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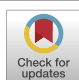

Long segment detection of HTLV-1 genome based on the fluorescence quenching technique

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Abstract

Detecting fluorescence changes due to energy transfer between a quencher and fluorophore is a common method used for the fluorescence-based biosensors. In the present report, a new biosensor for long segment detection of the human T cell-lymphotropic virus 1 genome was constructed based on the fluorescence quenching of graphene oxide by gold nanoparticles. The fluorescence signal of unmodified graphene oxide was measured before and after hybridization of target and probes functionalized with gold nanoparticles. The limit of detection of the biosensor was determined to be around 10 pg/mL. The specific design for long segment of target assures the selectivity of biosensor. Our results proposed that further development may be useful to detect other viruses.

Keywords: Virology, Biotechnology, Analytical chemistry

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1. Introduction

Viral biosensors development has high importance to early detection of viruses [1]. The common techniques including real-time polymerase chain reaction (RT-PCR) and Enzyme-linked immunosorbent assay (ELISA) are highly selective and sensitive; however, they are expensive, time-consuming, and dependent on professional operators [2]. Nanomaterial-based biosensors are capable of detecting biological species by means of different techniques such as spectrofluorimetry, electrochemistry, and spectrophotometry [3, 4, 5]. Recently, the nanomaterials with unique optical absorptions and fluorescence emissions have been used to detect biomolecules [6, 7, 8].

Graphene oxide (GO) denotes a two-dimensional honeycomb lattice composed of single-layer carbon atoms. It can adsorb proteins and deoxyribonucleic acid on its surface. Due to the optical properties of GO, it has been utilized to detect DNA hybridization based on the fluorescence quenching of the fluorophore-labelled single-stranded DNA and then restore the fluorescence emission in the presence of complementary target DNA. The photoluminescence property of GO belongs to the recombination of localized electron—hole pairs within the domain of sp^2 carbon embedded in a sp^3 matrix [9].

Gold nanoparticles (AuNPs) with inimitable optical properties have been broadly applied in different research areas. AuNPs can quench the fluorescence of a fluorophore via energy transfer [10, 11]. Also, it has been shown that the AuNPs operate as a quencher for carbon nanomaterials like graphene oxide and carbon nanotube [12].

Human T cell-lymphotropic virus 1 (HTLV-1) is known as an enveloped retrovirus which comprises two equal copies of single-stranded RNA virion genome with positive polarity. HTLV-1 is able to escape from hosts defense mechanisms. The majority of HTLV-1-infected subjects remain asymptomatic carriers [13, 14], while only the low percentage of infected cases can develop two diseases of adult T-cell leukemia/lymphoma (ATLL) or HTLV-I- associated myelopathy/tropical spastic paraparesis (HAM/TSP). Several proteins like Tax have key functions in HTLV-1 viral pathogenesis through their functions in the start of the leukemogenic process [15]. Therefore, the genome region responsible for its coding can be a proper target to design efficient and selective probes. Herein, we developed a new biosensor for identification of long genome segment through designing proper probes and tracing the fluorescence energy transfer between graphene oxide and gold nanoparticle before and after presence of the target genome.

2. Materials & methods

2.1. Synthesis of AuNPs

The synthesis of 12 nm AuNP was carried out according to Turkevich method [16]. Briefly, 90 μL of solution of 1 mM $HAuCl_4$ was added to 50 mL of deionization

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water. Then, 1 mL of 1% trisodium citrate solution was added instantly after boiling started. The reaction mixture was stirred and heated until the solution color was changed. Afterward, it was cooled down to room temperature under stirring. The maximum wavelength of the AuNPs surface plasmon resonance was 520 nm.

