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Detection of radon with biosensors based on the lead(II)-induced conformational change of aptamer HTG and malachite green fluorescence probe

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ABSTRACT

The aim of this paper is to assemble a new biosensor for detecting the accumulated radon dose in the environment to achieve rapid monitor of radon. Based on the correlation between radon and its stable decay daughter ^{210}Pb, a biosensor using the lead-induced specific aptamer HTG conformational changes, and the organic dye malachite green (MG) as a fluorescent probe was assembled. In these studies, we explored a novel, sensitive, label-free, fluorescence biosensing method for the detection of both radon and lead. The fluorescence intensity difference has a linear relationship with Pb^{2+} and the accumulated radon concentration from 6.87×10^3 Bq·h/m^3 to 3.49×10^5 Bq·h/m^3. The lead and radon detection limits of this method are 6.7 nmol/L and 2.06×10^3 Bq·h/m^3, respectively. The student's t-test results indicated that the new method was reliable and stable. The detection method is sensitive, accurate, easy to operate, has a wide linear range and is highly selective. In the sampling and determination processes of radon, the radiation harm to human health can be effectively avoided.

1. Introduction

Radon is a colourless, tasteless, odourless, radioactive inert gas, which is known as an “invisible killer”. Radon includes three isotopes of ^{222}Rn, ^{220}Rn, and ^{219}Rn, which are derived from the uranium series, thorium series and actinides, respectively. The common, hazardous radon refers to the ^{222}Rn isotope (having a half-life of 3.825 years) is generated from a long-life radionuclide Ra($T_{1/2}$ = 1600a) in the uranium series. After the decay, a series of nuclides are produced, and the stable isotope ^{210}Pb is formed as the final step(Cheng, 2008). Radon exists in rocks and soil under buildings, decorative marble, water sources, natural gas and coal combustion products, etc(Noh et al., 2016; Chang, 2002). The harm to human health from radon radiation accounts for more than 55% of the total radiation harm in one's life(Zhang et al., 2011). Radon has a high penetration performance, can be intercepted by the respiratory system and is deposited in the lungs (Torres-Durán et al., 2014, Giri and Pant, 2018). Hence, radon and its decay daughters are believed to be the second leading risk factor for lung cancer. The WHO ranks radon as one of the nineteen environmental carcinogens(IPCS, 1999), and the international cancer organization (IARC) designates radon as a first class carcinogenic factor (USEPA, 2016). Additionally, radon and its decay daughters can penetrate the respiratory mucosa and the blood-air barrier and spread through different body tissues, resulting in radiation harm to organs and blood. In particular, radon can be accumulated in the fat cells of bone marrow for a long time, causing α and β radiation harm to haematopoietic stem cells(Zhang et al., 2011). Consequently, skin cancer, leukaemia, functional damage to the central nervous system, and oesophageal cancer can develop(Salgado-Espinosa et al., 2015). With the development of the nuclear industry, the probability of nuclear accidents has increased, making the public pay more attention to the detection and monitoring of radioactive substances, such as radon. Therefore, it is of great significance to determine the concentration of radon in the environment.

In the decay process, radon radiates alpha- and beta-rays. Typically, physical methods based on the intensity of radiation and chemical methods based on the response of reagents to radiation are applied to detect radon. However, the physical methods are ineffective to detect the cumulative radiation amount, and the chemical methods are ineffective to detect radon at low concentrations. The traditional physical methods are divided into three categories: instant sampling methods (Ding et al., 2009) such as the double-filter film method(Wu et al., 2011), the scintillation method(Liu and Li, 2011), the balloon method (Zhu et al., 2014) and the activated carbon adsorption methods(Fu and

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Liu, 2007); cumulative sampling methods such as the activated carbon spectroscopy(Wang et al., 2015) and the solid track method(Zhao et al., 2008); and continuous measurement methods such as the electrostatic-collection-type continuous radon monitor(Song et al., 2002). These methods have a series of limitations including expensive instruments, high detection cost, harm to human health, low sensitivity, many interference factors, and so forth. To date, some new radon determination approaches have been developed, such as gas chromatography, photometric analysis, electrochemical analysis, Monte Carlo computer simulation, and others. These methods have new features, but it is still necessary and challengeable to improve existing radon detection technologies in the field of new analytical methods research.

Lead is a common environmental contaminant(Zhang et al., 2012). In recent years, the nucleic acid adaptation theory for lead detection has been well developed. An aptamer biosensor employs aptamer to specifically recognize target molecules and produce response signals. Combining the selectivity of aptamers to target molecules with traditional instrumental analysis methods has allowed for the emergence of new biochemical detection methods(Meng et al., 2016; Saidur et al., 2017). Compared to traditional antibodies, aptamers have the advantages of simple synthesis in vitro, low cost, no animal or cellular experiments, high thermal stability, high affinity, high specificity, and low toxicity(Wu and Kwon, 2016). Resulting from electrostatic interactions, hydrogen bonding, stacking effects, and shape matching between the aptamer and target molecule, the aptamer will form a specific secondary structure from a random coiling state into a stable spatial structure(Debnath et al., 2017). This change can be verified from the circular dichroism spectra (CD spectra)(Zhang et al., 2008). The affinity to organic dye is related to the type of spatial structure, and the changes in the response signals are related with the analyte concentration (Nguyen et al., 2017).

