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A carbon nanotubes based fluorescent aptasensor for highly sensitive detection of adenosine deaminase activity and inhibitor screening in natural extracts

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ABSTRACT

A carbon nanotubes (CNTs) based fluorescent aptasensor was developed for adenosine deaminase (ADA) activity detection and inhibitor screening by using adenosine (AD) as the substrate. This sensing system consists of CNTs, AD, split anti-AD aptamer fragment and dye-labeled aptamer fragment. In the absence of ADA, two aptamer fragments bind simultaneously with AD to form an AD-aptamer complex. This AD-aptamer complex cannot adsorb onto CNTs, and has high fluorescence intensity. When ADA is introduced into this system, ADA can convert AD into inosine, which has not affinity to the split anti-AD aptamer fragment. Thus, the split anti-AD aptamer fragments were adsorbed onto CNTs via strong π–π stacking interactions, resulting in the quenching of the fluorescence of the dye-labeled aptamer fragment. The proposed aptasensor can detect ADA activity from 0.005 to 0.2 U/mL with a low detection limit of 0.002 U/mL. Moreover, it has been also demonstrated that this CNTs-based fluorescence aptasensor is suitable for ADA inhibitor screening from traditional Chinese medicine (TCM). Considering the superior sensitivity and specificity, the proposed CNTs-based fluorescent aptasensor can be expected to provide a simple, cost-effective and sensitive platform for the detection of ADA activity and screening of potential drugs.

1. Introduction

Adenosine deaminase (ADA), a key hydrolytic enzyme for purine metabolism, can catalyze the conversion of adenosine (AD) to inosine by the removal of an amino group [1]. It plays a key role in the differentiation and maturation of the lymphoid system. Moreover, accumulating evidence indicate that the dysfunction of ADA in human body is closely related to a number of important diseases, such as tuberculosis, sarcoidosis, cancer and severe combined immunodeficiency (SCID) [2,3]. The significance of ADA in pathology makes it an important target for drug development and diseases detection. Traditional methods including the measuring ammonia produced [4], high-performance liquid chromatography (HPLC) [5], and colorimetric assay [6] have been described to be effective for monitoring ADA activity. Although each method has its advantages, many reported techniques still suffer the drawbacks, such as time-intensive, laborious, and low sensitivity. Thus, the search for simple and sensitive assay of ADA is ongoing.

With rapid development in the field of DNA biotechnology, the oligonucleotides have emerged as attractive recognition units for monitoring enzymes activities. A series of DNA-based probes have been developed for sensitive activity assays of various enzymes, such as DNA methyltransferases [7], endonucleases [8], RNase H [9] and DNA ligase [10]. In recent years, the aptamers have received tremendous attention in sensing applications because of their relative ease of isolation and modification, high affinity and specificity toward targets, and resistance against denaturation [11]. Up to now, several methods based on the use of aptamers as recognition units have been developed for the quantitative determination of ADA activity. These include the electrochemical aptasensor [12], colorimetric aptasensor [13] and fluorescence sensor [14]. Although these techniques can be quite powerful, a simpler and more sensitive method for ADA detection is still required.

The carbon nanomaterials have a significant role to play in new developments in each of the biosensor size domains. This significance arises as nanomaterials can help address some of the key issues in the development of all biosensors [15]. For

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example, a series of graphene oxide-based aptasensors have been developed for diverse biomolecules detection [16–18], the carbon nanoparticles and carbon nanodots have also extensively been used to develop biosensors [19,20]. Recently, carbon nanotubes (CNTs) have been extensively studied due to their unique optoelectronic properties and excellent biocompatibility [19]. Specifically, the extraordinary fluorescence quenching property of CNTs has been employed to develop nanosensors for diverse biomolecules in homogeneous solution. For example, the CNTs has used as a biosensing platform for the detection of DNA based on the quenching effect on the dye-labeled DNA probe [21]. Similarly, the CNTs-based aptasensors were also used for sensitive detection of various biomolecules [22,23]. However, to the best of our knowledge, no study has been reported the use of CNTs for homogeneous assay of ADA assay.

In the present work, we developed a sensitive and selective fluorescent aptasensor based on multi-walled carbon nanotubes (MWCNTs) using AD as the substrate for ADA activity detection and inhibitor screening. This aptasensor relies on the high fluorescence quenching property of MWCNTs and the different interaction ability of aptamer, AD-aptamer complex with MWCNTs. Compared with the traditional fluorescence resonance energy transfer (FRET)-based aptasensor [14], the proposed assay only requires the labeling of the oligonucleotide probe with one dye, which is very simple and cost-effective. Moreover, this aptasensor exhibits high sensitivity and specificity toward ADA over other non-specific enzymes, with a detection limit of 0.002 U/mL for ADA. In addition, the suitability of this MWCNT-based fluorescence aptasensor for ADA inhibitor screening from TCM has also been demonstrated.

