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Amplified colorimetric sensor for detecting radon by its daughter lead based on the free-fixed auto-assembly structure of Duplex-hemin/G-quadruplex

Guiying Yang, Chunli Song, Qiwei Shi, Hongwen Liu, Shiya Li, Ran Liu, Shimeng Liu, Changyin Lv*

College of Public Health, University of South China, Hengyang, 421001, PR China

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ABSTRACT

A novel signal amplification strategy based on a Pb^{2+}-dependent DNAzyme is proposed for detecting Pb^{2+} and radon through Pb^{2+}-induced DNAzyme cleavage and the auto-assembly of a duplex-hemin/G-quadruplex with two loop-stem structures and enzyme-mimicking catalytic activity. First, the Pb^{2+}-specific DNAzyme cleaves a primer sequence, which induces assembly of the hairpin probe Hp1/Hp2 into a double helix structure. Subsequently, a G-quadruplex forms after the insertion of hemin into the free G-rich sequences; this G-quadruplex possesses the catalytic activity of horseradish peroxidase and changes colorless TMB to its deeply colored oxidized state. lead-dependent DNAzymes were constantly sheared by Pb^{2+}, and the free primer strands were continuously assembled into double chains by hybridization with hairpin probes, providing amplification for the detection of lead (II) and radon. Under the optimum conditions, there was a good linear relationship between ΔA and the lead concentration for Pb^{2+} concentrations ranging from 2.58 to 18 nM, and the detection limit was 0.77 nM. Analysis of actual samples indicated that when the radon concentration was in the range of $5.41 \times 10^{3-}$ 1.65×10^{5} Bq·h/m^3, the radon concentration and the absorbance were linearly correlated with a detection limit of 1.62×10^{3} Bq·h/m^3. During the process of radon sampling and detection, radiation damage from the radioactive gas radon can be avoided.

In this study, the primer dissociated by the DNAzyme was in a free state. Compared with the fixed-state primer chain, this system will be more convenient for the biological analysis of ultratrace metal ions. Therefore, this strategy has good application prospects for biosensors and is expected to become a novel platform for the amplification and detection of metal ion signals.

1. Introduction

Radon is the product of radium decay. This colorless, tasteless, and odorless radioactive inert gas can emit α-rays and β-rays upon decay [1]. Radon, which is a major indoor radioactive gas pollutant that is commonly found in our living environments and workplaces, mainly originates from building materials, decoration materials, water, fuel, etc. [2]. Radon is one of the 19 carcinogens identified by the World Health Organization, and exposure to high levels of radon may cause lung cancer [3], leukemia, infertility, fetal malformation and genetic abnormalities, and other diseases.

At present, physical methods are the main means of detecting radon radiation [4]. With the development of detection technologies, some new methods for detecting radon itself or radon radiation have been reported, such as gas chromatography, spectrophotometry, electrochemical methods, and Monte Carlo computer simulations. However, most of these methods require the use of detection instruments, which have low detection sensitivity and are difficult to operate, time consuming, and susceptible to strong interference from environmental factors such as temperature and humidity. Additionally, during the detection process, operators are exposed directly to the radioactive environment. To overcome the shortcomings of those methods, a new detection strategy is proposed in our research. Radon (^{222}Rn) that emits α- and β-rays is transformed into the daughter lead (^{210}Pb), and we achieved rapid and accurate chemical detection of radon by detecting the daughter lead. Based on the research foundation that

* Corresponding author.
E-mail address: Lchy1955@163.com (C. Lv).

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lead-induced conformational change of aptamer can interact with fluorescent dye to achieve radon detection [5], It's feasible to determine radon through detecting lead, but these methods are only true first order reactions, it's hard to achieve amplification [6]. And we also utilized gold nanoparticles as signal probe to detect radon [7], while the aggregation of AuNPs is not stable enough.

In recent years, there are many meaningful strategies for detecting metal ions, such as nanotechnology, signal amplification strategy, DNAzyme and aptamer. Zhou [8] synthesized a water-soluble GPPCN nanostructure which was utilized as a photoluminescence probe for detecting Fe^{3+} with FRET. Signal amplification strategy has been widely used to improve the detection sensitivity of biosensors [9]. DNAzyme [10] is a special functional nucleic acid with specific recognition ability for metal ions that can shear complementary chains at their specific position. This feature is widely applied to biosensors research of metal ions [11], such as Pb^{2+} [12], Cu^{2+} [13], Hg^{2+} [14], and UO^{2+} [15]. G-quadruplex, a special structure of DNA chains with a variety of spatial structures and chemical properties, can be designed for different signal output strategies and interact with a variety of small molecules, metal ions and proteins [16]. Hemin/G-quadruplex complexes were used as catalyzer and steric hindrance effector simultaneously for a multiple-mechanism electrochemiluminescent sensor, which greatly increased its application in biosensors [17]. Therefore, it is extensively applied for colorimetric sensing [18], fluorescence [19], electrochemiluminescent [17], and wireless sensing. Ting Fu [20] reported that, after Pb^{2+} specific splicing of the GR-5 enzyme, the combination of molecular beacons with zinc protoporphyrin lead to the formation of a fluorescent signal-enhanced G-quadruplex; the authors applied G-quadruplex/hemin [21] with horseradish peroxidase activity to a signal amplification system and studied the detection of Ag^{+}, uric acid and small-molecule DNA. Currently, the reported methods for signal amplification typically involved fixed primerämplification.