2.2. Preparation of probe−AuNPs

Thiol-modified DNA oligomers (sequence F1: 5′-TTCCGTTCCACTCAACCCT CAC-(T)10-(C3-SH)- 3′; sequence F2: 5'- (SH-C6)-(T)10-CAGCCATCT TTAGTACTACAGTCCTCCTCC- 3′) were purchased from Takapouzist Biotech Company (Iran). DNA target sequence was as follow:

5′-GGAGGAGGACTGTAGTACTAAAGATGGCTGGCCATCTTTAGGGCA GGGCCCGGAAATCAT

AGGCGTGCTATCGGTAAATGTCCAAATAAGGCCTGGAGTGGTGAGGG TTGAGTGGAACGGAA-3′

The thiol-modified DNA probes were functionalized with AuNPs according to the following procedure: 500 μL AuNPs was mixed with 1 μM thiolated DNA for 16 h. Subsequently, the AuNP-DNA solution was centrifuged two times at 12,000 rpm for 10 min to remove excess AuNPs and unconjugated DNA. Finally, the precipitate was dispersed in a 0.01 M phosphate buffer solution at pH 7.0, then stored at 4 °C for further use.

2.3. Procedure for DNA detection

In order to detect DNA target, firstly 10 μL of 100 μg/mL GO, 5 μL from each DNA probe-modified AuNPs in 10 mM phosphate buffer (pH 7.0), 5 μL 0.1 M NaCl, and 5 μL 5 mM $MgCl_2$ were incubated for 10 min. Then, the DNA target was added and the mixture was heated at 90 °C for 5 min and incubated for 15 min. The GO was purchased from Graphenics, Iran. The final volume was 40 μL. Finally, the fluorescence intensities were measured before and after adding DNA target. GO was sonicated for about 30 min before use.

3. Results

Two thiolated oligonucleotides (probes 1, 2) which were complementary to two specific regions of HTLV-1, were designed by Gene Runner software (version 6.5.48). The nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was applied to confirm the specificity of the probes [17]. The designed probes were completely specified for the 122-base fragment of the tax region of the HTLV-1 genome. Probe 1 and probe 2 were made of 22 and 30 bases, respectively. After that, the probes were functionalized with AuNPs in average size of 12 nm. The excitation of GO at 400 nm

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causes the emission fluorescence peak around 460 nm. In the proximity of GO, the DNA-AuNPs were adsorbed on the surface which led to fluorescence energy transfer between AuNPs and GO (Fig. 1). The fluorescence emission of GO was quenched when probe-AuNPs were adsorbed on its surface. In order to enhance the quenching efficiency, both probes were functionalized with AuNPs, thus a high number of AuNPs were placed close to the GO sheets. In the presence of target, the probes could be desorbed from the GO surface and hybridized with the target. As a result, the fluorescence emission of GO nanosheets was recovered. The coating of AuNPs with thiolated probes was investigated. Fig. 2 shows that the absorption of AuNPs was slightly decreased after binding to the thiolated probe, which may due to alterations of plasmon resonance frequency [18]. To check the accuracy of designed probes, the hybridization of probes with target was followed based on aggregation of AuNPs. To this end, the AuNPs modified probes were incubated with target. As anticipated, they hybridized with target and constituted a stable duplex.

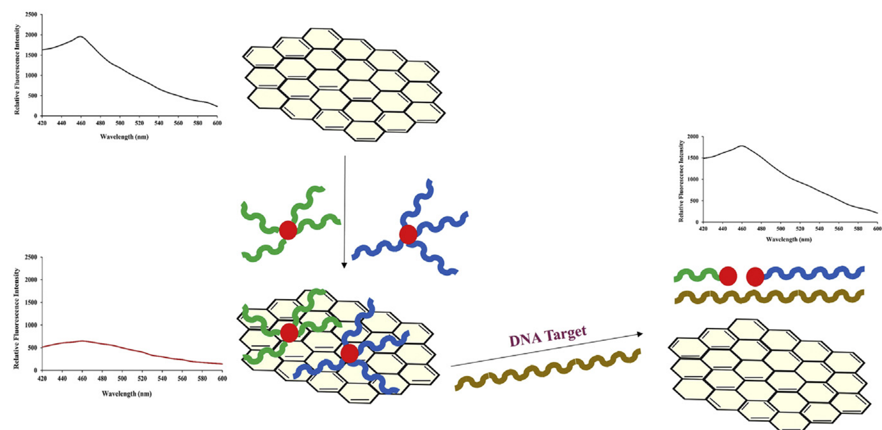

Fig. 1. Schematic representation of the biosensor for detection of DNA target.

