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A fluorescent biosensor based on molybdenum disulfide nanosheets and protein aptamer for sensitive detection of carcinoembryonic antigen

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ABSTRACT

Simple, rapid, sensitive detection of tumor biomarker carcinoembryonic antigen (CEA) is of great importance for the screening, diagnosis and prognosis evaluation of various gastroenteric tumor. This paper presents a "turn-on" fluorescent biosensor based on molybdenum disulfide (MoS_2) nanosheets and fluorophore labeled protein aptamer for rapid and sensitive detection of CEA protein. CEA aptamer probe can be adsorbed on the surface of MoS_2 nanosheets in close proximity via van der Waals force, triggering fluorescence resonance energy transfer, and consequently fluorescence signal of aptamer probe was quenched. While in the presence of CEA protein, the fluorescence signal was recovered because aptamer probe could detach from MoS_2 nanosheets with binding-induce conformation change. MoS_2 nanosheets with high quenching efficiency combined with well discrimination ability between aptamer and aptamer/protein afforded the biosensor easy construction, fast detection and high sensitivity. The sensing platform also exhibited good reproducibility, selectivity and showed high sensitivity for CEA protein in a broad range of 100 pg/mL–100 ng/mL with the detection limit of 34 pg/mL. The aptamer-MoS_2 based fluorescent biosensor may be an ideal mode for protein detection in clinical sample, pesticide detection and environmental monitoring.

1. Introduction

Cancer is a major worldwide public health problem and the second leading cause of death globally [1]. The cure rate is very low in the middle and late stage, however, it is high at the early stage [2–4]. Therefore, early detection and treatment are significantly important strategy for conquering cancer. Highly sensitive detection of tumor markers may dramatically improve the accuracy rate of cancer early diagnosis. Carcinoembryonic antigen (CEA) is one of the most common tumor biomarkers in clinic, which used for screening, diagnosis and prognosis evaluation of various gastroenteric tumor including colorectal cancer, Esophageal cancer, gastric carcinoma, pancreatic carcinoma [5–8]. So rapid and sensitive detection of CEA is considerably essential for cancer diagnosis and treatment.

At present, the clinical methods of CEA protein detection mainly include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and chemiluminescent immunoassay (CLIA). The electrochemical [9], photochemical [10] and fluorescence analysis method were employed for the development of ultrasensitive CEA immunosensor with excellent sensing properties. However, anti-CEA antibody used in immunoassay has the nature of relatively high cost and easy denaturation. Aptamers as promising alternative of antibody are attractive for the construction of biosensor due to their good stability, ease of synthesis and modification, low cost, fast tissue penetration and low toxicity [11,12]. Therefore, it is highly desirable to develop aptamer-based biosensors for tumor biomarkers detection with easy construction, high sensitivity and stability, as well as low cost. Besides, fluorescent biosensor has drawn much attention and was widely employed to detect protein because of its simplicity, rapid analysis, low-cost and high sensitivity [13–15].

In the past few years, much focus has been paid to graphene oxide (GO) in the construction of fluorescent biosensor due to its high quenching ability and water solubility [16,17]. The sensing platform based on GO has been used to detect many biomolecules [18,19]. Recently, other 2D nanomaterials were continuously discovered and synthesized such as transition metal dichalcogenides (TMDs), transition metal oxides (TMOs), silicate clays, layered double hydroxides (LDHs) [20]. Among them, MoS_2 nanosheets share some common features with graphene in structural and physical/chemical properties [21]. MoS_2 nanosheets have some special and fascinating properties [22]. First, MoS_2 nanosheets have ultrahigh surface-to-volume ratio and can load various and large amounts of biomolecules [23]. Second, MoS_2

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nanosheets exhibit super quenching ability [24]. Third, due to lack of dangling bonds, MoS_2 nanosheets show high stability in aqueous solution without the process of surfactants or oxidation treatment [23–28]. All above characteristics make it an ideal candidate as a new nanomaterials in the construction of fluorescent biosensor. In addition, much focus has been also paid on their applications in the biomedical field, including ultrasensitive biosensing, biological imaging, cancer therapy, antibacterial treatment and drug delivery [29–32].

In order to simple, rapid, sensitive and selective detection of CEA, a fluorescence turn-on biosensor based on aptamer-MoS_2 nanosheets was established. The sensing platform has a high sensitivity and selectivity for CEA protein, due to the super quenching ability of MoS_2 nanosheets and high selective aptamer. The system has merits of fast detection, low cost and easy construction, which make it a promising sensing platform for protein detection in vitro applications.

2. Materials and methods

2.1. Materials and apparatus

Molybdenum disulfide (MoS_2) nanosheets were purchased from Nanjing XF Nano Material Tech Co., Ltd. (Nanjing, China). Carcinoembryonic antigen (CEA) was purchased from Shanghai Linc-Bio Science Co., Ltd (Shanghai, China). Bovine serum albumin (BSA) and immunoglobulins G (IgG) were purchased from Dingguo Biotech Co., Ltd (Beijing, China). Tris-HCl was purchased from Dingguo Biotech Co., Ltd. CEA aptamer probe (CA) and ssDNA were synthesized and purified by Sangon BiotechCo., Ltd (Shanghai, China). The sequence used as follows: CEA aptamer probe (CA) 5′-Texas Red –ATACCAGCT TATTCAATT-3′, random ssDNA 5′-TCATTACATGTTTCCTTACTTC CAG-3′.

