Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

Cite this: *Anal. Methods*, 2020, **12**, 4541

An electrochemical biosensor based on a graphene oxide modified pencil graphite electrode for direct detection and discrimination of double-stranded DNA sequences†

Mehran Ahmadi and Fatemeh Ahour *

Received 6th June 2020
Accepted 3rd August 2020

DOI: 10.1039/d0ay01128b

rsc.li/methods

The ability to directly recognize double-stranded DNA (ds-DNA) is a major challenge in disease diagnosis and gene therapy because DNA is naturally double-stranded. Herein, a novel electrochemical biosensor for the sequence-specific recognition of ds-DNA using a peptide nucleic acid (PNA) probe and graphene oxide (GO) modified pencil graphite electrode is reported and applied for the direct detection of the desired sequence in plasmid samples. For this purpose, GO was assembled onto the pencil graphite electrode surface (GO/PGE) by a simple casting method and applied for PNA probe immobilization (PNA-GO/PGE). Upon addition of ds-DNA, the interaction of the PNA probe with ds-DNA induces probe detachment from the electrode surface which results in a guanine oxidation signal decrease. Under optimized conditions, the guanine oxidation signal decreased linearly with the ds-DNA concentration increasing in the range from 30 pM to 10 nM, with a detection limit of 1.3 pM. Moreover, the proposed biosensor was applied for the sensitive and selective detection of double-stranded target DNA in plasmid samples. This proposed method could be used as a platform for direct detection of various sequences in double-stranded genomic DNA.

Introduction

Peptide nucleic acid (PNA) is an uncharged DNA mimic that can hybridize with its complementary ss-DNA (com ss-DNA) obeying Watson–Crick base-pairing rules.[1–4] In addition, the formation of higher-order complexes between PNA and ds-DNA in a sequence specific manner based on Watson–Crick base pairing and Hoogsteen hydrogen bonding is possible.[4–10] PNA has been used as a sensing probe in biosensors for DNA detection and shows better properties than its counterpart DNA sequences basically due to the neutral backbone of the PNA.[11–14] The performance of PNA as a sensing probe has been compared to that of a DNA probe in some studies.[15–22]

As genomic DNA is naturally in the double-stranded form, there has been extensive attention on the direct detection of target ds-DNA without further pre-treatment involving its denaturation (conversion to ss-DNA). DNA biosensors were extensively studied by different groups based on various techniques and summarized in published review papers completely.[23–28] In spite of the significance of ds-DNA detection, there are limited reports for direct detection and sequence-specific recognition of ds-DNA by various methods.[9,29–42] Electrochemical DNA detection has attracted growing interest because of the simple operation, portable testing, short response time, high sensitivity, miniaturization, *etc.* In electrochemical DNA biosensors, the signal is obtained directly from the electroactive oligonucleotides or indirectly from an electroactive label. Hybridization detection based on guanine oxidation signals seems to be a simpler, faster, and more practical strategy than others. Almost all of the studies reported for ds-DNA detection are based on the gold electrode and electroactive labels.[29–39] This is the first report for label free and direct electrochemical detection of ds-DNA without requiring denaturation. Recently biosensors based on nanomaterials have opened new avenues in detection and have some advantages over common materials.[43–46]

Graphene oxide (GO), a derivative of graphene (GN) with better water dispersion, has excellent properties and shows unexpected behaviour in biosensors. Agglomeration of graphene due to van der Waals forces limits the application of graphene materials.[47] Reduced graphene oxide (RGO) simply prepared by chemical reduction of GO using toxic and dangerous reducing agents agglomerates easily too. But GO overcomes this disadvantage due to its oxygen-containing moieties and is easy to prepare. Superior binding of GN and GO to ss-DNA and PNA over rigid ds-DNA opens a door for the construction of biosensors for sensing single-stranded nucleic

Nanotechnology Research Center, Faculty of Science, Urmia University, Urmia, Iran. E-mail: f.ahour@urmia.ac.ir; Fax: +98 44-32752746

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0ay01128b

acids,[48–53] proteins,[54–57] small molecules,[58,59] and double-stranded nucleic acids.[42] Lee *et al.* reported sequence-specific detection of ds-DNA using the PNA probe and GO as a fluorescence quencher.[42]

As single stranded oligonucleotide sequences are adsorbed on a graphene sheet surface with all nucleobases lying nearly flat, hybridization efficiency and sensitivity may be decreased. Based on the above illustrations, in this paper, a simple label-free electrochemical biosensor for direct detection of double-stranded DNA was explored based on a GO modified PGE (GO/PGE) and PNA probe and used for identifying the universal sequence of the hepatitis C virus (HCV) in plasmid samples.