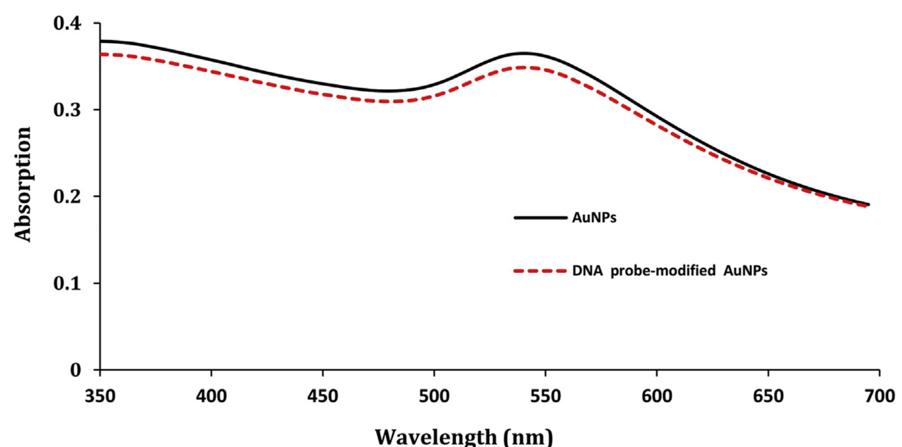

Fig. 2. UV-Vis spectra of AuNPs and DNA probe-modifies AuNPs.

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This process led to a red-shifting and attenuation of the AuNPs plasmon resonance (Fig. 3) [19].

The optimum concentration of GO should be found because the excess amount has a high effect on the sensitivity and background signal. To this purpose, various concentrations of GO were incubated with two probes-AuNPs. The quenching efficiency is represented as $Q=(F_0-F_q)/F_0$, in which F_0 is the primary fluorescence intensity of GO and F_q is the fluorescence intensity of GO after quenching [20]. The maximum fluorescence quenching was observed in the presence of 25 μg mL^{-1} of GO (Fig. 4). So, it was elected as the optimum amount of GO in the further experiments.

The pristine GO showed a strong fluorescence emission spectrum at 460 nm with excitation of 400 nm, while the GO fluorescence emission was diminished after adsorption of the probe-AuNPs (Fig. 5). The quenching effect of probe-AuNPs adsorbed on the GO sheets was followed through the comparison of the fluorescence

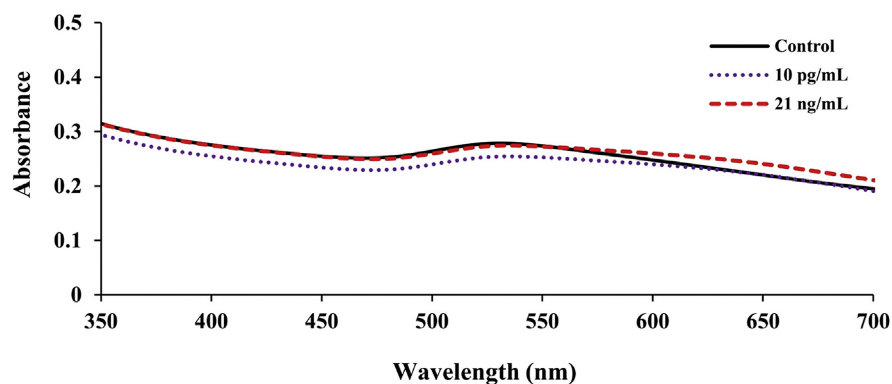

Fig. 3. Absorption spectra of probes-AuNPs in the absence (control) and presence of 10 pg/mL and 21 ng/mL target.

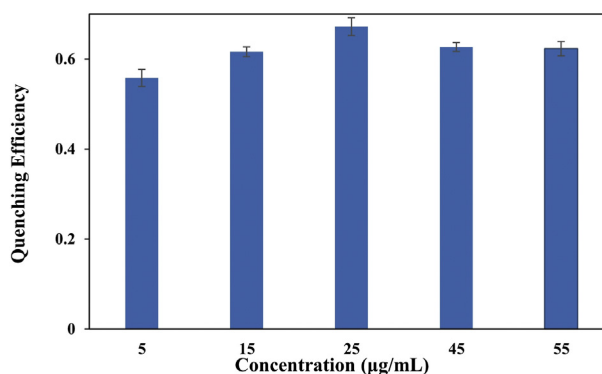

Fig. 4. Quenching efficiency of solutions containing 5, 15, 25, 45, 55 μg/ml GO after treatment with two probes-AuNPs.

