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RESEARCH PAPER

A Dual-signal Amplification Method for DNA Detection Based on Exonuclease III and Fluorescence Quenching Ability of MoS_2 Nanosheet

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Abstract: A highly sensitive and selective DNA biosensor was described based on the fluorescence quenching ability of MoS_2 nanosheet and exonuclease III (Exo III) assisted dual-signal amplification. In the absence of target DNA, two single oligonucleotides linked fluorophores (HP1 and HP2) resisted the digestion by Exo III due to the 3'-termini protrusion, resulting in low fluorescent signal by the adsorption and the quenching ability of MoS_2 nanosheets toward single oligonucleotides and fluorophores. In the presence of target DNA, HP1 and HP2 were digested by Exo III because of the target DNA-induced two-step hybridization, and desorbed from the surface of MoS_2 nanosheets, trigging dual-signal amplification. As a result, a large amount of fluorescent fragments and high fluorescent signal were generated. Under the optimal conditions, the proposed strategy could detect target DNA with a detection limit of 0.28 pM. In comparison with single amplification method, this method provided an improvement in the sensitivity and discrimination of single-base mismatch.

Key Words: DNA biosensor; Molybdenum disulfide nanosheet; Exonuclease III; Dual-signal amplification method; Quenching ability

1 Introduction

Detection of specific sequences of DNA is vital in diagnosis of pathogenic infections and genetic diseases, forensic analysis, and modern life sciences[1–5], thus great attention is paid to the highly sensitive method for DNA detection[6,7]. Currently, various amplification techniques have been developed for sensitive detection of DNA, including exonuclease III (Exo III)-aided amplification[8–10], strand-displacement amplification (SDA)[11–13], hybridization chain reaction (HCR)[14–17] and rolling circle amplification (RCA)[18–22]. Exo III is widely used for signal amplification based on the unique property by which it can remove the mononucleotides of duplex DNA from its 3'-terminus without ignorable effect on by the sequences[23,24]. Therefore, Exo III was evoked in developing a variety of highly sensitive detection of nucleic acids[8–10,23,24], proteins[25,26] and other targets[27,28]. Recently, Cai *et al*[10] proposed a novel exonuclease III-based dual-signal amplification method for DNA detection. Compared with Exo III-based single signal amplification, this method could not only enhance the sensitivity, but also obtain good selectivity in the discrimination of single base mismatched targets.

MoS_2 nanosheets, a 2D layered nanomaterial analogous to graphene, attract great attention as an energy acceptor in resonance energy transfer because of their excellent nanoelectronics, optoelectronics, and energy harvesting properties[29,30]. In comparison with graphene, MoS_2 nanosheets can be facilely synthesized in large scale and directly dispersed in aqueous solution without extra treatment with surfactants or oxidation[31,32]. Furthermore, MoS_2 could adsorb dye-labeled single-stranded DNA (ssDNA) probe

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between nucleobases and basal plane of MoS_2, and then quench the fluorescence of the dye. This unique optical properties, large specific surface area, and potential for surface modification could be applied in the field of constructing sensitive, rapid and cost-effective biosensor[33−35]. However, the use of MoS_2 nanosheets as an indicator probe has rarely been performed[36]. It remains a challenge for MoS_2 nanosheets application to biosensors.

Herein, we developed a dual-signal amplification strategy for detection of DNA based on Exo III and efficient quenching ability of MoS_2 nanosheets, taking human haemochromatosis protein (HFE) gene as the target DNA. Two single oligonucleotides linked fluorophores (HP1 and HP2) were designed as probes. In the presence of the target DNA, HP1 and HP2 probes were desorbed from the surface of MoS_2 nanosheets owing to the target DNA-induced two-step hybridization, leading to a large amount of fluorescent fragments by the digestion of Exo III and triggers dual amplified fluorescence signal. Additionally, the introduction of MoS_2 nanosheets not only reduced the fluorescence background, but also decreased the detection cost.