One of the protected areas of the HCV genome, which has no sequence variation between different genotypes, is the core gene of the HCV and this sequence is an ideal candidate for inclusion in a DNA-based vaccine. Although the hepatitis C genome is a single-stranded RNA, recombinant proteins are used in vaccine production, which are made by recombinant organisms and recombinant plasmids containing the hepatitis C core/E1 encoding cDNA insert. The core/E1 recombinant protein of the HCV is produced using the pBKC84 recombinant plasmid cloned by Kazemi *et al.* and can be used to produce anti-HCV antibodies.[60] Since the DNA in plasmid molecules is double stranded, it is important to quickly and easily recognize target ds-DNA in the plasmid without the need for multiple steps. There are some reports on the diagnosis of hepatitis *via* nanomaterials reviewed previously.[61]

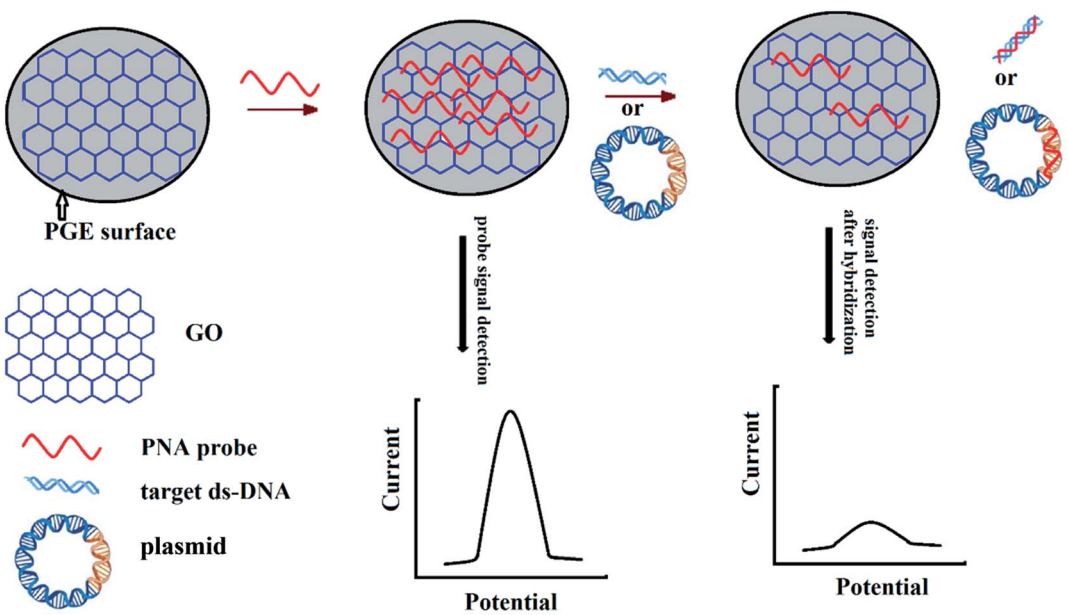
Herein, we report for the first time the use of a PNA probe and GO modified electrode for the label free and direct electrochemical detection and discrimination of ds-DNA. The novelty of the present work is the application of graphitic electrodes for direct and label free detection of ds-DNA. The present label-free biosensor with a wide linear dynamic range and high sensitivity is achieved *via* non-covalent immobilization of the PNA probe on the GO modified PGE. After the hybridization of the immobilized PNA probe with ds-DNA, desorption of the hybrid takes place from the electrode surface and the guanine oxidation signal decreases (Scheme 1). The proposed biosensor was used for the sequence-specific recognition of hepatitis C core/E1 encoding cDNA in the ds-plasmid (ds-Pl) as a real sample. Indeed this protocol could be used as a platform for preparation of single use DNA kits using GO and screen printed carbon electrodes for sequence-specific fast detection of genomic ds-DNA.