Based on the decay of ^{222}Rn to produce lead, we proposed a new label-free fluorescence biosensing method to detect radon based on its decay daughter lead(Deng et al., 2016; Long et al., 2016). The lead ions induce the aptamer T30695 to form a stable G-quadruplex, and hence, the single strand T30695 can no longer form a double helix structure with its complementary strand. Therefore, the positively charged fluorescent dye PG could not insert itself into the double strand plane and the minor groove of the double helix, producing a weaker fluorescence. Meanwhile, the fluorescent dye PG is added, and the single-strand T30695, which not affected by the lead ions, is combined with its complementary strand to form a double helix structure, producing a strong fluorescence.

Malachite green (MG) is a green crystalline triphenylmethane dye with a metallic lustre. Bhasikuttan et al. (2007) found that the interaction between malachite green and the G-quadruplex could produce fluorescence. Kong et al. (2009) believed that crystal violet could distinguish the anti-parallel and parallel structures in the G-quadruplex, and triphenylmethane dyes could serve as the fluorescence probe for the detection of G-quadruplex. Guo et al. (2009) showed that the energy transfer fluorescence spectroscopy of malachite green could identify the intramolecular and intermolecular G-quadruplex and identify single- and double-strand DNA. Ryoko et al.(Uda et al., 2017) reported that malachite green could preferentially combine with G-quadruplex in the form of terminal stacking or in situ insertion, and the combination could produce a strong fluorescence.

Based on the specific recognition ability of the aptamer and the fluorescence produced by the combination of malachite green and the anti-parallel G-quadruplex, we constructed a new fluorescence biosensor platform to detect radon and its decay daughter, lead.

2. Materials and methods

2.1. Equipment and reagents

This study was conducted in a radon chamber at the Sixth Research Institute of Nuclear Industry. The radon concentration was determined using a RAD7 electronic radon detector (Durridge, USA). The experiments utilized a Thermo Fisher Lumina fluorescence spectrophotometer (Thermo Fisher Scientific Incorporation, USA) with a scanning speed of 1200 nm/min. The slits for excitation and emission wavelengths were 10 nm and 10 nm, respectively. We also used a circular dichroism JASCO-815 instrument (JASCO Corporation, Japan) with a scanning speed of 100 nm/min and scanning range of 230–350 nm.

PAGE-purified oligonucleotide: HTG (5′-AGGGTTAGGGTTAGGGTTAGGG-3′) diluted with ultrapure water to a concentration of 100 μM was stored at 4 °C. The organic dye malachite green was diluted with ultrapure water to a concentration of 0.5 mM and stored at 4 °C. The standard lead solution used was diluted with ultrapure water to a concentration of 100 mg/L of Pb^{2+} in the working solution. Trisaminomethane (Tris) with a purity of at least 99.9% was used to form a Tris-HAc buffer for the radon samples and a pH of 6.5. All reagents were diluted with ultrapure water with a resistivity of 18.25 MΩ cm.

2.2. Sampling method of the decay daughter lead

10 mL of 0.2% acetic acid was placed in a petri dish, which was then covered with a mixed-cellulose microporous membrane to prohibit airborne lead-containing pollutants into the petri dish. The schematic diagram of chemical sampling radon is shown in Fig. 1. In addition, sampling times of 2, 4, 8, 12, 20, 24, 42, 60, 72 and 84 h were set. The petri dish was placed in the radon chamber for accumulative irradiation, and the lid was removed. Then, the petri dish was placed at room temperature for more than three and a half days, which is longer than the half-life of radon. The decay daughter lead was absorbed with the dilute acetic acid and was converted to lead ions. After the sampling process, the sample was diluted with the 0.2% acetic acid until the total volume reached 10 mL. The details of operating standard are shown in Table S1(See Supplementary material).

2.3. Fluorescence detection method for standard Pb^{2+} solutions

Two-millilitre EP tubes were used to contain 5 mM of Tris-HAc (trisaminomethane-acetic acid buffer solution, pH = 6.5), a certain amount of standard Pb^{2+} solution, and 0.6 μM of aptamer HTG. After incubation at 37 °C for 90 min, 3.0 μM of malachite green was added, and the total volume was increased using ultra-pure water to 200 μL. The incubation of these sample solutions continued for another 10 min. Meanwhile, the corresponding control group was prepared. The fluorescence intensity F values of the sample solutions and F_0 values of the blank solutions identified an excitation wavelength of 645 nm and an emission wavelength of 680 nm. Then, the fluorescence intensity difference was calculated as $\Delta F = F - F_0$.

2.4. Fluorescence detection method for samples after radon radiation

Likewise, the decay daughter lead was absorbed with 0.2% acetic

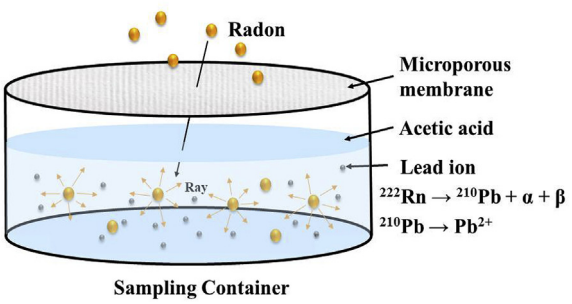

Fig. 1. Schematic diagram of chemical sampling radon.