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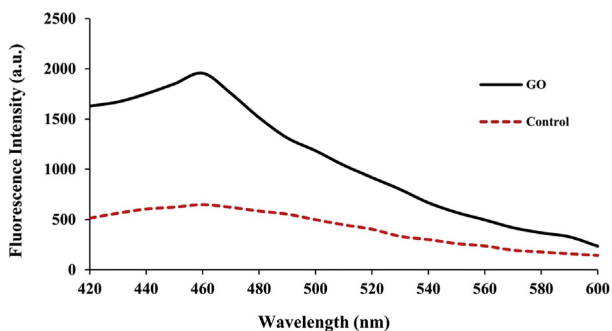

Fig. 5. Fluorescence intensity of GO before (GO) and after treatment with two probes-AuNPs (control).

emission intensities. Afterward, the analytical parameters of biosensor were obtained by varying the concentration of target.

The GO fluorescence emission gradually increased with the increase of the target concentrations and it reached a value of 90% in the presence of 21 ng/mL target. Then, the fluorescence emission has remained unchanged after further increase of the target concentration. Fig. 6a shows the recovery of GO fluorescence emission

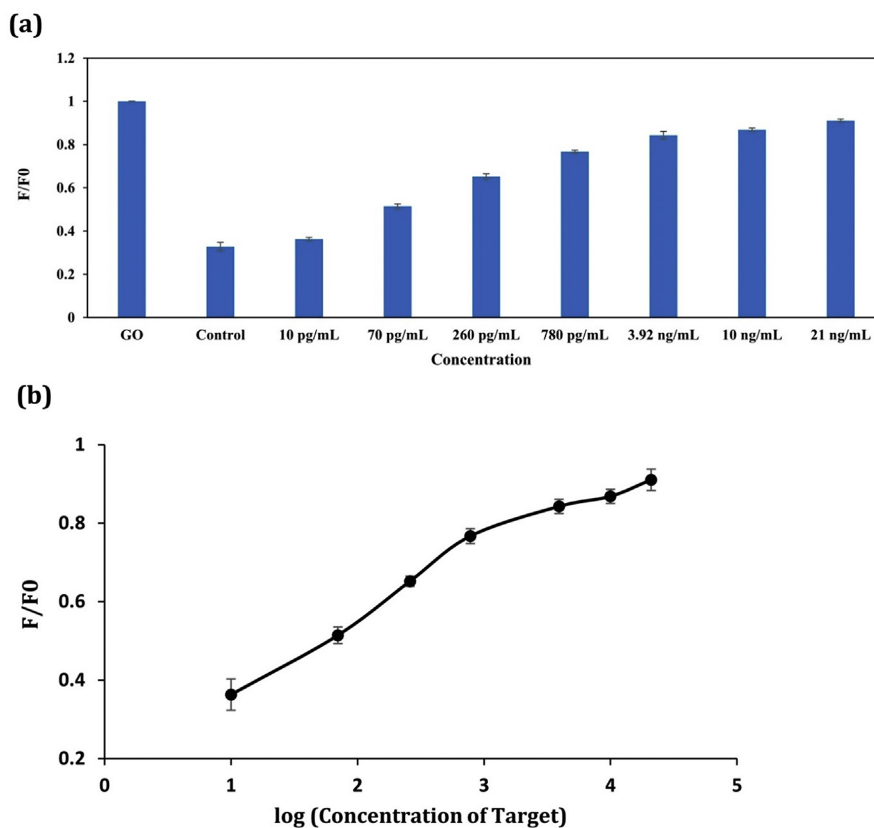

Fig. 6. (a) Relative fluorescence intensity of GO (F/F0) after treatment with two probes-AuNPs and various concentrations of target. (b) Relative fluorescence intensity of GO (F/F0) as a function of log (Concentration of Target). Error bars show standard deviation for three replicates.