Experimental

Materials

The pencil graphite of type H and a diameter of 2.0 mm was obtained as pencil lead from Rotring Co. Ltd, Germany. A 20-mer PNA chain corresponding to the universal segment of all HCV genotypes was used as the probe. The sequence of the used ss-DNA was the same as that of the PNA probe and all of them are presented in Table 1S.† The sequence of the target ds-DNA corresponds to those of all the HCV types (universal segment) and is composed of two complementary sequences. As core/E1 recombinant protein producing anti-HCV antibodies can be produced by the plasmids encoding the core/E1 sequence, the detection of plasmids containing this sequence by fast methods is of interest. 0.1% trifluoroacetic acid was used for the PNA probe stock solution (500 μM) preparation and frozen at −20 °C. The stock solution of DNA (500 μM) was prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 7.00) and kept frozen. Afterward, 0.1 M phosphate buffer solution with pH 5 (PBS) was used for preparation of the dilute solutions of the probe.

The complementary (Com) target recombinant plasmid comprising core/E1 encoding cDNA with 4568 base pairs named



Scheme 1 Schematic illustration of the GO based electrochemical detection of ds-DNA.

pBKC84 and the non-complementary (NC) plasmid with 5443 bp named pET21a (+) have a concentration of 1.5 μg μL^{-1} 10 mM Tris–HCl buffer solution (pH 7.0) was used to prepare dilute solutions of the plasmids. All chemicals used in performing the experiments were of analytical grade and dissolved in distilled, deionized and sterilized water ($\leq$4 μS m^{-1}). The prepared solutions were poured into glass containers (max 2/3 full, Loosen caps) and placed in an autoclave maintaining a temperature of 121 °C for at least 30 minutes. The autoclaved materials were cooled to room temperature before transport and then sealed using their caps and kept at room temperature until use.

Apparatus

An AUTOLAB PGSTAT 30 electrochemical analysis system and the GPES 4.7 software package (Eco Chemie. The Netherlands) were used for performing the electrochemical experiments. The experiments were conducted in a three electrode system with pencil graphite with a 2 mm diameter as the working electrode, a platinum wire as the counter electrode and saturated calomel (Hg/Hg_2Cl_2, KCl 3 M) as the reference electrode. A modified Hummers method was used for the synthesis of GO according to previous reports.[62,63]

Design of the GO modified PGE

The biosensor was constructed on a PGE. Before the immobilization of the PNA probe, the PGE surface was polished on a weighing paper followed by sonication in nitric acid for 3 minutes, successive washing with double distilled water and then drying.

After the above mentioned pre-treatment, the body of the pencil lead was covered tightly with a Teflon band and a copper wire placed under Teflon coverage to establish electrical contact. For preparation of the working electrode, 1.0 mg graphene oxide was dispersed in 1.0 mL H_2O by ultrasonication and then 3 μL of this homogeneous suspension (1.0 mg mL^{-1} GO) was dropped on the surface of the vertically fixed PGE and dried in the air. The electrodes were stored at 4 °C until application for detection.

PNA probe immobilization

PNA probe (PHCV) immobilization on the electrode surface was carried out by dipping the GO modified electrode into 1.5 μM PHCV1a while stirring for at least 20 min and then rinsing with water thoroughly unless mentioned otherwise. This electrode was named PNA/GO/PGE.

DNA immobilization

Immobilization of ss-DNA and ds-DNA at the surface of the bare PGE and GO/PGE was performed by 1 h dipping of these electrodes in a 1.5 μM solution of ss-DNA or ds-DNA in PBS with pH 5 while stirring.

Hybridization

For hybridization of the PNA probe with the ds-target, PNA/GO/PGE was dipped into 1 mL stirred ds-target DNA solution with a desired concentration for 30 min and then rinsed with PBS solution.