2 Experimental

2.1 Apparatus and materials

The luminescence intensity was detected on a RF-5301PC fluorescence spectrophotometer (Hitachi High-Technology Co., Ltd., Tokyo, Japan) at excitation wavelength of 480 nm and emission wavelength of 500−700 nm. The slits for excitation and emission were all set at 10 nm. The pH measurement was carried out by a pH meter (Sartorius). SPA-400 atomic force microscopy (AFM) was controlled by a SPI-3800 software (Seiko Instruments Industry Co., Tokyo, Japan). Transmission electron microscopy (TEM) images were taken by JEM-1200EX.

MoS_2 nanosheets were purchased from Nanjing Xfnano Materials Tech Co., Ltd. Exo III was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Tris (hydroxymethyl) aminomethane hydrochloride (Tris) and $MgCl_2$ were purchased from Sigma-Aldrich (USA). All other chemicals not mentioned here were of analytical-reagent grade or better. The oligonucleotides were purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), and the sequences were shown in Table 1.

2.2 Procedure for DNA detection

HP1 (2.0 nM), HP2 (8.0 nM), Exo III, and different concentrations of the target were mixed together and Tris-HCl buffer (20 mM Tris, 300 mM NaCl, 10 mM $MgCl_2$, pH 8.0) was added to a final volume of 200 μL, followed by incubating at 37 °C for 80 min and then cooling to room temperature. Finally, the reaction solution was added with MoS_2 nanosheets and Tris-HCl buffer solution to a total volume 400 μL. The fluorescence intensity was measured at excitation wavelength of 480 nm and emission length of 520 nm. All the experiments were repeated three times

3 Results and discussion

3.1 Detection mechanism

The principle of the proposed biosensor is illustrated in Fig.1. Two hairpin DNA probes, HP1 and HP2, were designed with 3'-termini protrusion for resistance to degradation by Exo III. There were two amplification reactions in this biosensor. In the absence of target DNA, HP1 and HP2 were protected from the digestion by Exo III. As a result, low fluorescence signal output appeared due to the strong absorption of HP1 and HP2 toward MoS_2. With the addition of target DNA, the hybridization between the HP1 and the target DNA occurred, and the HP1 was degraded stepwise into fluorophore, the black part of HP1 (T1) was from the 3'-to 5'-end by Exo III. As a result, the target DNA was released and rolled into the next signal generation cycle (recycle 1), releasing more HP1 from the surface of MoS_2 as well as sending out the high fluorescence signal. Meanwhile, HP2 was hybridized with T1 and degraded by Exo III, leading to the release of T1 and trigging the second recycle (recycle 2). More HP2 were desorbed from the MoS_2 surface and induced another high fluorescence signal. Thus, a small quantity of the target DNA could generate a large amount of fluorescent fragments from the recycle 1 and recycle 2, causing dual- signal amplification for the detection of DNA and effectively enhancing the sensitivity with the aid of fluorescence quenching ability of MoS_2.

Table 1 DNA sequence

DNA name	DNA sequence
Hairpin 1 (HP1)	5'-CC**AGATATACACGTCTCCTC**GCAGCCTTTTTTTTTTGGCTGCGAGGAGACGT*CACCTGGCACGTATAT CTGGACTATG*-FAM-3'
Hairpin 2 (HP2)	5'-ACACGT**GAGGAGACGTGTATATCT**-FAM-3'
Target DNA	5'- CaATAGTCCAGATATACGTGCCAGGTGGAGTACG-3'
Single-base mismatched DNA (MT1)	5'- CATAGTCCAGATATAC***A***TGCCAGGTGGAGTACG-3'
Two-base mismatched DNA (MT2)	5'- CATAGT***A***CAGATATAC***A***TGCCAGGTGGAGTACG-3'

The underlined portion in HP1 is T1, the italic portion in HP1 is complementary to the target DNA. The bold bases in HP1 and HP2 are complementary. The italic and bold bases are mismatched bases.