The buffer used in this work was 10 mM Tris-HCl (pH 7.4), containing 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$. All chemicals were of analytical grade and used without further purification. All solutions were prepared in Milli-Q water (resistance > 18 MΩ cm). All fluorescence measurements were carried out on a RF-5301PC fluorophotometer (Shimadzu, Japan). Time decay photoluminescence measurements were recorded with an Edinburgh FLS920 phosphorimeter (Edinburgh Instruments, U.K.).

2.2. Optimization of detection conditions

Firstly, the content of MoS_2 nanosheets employed in sensing platform was optimized. The typical procedure was conducted as follow. A certain concentration of MoS_2 nanosheets was added to the 5 nM fluorophore-labeled CEA aptamers, followed by the incubation for 10 min at room temperature. The fluorescence spectra were recorded immediately by RF-5301PC fluorophotometer. Meanwhile, 5 nM fluorophore-labeled CA was incubated with 10 ng/mL CEA at 37 °C for 2 h prior to the addition of different concentrations of MoS_2 nanosheets. After the incubation for 10 min at room temperature, the fluorescence spectrum was recorded immediately. Each experiment was repeated three times.

In order to determine the quench time of MoS_2 nanosheets for CA and CA/CEA, the fluorescence spectra were recorded at different time after the MoS_2 nanosheets (200 μg/mL) were added in the 5 nM CA and the mixture of 5 nM CA and 10 ng/mL CEA respectively.

All the experiments were conducted in a buffer solution (10 mM Tris-HCl, containing 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$).

2.3. Procedures for CEA detection

For CEA detection, 5 nM CA was incubated with different concentrations of CEA at 37 °C for 2 h, and then 200 μg/mL MoS_2 nanosheets was added in the same solutions. After the incubation for 10 min at room temperature, the fluorescent intensity changes were recorded on a RF-5301PC fluorophotometer. Fluorescence measurements were performed under the same conditions at the room temperature. The excitation/emission wavelengths were fixed at 595 and 608 nm respectively. To examine the specificity of fluorescent biosensor for CEA protein detection, BSA (10 ng/mL and 40 ng/mL), IgG (10 ng/mL and 40 ng/mL), ssDNA (10 nM) were added into the system instead of CEA respectively followed by the same CEA detection procedure.

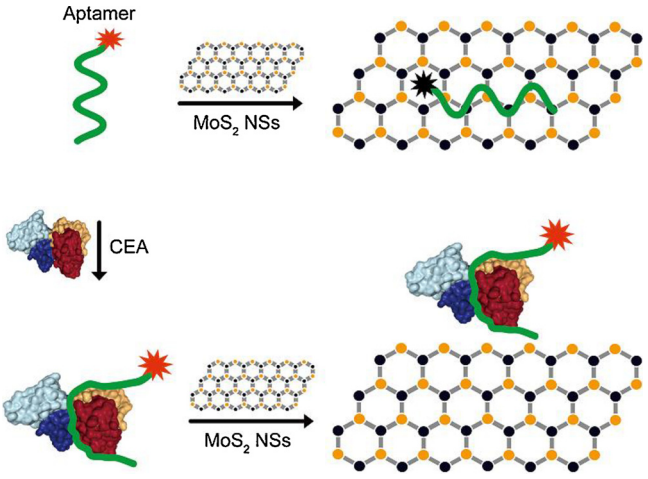

Fig. 1. Schematic illustration of fluorescent biosensor based on MoS_2 nanosheets for CEA protein detection.

3. Results and discussion

3.1. Mechanism of fluorophore-labeled aptamer/MoS_2 based FRET biosensor

The mechanism of fluorescent bio-sensing system biosensor for CEA detection in this work is illustrated in Fig. 1. Fluorophore (Texas Red) was used as fluorescence resonance energy transfer (FERT) donor, which can emit intense blue light at 608 nm under light excitation of 595 nm. MoS_2 nanosheets were served as FERT acceptors owing to high fluorescence quenching ability. Fluorophore-labeled aptamer can adsorb on the surface of MoS_2 nanosheets in close proximity via van der Waals force between nucleobases and the basal pane of MoS_2 nanosheets [24,25,33], furthermore, FRET between fluorophore and MoS_2 nanosheets was triggered, resulting in the subsequent fluorescence quenching of the fluorophore. In contrast, when CEA existed in the reaction system, they would bind with CA, resulting in the change of conformation of CA/CEA complex. Therefore, the interaction between CA/CEA complex and MoS_2 nanosheets became so weak that CA detached from the surface of MoS_2 nanosheets, leading to the restoration of fluorescence signal. Consequently, the target CEA protein can be detected by monitoring the variation of fluorescence signal.

3.2. Construction of biosensor based on MoS_2 nanosheets and protein aptamer

To investigate the feasibility of fluorophore-labeled aptamer/MoS_2 based FRET biosensor, the fluorescence spectra of CA and CA/ CEA in the absence or presence of MoS_2 nanosheets were recorded respectively. As shown in Fig. 2, CA and CA/CEA exhibit the similar fluorescence in the absence of MoS_2 nanosheets, which demonstrates that the interaction between CA and CEA hardly had an effect on the fluorescence intensity of CA. Moreover, the fluorescence intensity of CA could be quenched 99.1% by optimal concentration of MoS_2 nanosheets (200 μg/mL) (green curve) and restored dramatically by addition of target CEA protein (blue curve). These results indicate that MoS_2 nanosheets not only possess high fluorescence quenching ability to CA but also have