In this paper, a new strategy for signal amplification is proposed, which is established by using the free primer chainto induce the hairpin probe to form a double helix structure. Although results for the determination of lead and radioactive gas radon are good, there are no related reports to date. We designed a lead-specific DNAzyme by ensuring that the active center of the GR-5 enzyme, lead ions or daughter lead was used to trigger an enzyme-mimicking reaction when the substrate chain was cut and the primer chain was cut off in solution. Then, under the presence of the probes Hp1 and Hp2 with a G-rich sequence at the end of the stem ring, the complexes of duplex-hemin/G-quadruplex being formed to trigger the chromogenic reaction. Thus, we established a novel method for the quantitative detection of both Pb^{2+} and accumulated radon.

2. Experimental

2.1. Reagents

The specific lead (II) DNAzyme substrate chain, polymerase chain, and hairpin probes Hp1 / Hp2 designed for this study were synthesized by Bioengineering (Shanghai) Co., Ltd. and were purified by high-performance liquid chromatography (HPLC) and denatured polyacrylamide gel electrophorEAs (ULTRAPAGE). Their base sequences are shown in Table 1. Standard lead solution (100.0 μg/mL) was purchased from the Chinese Academy of Metrology and diluted to the proper concentration with ultrapure water before use. Tri (hydroxymethyl) aminomethane (Tris) was purchased from Shanghai Aladdin Biochemistry Technology Co., Ltd. Chlorhematin (hemin) and 3,3′,5,5′-tetramethyl benzidine (TMB) were purchased from Shanghai Sigma Aldrich Trading Co., Ltd.

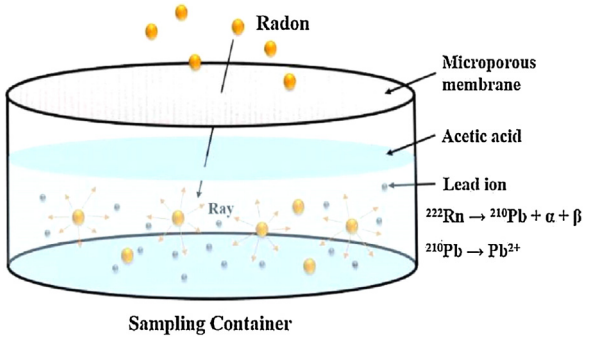

Fig. 1. Sampling apparatus.

Glacial acetic acid (HAc) was purchased from Tianjin Fuchen Chemical Reagent Factory. Hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were purchased from Guangdong Guanghua Technology Co., Ltd. Mixed cellulose membranes were purchased from Merck Millipore. The water used in experiments was sterilized ultrapure water (resistivity > 18.25 MΩ cm @ 25°) obtained from an ultrapure water apparatus (Hunan Zhongwo Water Environmental Protection Technology).

2.2. Apparatus

This research was carried out in a radon chamber at South China University's Institute of Nuclear Industry No. 6. UV–vis absorption spectra were measured on a UV-2550 ultraviolet-visible spectrophotometer (Japan, Shimatsu). Circular dichroism measurements were conducted at a scanning speed of 200 nm/min in a J-1500 circular dichroism spectrometer (Japan, Spectroscopic). The pH of the buffer solution was checked by a PB-20 (PB-S) Precision Acidity meter (Germany, Sedorius). Gel electrophoresis images were obtained on a gel image analysis system (USA, Kodak). Polyacrylamide gel electrophoresis was performed on a DYY-6 L electrophoresis apparatus (China, Beijing 61 Company). The RAD7 electronic radon detector (Durridge, America) was used for radon determination. The reaction temperature was controlled precisely by a MS-100 THERMO-SHAKER (Hangzhou, Aosheng).

Radon is a radioactive gas which can decay α and β particles, with the characteristics of soluble, adsorptive and diffusible, often causes respiratory system diseases. For safety, under the process of sampling, we should pay attention to: Following the operate rules strictly in the radon chamber; Don't touch the radon seed bare-hands; Wearing the special radiation protective clothing and breathing mask when entering the radon chamber environment.

2.3. Sample preparation

We added 10 mL of 0.2% HAc to a series of disposable plastic Petri dishes (70 mm diameter and 17 mm height), as shown in Fig. 1, which were covered with 0.8 μm pore-size mixed cellulose membranes to prevent lead-containing aerosols from entering the dishes. In addition, sampling times of 1 h, 2 h, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, 28 h and 32 h were used, the sampling dish was placed in the radon chamber, and the radon samples were passively collected. Then, the tubes were sealed at room temperature for four days, which is longer than the half-life of radon, and 0.2% HAc was used to obtain a final volume of 10 mL. Afterwards, the sample was fully mixed and then stored in a 4° refrigerator before measurement.