayan culture [13,14], has attracted considerable attention because of its extraordinary stability. The ancient Maya Blue is recognized as a hybrid of attapulgite (Atta) and indigo [15–18]. Atta is a hydrated magnesium aluminum silicate present in nature as a fibrillar silicate clay mineral, with high adsorption properties and a large surface area. In contrast to other nano-sized synthetic supports, natural clay is very inexpensive and readily obtainable as China possesses it in large reserves. These factors make the Atta-doped fluorescent dye an optimal candidate as a reference. Meanwhile, the Atta clay's surface is rich in silicon hydroxyl, which is easily functional. Nanosensor selectivity for DPA can be improved by bonding with ethylenediaminetetraacetic acid dianhydride (EDTAA), which then chelates lanthanide ions [4]. EDTA can occupy six lanthanide coordination sites in an aqueous solution, leaving three adjacent sites available for DPA coordination. Based on the advantages afforded by its structural properties, Atta can be used as a platform for covalent grafting with terbium complex to produce a multicolor fluorescent nanosensor.

Hence, we design and assemble a novel Atta-based ratiometric fluorescent nanosensor for the biomarker of bacterial spores using dye-doped Atta as a reference (Fig. 1). Such a hybrid internal reference with an orange fluorescence shows the same structural features and stability of the famed Maya Blue. With increasing DPA concentrations, energy transfer from DPA to Tb^{3+} gradually enhanced, and the emission intensity of Tb^{3+} increased, thereby resulting in strong and predominant green fluorescence. Changes in the multicolored fluorescence can also be observed by the naked eye under UV light. This is a highly sensitive and selective strategy for the ratiometric fluorescent detection of bacterial spore biomarkers with a response time of 10 s. We believe that this ratiometric lanthanide-functionalized Atta-based strategy may be useful in the detection of various biomarkers of bacterial spores and biological molecules.

2. Experimental section

2.1. General

3-aminopropyltriethoxysilane (APTES, 99%), tetraethoxysilane (TEOS) and terbium nitrate ($Tb(NO_3)_3 \cdot 6H_2O$) were purchased from Adamas Reagent Co., Ltd (China). Kaiser test reagents were obtained according to the literature [19]. Attapulgite (Atta, Jiangsu Autobang Co., Ltd, China) was purified through suspending into $(NaPO_3)_6$ aqueous solutions, and then treating with HCl and H_2O_2 in order to remove the impurities and activate hydroxyl groups on its surface. All the other chemical reagents were of analytical grade and purchased from China National Medicines Co., Ltd (China).

Transmission electron microscopy (TEM) was determined by a Tecnai-G2-F30 at acceleration voltages of 300 kV. The UV-vis absorption spectra were performed on a Perkin Elmer Lambda 950 spectrophotometer. Nitrogen (N_2) adsorption-desorption isotherms were carried out by an ASAP 2010 analyzer with nitrogen. Powder X-ray diffraction patterns (PXRD) were obtained by Rigaku-Dmax 2400 diffractometer using Cu Kα radiation over the 2θ range of 5-60°. FTIR spectra of the materials were determined using a Bruker V70 FTIR spectrometer within the 4000–400 cm^{-1} wavenumber range with the KBr pellet technique. The content of Tb^{3+} ion was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using an IRIS Advantage ER/S spectrophotometer. The luminescence spectra were measured on a Varian CARY Eclipse fluorescence spectrometer.

2.2. Preparation of dye doped atta

Rhodamine B (RhB) was employed to be an internal dye molecule. The Atta–RhB pigment can be prepared according to the

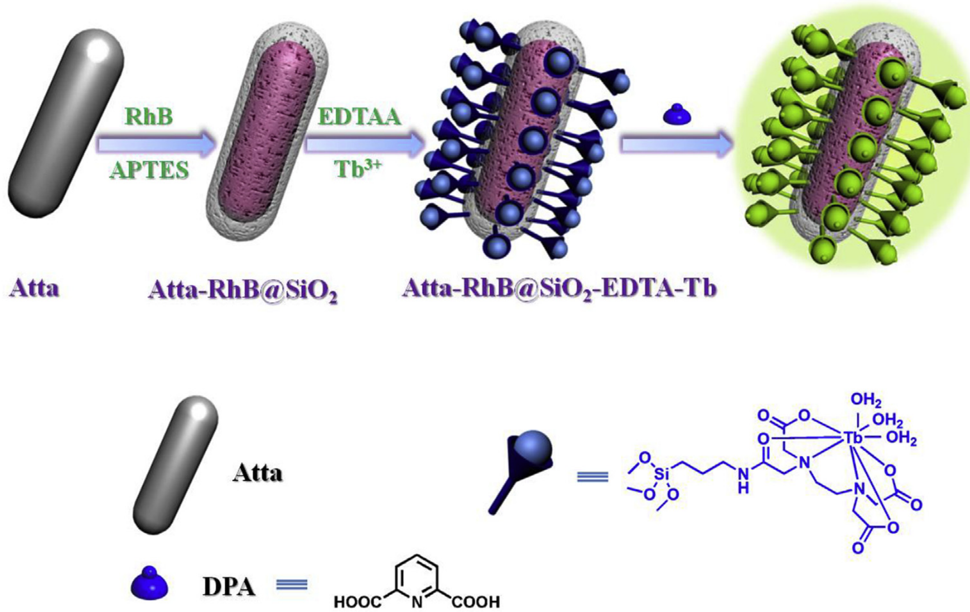

Fig. 1. Schematic diagram for fabrication and sensing process for DPA of Atta–RhB@SiO_2–EDTA–Tb nanosensor.