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versus a series of target with concentrations from 10 pg/mL to 21 ng/mL. Moreover, the calibration curve based on the relative fluorescence intensity of GO (F/F0) as a function of log (Concentration of Target) was plotted (Fig. 6b). The limit of detection (LOD) was determined as the minimum concentration of target that can identify from control, which was as low as 10 pg/mL. In addition, the reproducibility of the proposed biosensor for detection of the oligonucleotide targets at concentration of 780 pg/mL was investigated by measuring fluorescence recovery of graphene oxide in three sensing platforms constructed under similar conditions. The relative standard deviation (RSD) was 9.4 %, which is acceptable reproducibility. Furthermore, the biosensor response versus the genes of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) were also investigated. Fig. 7 shows the preferable selectivity of the biosensor against other disease types. In fact, the fluorescence recovery of GO decreases considerably in the attendance of other virus types.

4. Discussion

The effective fluorescence energy transfer was observed between AuNPs and GO to detect HTLV-1 target. The detection of long segment of a target was selected to assure from selectivity of the biosensor. Also, the nucleotide BLAST tool guaranteed the specificity of designed probes. A BLAST search helps us to compare the query sequence with all databases of sequences, and identify the regions which are complementary with the query sequence. The probes were thiolated such that they were placed as head-to-head to confirm the hybridization with target through tracing the AuNPs plasmon resonance.

The surface of graphene oxide is extremely heterogeneous and functionalized with groups such as carboxyl and hydroxyl groups [21]. So, the oligonucleotides can adsorb onto its surface with different adsorption affinity via two main mechanisms: i) electrostatic interaction with some groups, such as oxygen and carboxyl group on

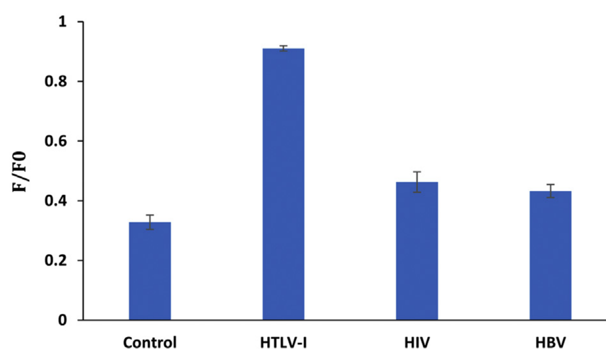

Fig. 7. Specificity of the suggested sensor for human T-lymphotropic virus-1 (HTLV-1) rather than human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Error bars show standard deviation for three replicates.

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the planes and edges of GO and ii) π-π stacking between aromatic groups of oligonucleotides and GO sheets [22, 23]. The GO is a long-ranged fluorescence quencher, so the efficient design can lead to construction an effective biosensor. Accordingly, a GO-based immuno-biosensor was constructed for detecting a rotavirus. Firstly, the antibodies were immobilized on the GO and the rotavirus cell was captured by specific antigen−antibody functionalized with AuNPs interaction. Fluorescence quenching of GO in the proximity of AuNPs was followed to investigate the capture of a target cell [12]. In another report, the GO sheets were functionalized with the probe DNA by using carbodiimide chemistry. After hybridization of GO-probe with an AuNP-labeled complementary DNA, the fluorescence emission intensity of the GO array was quenched [24].

Herein, the quenching quantum efficiency of GO was improved by functionalization of both probes with AuNPs in addition the sonication of GO before adsorption of probes. The proposed biosensor omits the chemical modification of GO and simplifies the detection procedure. Development of such biosensing structure can bring about the capability of miniaturizing the biosensor and point of care devices.

5. Conclusion

In conclusion, we have presented a novel GO-based sensor for rapid and sensititive detection of HTLV-1. We utilized the photoluminescence quenching of GO in the proximity of AuNPs as the quencher. Our outcomes proposed that the further improvement of the system can be applied in other research areas such as immunoassay, drug delivery, and nanobiotechnology.

Declarations

Author contribution statement

Mohadeseh Z. Ghobadi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sayed-Hamidreza Mozhgani: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Fatemeh Hakimian: Performed the experiments.

Hedayatollah Ghourchian: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Mehdi Norouzi, Seyed A. Rezaee: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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