2. Experiment

2.1. Chemical and reagents

The adenosine specific aptamer was splited into two single-stranded oligonucleotides (Apt-1 and Apt-2) [24], and their sequences were shown below: Apt-1: 5′-ACCTGGGGGAGTAT-3′; and Apt-2: 5′-ATGCGGAGGAAGGT-3′. The Apt-1 was modified at 5′-ends with 6-carboxyfluo rescein (FAM). And these aptamers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, Shanghai, China) and purified using high performance liquid chromatography. Multi-walled carbon nanotubes (MWCNTs) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, Guangdong, China). Adenosine, adenosine deaminase and inosine were purchased from Amresco Co., Ltd. (Solon, OH, USA). Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, DE, USA). Traditional Chinese medicine (TCM) was obtained from Guilin Pharmaceuticals Group of China (Guilin, Guangxi, China). Milli-Q water (18.2 MΩ cm) was used throughout this work. All other reagents used in this work were of analytical grade.

2.2. Pretreatment of MWCNTs

The commercial MWCNTs were purified and oxidized according to the procedure described by He and Bayachou [25]. The MWCNTs (200 mg) was first refluxed in HNO_3 (150 mL, 2.0 mol/L) for two days. After being kept overnight, the suspension was centrifuged at 14,000 rpm for 30 min, and the clear solution was removed. The purified precipitates were further oxidized by 40 mL of HNO_3/H_2SO_4 ($V_{HNO_3} : V_{H_2SO_4} = 1 : 3$) solution in an ultrasonic bath for 4 h. Then, the suspension was diluted 10-fold with water and allowed to stand for 12 h at room temperature. After removal of the clear solution over the precipitates, the remaining suspension was filtered through a 0.45 μm filtration membrane and then washed with water to obtain a neutral pH. The resulting precipitates were dried in a vacuum drier about 12 h to remove the water. The obtained MWCNTs with a carboxyl group were re-dispersed in water to give a final concentration at 5 mg/mL.

2.3. Preparation of natural extracts

Air-dried TCM was ground into a fine powder with a pulverizer. 5.0 g of powder was extracted with 50 mL of isopropanol in an ultrasonic cleaning bath for 0.5 h at 60 °C, and this process was repeated three times. The solvent was evaporated using rotary evaporator at 40 °C. Finally, these extracts were dried in vacuum drier for about 5.0 h. The natural extracts were dissolved with 2-propanol aqueous solution giving a final concentration of 5.0 mg/mL. Other natural extracts were prepared in the same way.

2.4. ADA activity detection

The Apt-1 (10 μL, 1 μM) was mixed with Apt-2 (10 μL, 1 μM), and then 10 μL of 5 mg/mL MWCNTs was added into the above solution in Tris–HCl buffer (10 mM, pH = 7.4), and allowed to incubate for 15 min. The fixed concentration of adenosine (1.0 μM) was treated with different activity of ADA (0, 0.002, 0.005, 0.01, 0.015, 0.05, 0.1, 0.15, 0.20, and 0.30 U/mL), and the resulting solution was added to the aptasensor/MWCNTs complex solution in Tris–HCl buffer (10 mM, pH = 7.4). After 50 min of incubation at 37 °C, the fluorescence emission spectra the solution were measured with a LS-55 spectrofluorometer (Perkin-Elmer, USA) at 520 nm with excitation at 480 nm. All experiments were repeated three times. Each sample was measured five times.

2.5. Inhibition screening procedures

For comparison of the inhibition ability of test compounds, different concentration EHNA or 0.20 mg/mL of each TCM was mixed with 1.0 μM adenosine solution before the addition of 0.20 U/mL ADA, and incubated 50 min at 37 °C. Other assay steps were the same as that of the ADA activity assay.

3. Results and discussion

3.1. Assay principle

Fig. 1 depicts the principle of the MWCNTs-based fluorescence aptasensor for ADA activity detection and inhibitor screening. This sensing system consists of two anti-AD aptamer fragments (FAM-labeled Apt-1 and Apt-2), AD and MWCNTs. In the absence of ADA, AD binds with both FAM-labeled Apt-1 and Apt-2 to form the AD/aptamer complex that cannot bind to MWCNTs stably. In this case, the FAM dye exhibits high background fluorescence (Fig. 1a). When ADA is introduced into the system, ADA can convert AD into inosine, which has not affinity with two anti-AD aptamer fragments. Then, the free anti-AD aptamer fragments can be adsorbed onto MWCNTs by means of strong π–π stacking interactions between nucleotide bases and the MWCNT sidewalls [21], resulting in the quenching of the fluorescence of the FAM dye (Fig. 1b). The fluorescence intensity should decrease with increased amount of ADA. In addition, since the conversion of AD into inosine by the ADA-catalyzed is restrained in the presence of inhibitors, the proposed aptasensor can be adapted to screen the inhibitors of ADA (Fig. 1c).

3.2. Feasibility study

To investigate the feasibility of the proposed sensing strategy, the fluorescence spectra under different conditions were measured,