Preparation of double-stranded oligonucleotides

The ds-oligonucleotides were prepared by mixing the same concentrations (50 μM) of the desired pair of oligonucleotides (PHCVuni and HCVuni) in a microtube, heating at 95 °C in a Bain Marie for 10 min, and then cooling the tubes gradually to room temperature.[33–35]

Hybridization detection by the voltammetric method

Differential pulse voltammetry (DPV) by sweeping the electrode potential between 0.50 and 1.2 V was applied for studying the electrochemical behaviour of the electrode surface in 0.1 M PBS (pH 5). The DPV parameters, such as a step potential of 5 mV, modulation amplitude of 25 mV, modulation time of 50 ms, interval time of 500 ms, and scan rate of 10 mV s^{-1}, represent the optimum values with suitable height and sharp peaks. The Savitzky and Golay filter (level 2) of GPES software was used for treating the raw data, after which baseline correction is carried out based on the moving average. For each measurement, the

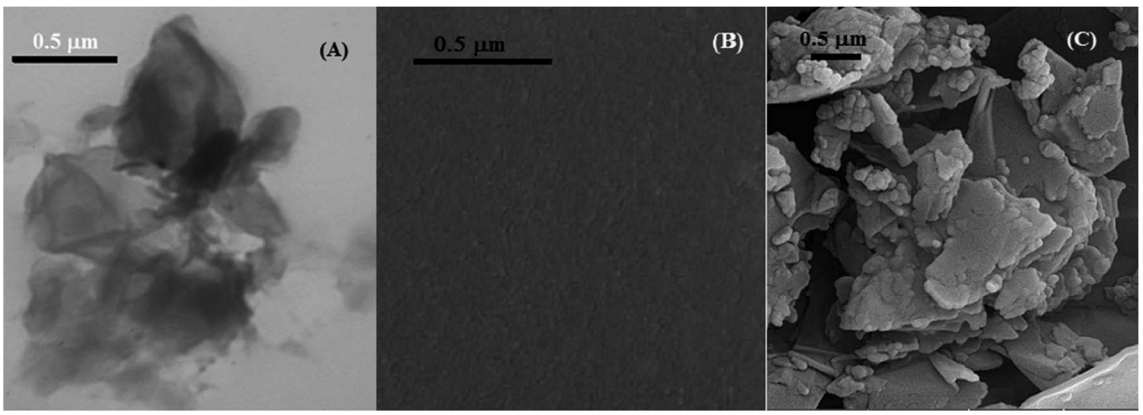


Fig. 1 (A) TEM image of GO; SEM images of (B) the bare PGE and (C) GO/PGE.

PGE surface was renewed by complete polishing of the electrode.

Results and discussion

Initial evaluation of the biosensor

An effective method to characterize the morphology of graphene oxide is TEM. Obviously, from Fig. 1A it can be seen that exfoliated GO sheets have a smooth surface.

The SEM images of the bare PGE (Fig. 1B), GO modified PGE (Fig. 1C) and GO (Fig. 1SA†) indicate that the GO nanosheets with a flake-like shape were prepared and immobilized on the electrode surface successfully.

The X-ray diffraction (XRD) patterns of graphite and GO are displayed in Fig. 2A and are in agreement with those reported in the literature.[64,65] The XRD peak of GO is formed at $2\theta = 11^{\circ}$ (d ~0.81 nm) owing to the introduction of oxygen containing functional groups which increases the interlayer spacing compared to that for graphite.

The FT-IR spectra of graphite and GO are presented in Fig. 2B. The obvious differences between these two spectra and the characteristic peaks of –OH (3426.45 cm^{-1}), C=O (1720.20 cm^{-1}), skeletal vibrations for unoxidized graphitic domains (1589.51 cm^{-1}), and C–O stretching of alkoxy groups (1032.22 cm^{-1}) indicated that graphene oxide was synthesized successfully. Also, the TGA analysis results of graphite and GO (Fig. 1SB†) and the EDS spectra of GO (Fig. 2S†) were recorded. The presence of three degradation stages in GO, indicating a high degree of oxidation and weight percent of constituent elements, proved that GO was synthesised successfully.

In order to make the construction process of the sensor clear, the electrochemical behaviour of bare PGE and GO modified PGE was studied. The electrochemical performance of the bare and modified PGEs was studied by cyclic voltammetry (CV) in 5 mM $[Fe(CN)_6]^{3-/4-}$ as the redox probe. As can be seen from Fig. 3A, the GO modified PGE exhibits higher peak currents and small peak separation for $[Fe(CN)_6]^{3-/4-}$, probably due to the large electroactive surface area and fast electron transfer rate of GO/PGE. Secondly, it can be clearly seen from Fig. 3A that the electron-transfer resistance increases after immobilization of the probe which proves probe immobilization, and this resistance for the DNA probe is higher than that for the PNA probe probably due to the negative charge of ss-DNA which restricts electron transfer.

Moreover, immobilization of the PNA probe, ss-DNA and ds-DNA at the bare and GO modified PGE surface was examined using the guanine oxidation signal. For this purpose, the bare PGE and GO/PGE were immersed in the PNA or its counterpart ss-DNA containing solution for 1 hour and then applied for guanine oxidation signal recording (Fig. 3B). The results showed that PNA probe and ss-DNA immobilization takes place successfully at the surface of GO/PGE and suitable oxidation

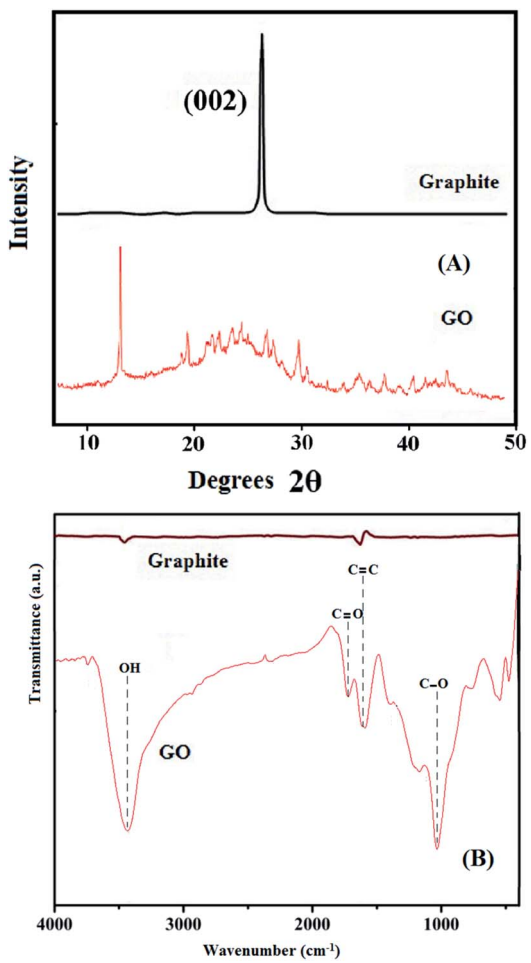


Fig. 2 (A) XRD patterns and (B) FT-IR spectra of graphite and GO.

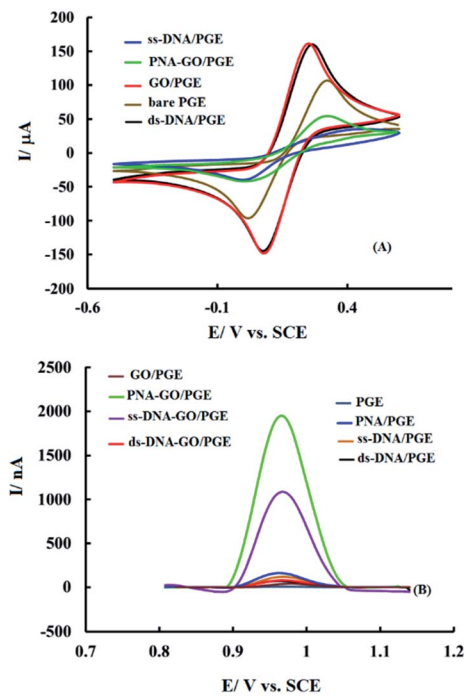


Fig. 3 (A) CV results of the bare and modified electrodes in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.5 M KCl; (B) DPV responses of the bare PGE and GO/PGE before and after 1 h of dipping for immobilization of the PNA probe, ss-DNA, and ds-DNA in 0.1 M PBS, pH 5.

peaks for guanine appear, but at the surface of the bare PGE, a very small peak was observed that proves that PNA and ss-DNA don't immobilize at the surface of the bare PGE properly maybe due to the compact structure of the PGE and lack of suitable interactions between PNA and the PGE. Indeed some experiments were performed for ds-DNA immobilization and the results showed that ds-DNA doesn't immobilize properly at the surface of GO-PGE and the bare PGE. This result verifies that ds-DNA unlike ss-DNA doesn't exhibit suitable interaction and adsorption on GO.

In continuation, some experiments based on the guanine oxidation signal were carried out to study the influence of effective parameters such as GO concentration (as the electrode modifier), PNA probe concentration and immobilization time on the immobilized probe quantity.

The amount of graphene oxide fixed at the electrode surface is one of the factors affecting the surface parameters (area and conductivity), probe immobilization and biosensor efficiency and can be varied by using a different modifier volume at the electrode preparation stage. Because the best electrode surface coverage and the stability of the modified electrode are obtained with 2–6 μL of the modifier, experiments are performed in this range. As shown in Fig. 4a, by increasing the modifier value up to 3 μL, the amount of modifier fixed at the electrode surface increases and this results in improved electrochemical activity and better adsorption of the PNA probe at the electrode surface. The larger volume of GO reduces the guanine oxidation peak current and deforms the resulting peak probably due to the decrease in biosensor conductivity as a result of the higher modifier film thickness. Subsequently, 3 μL of GO was selected as the optimum concentration for electrode modification.

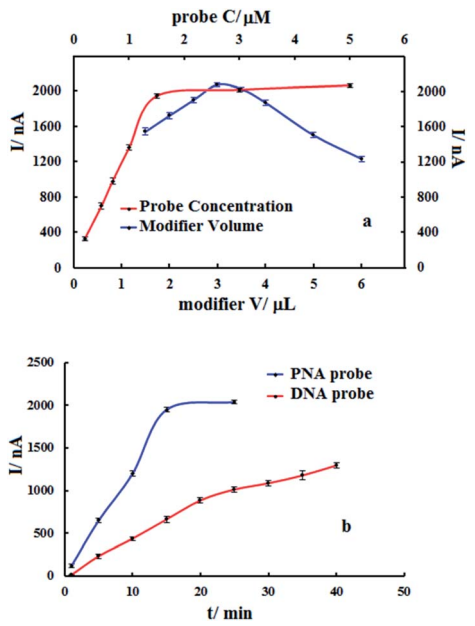


Fig. 4 (a) Effect of the GO amount and PNA probe concentration on the DPV response of the prepared electrode; (b) effect of the immobilization time of the PNA and counterpart DNA probe on the DPV response of the prepared electrode. Probe immobilization conditions: 15 min in a 1.5 μM probe solution with pH 5.

In addition, the results (Fig. 4a) showed that the amount of the immobilized probe increased when increasing the probe concentration up to 1.5 μM and a subsequent increase to higher values has no effect on the obtained signal probably due to the saturation of the electrode surface. Thus 1.5 μM was used as the optimum probe concentration in other experiments.

The effect of immobilization time was also studied and the results (Fig. 4b) showed that the amount of the immobilized PNA probe increases by increasing the immobilization time up to 15 min and then levels off. Indeed as presented in Fig. 4b, PNA adsorption occurs faster than that for the DNA counterpart, which may be due to the lack of electrostatic repulsion between the oxidized parts of GO and neutral PNA segments. Based on the obtained results, 15 min was selected as the optimum time for probe immobilization.

Based on the published reports, there is electrostatic repulsion among the oxidized parts of GO and negatively charged structure of DNA and hydrophobic physical adsorption and van der Waals attraction among the aromatic rings in the DNA structure and the hexagonal cells of GO.[49,66–69] But in the case of PNA, π–π attraction forces between the aromatic rings of nucleo-bases in PNA and the hexagonal cells of GO are present, but repulsion forces are absent due to the neutral backbone of PNA which results in facile binding of the PNA probe to GO even without electrolyte addition. Experiments conducted with probe solutions containing various NaCl concentrations verified this result.

There are some carboxylic acid groups with considerably diverse pK_a values in the GO structure. Since hydrophobic interactions are very necessary for the binding between PNA and GO, protonation of carboxylic acid groups causes the GO surface to become less polar which may result in stronger hydrophobic interactions and better PNA immobilization.

According to what has been mentioned, changing the solution pH can affect probe adsorption by changing the surface charge. As can be observed in Fig. 3S,† by decreasing the solution pH from 8 to 5, the PNA immobilization at the GO/PGE improved by approximately 35%; thus pH 5 was selected as the optimum pH for PNA probe immobilization. Subsequently, based on the obtained results, probe immobilization was conducted in phosphate buffer solution with pH 5 (PBS) containing 1.5 μM PNA probe for 15 min.

Hybridization detection

In the following, the applicability of the PNA probe modified electrode for ds-DNA detection was studied. Previous studies showed that the DNA probe was released from the electrode surface after hybridization due to the weak binding ability of ds-DNA to GO. In the present study, it is desired that the interaction of the PNA probe with ds-DNA results in probe detachment from the electrode surface as presented in Scheme 1.

For this purpose, some experiments were conducted with complementary ds-DNA (com-ds-DNA), non-complementary ds-DNA (NC-ds-DNA) and blank buffer; PNA-GO/PGE was dipped in these solutions for 30 min under stirring. The results showed that (Fig. 5) after interaction of the probe modified electrode

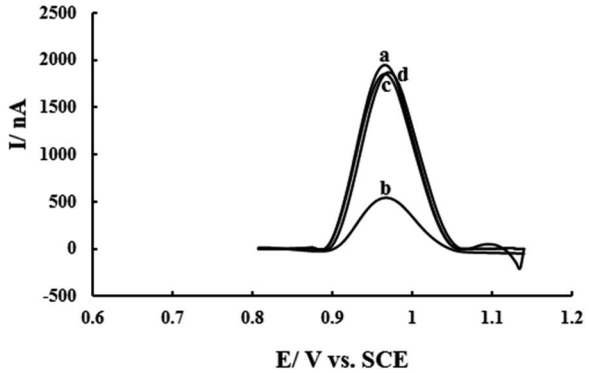


Fig. 5 DPV responses of the probe immobilized GO-PGE (a) before and after interaction with (b) com ds-oligonucleotides, (c) blank buffer and (d) NC ds-oligonucleotides. Probe immobilization conditions: 15 min in 1.5 μM PNA solution with pH 5; hybridization conditions: 1 nM ds-DNA with pH 7 for 30 min.

with com-ds-DNA, the guanine oxidation signal decreases, but the blank buffer and NC-ds-DNA sequence have a negligible effect on the initial probe oxidation signal. These results confirm that com-ds-DNA binds selectively to the PNA probe, induces PNA probe release from GO and results in a guanine oxidation signal decrease. But there is no loss of signal due to the NC-ds-DNA interaction or probe leaching in the buffer solution. Also, based on the experiments and obtained results (not shown), the signal of the ss-DNA modified electrode doesn't change properly after hybridization with ds-DNA which may be related to the different abilities of the PNA and DNA probe in hybridization with ds-DNA.

These consequences demonstrate the suitability of the PNA immobilized GO/PGE for biosensor construction and detection of ds-DNAs.

The recognition process in biosensors depends on some experimental conditions. Two important steps in this kind of biosensor are the hybridization between the PNA probe and target ds-DNA and then desorption of the hybridized probe from the GO modified electrode surface. Any factors related to these two steps can affect the results. Therefore, experimental conditions such as the probe immobilization method, probe concentration, pH value, and interaction time as the affective parameters for sensing efficiency were studied.

Effect of the probe immobilization method

Previous studies carried out using the PNA probe and PGE showed that activation of the PGE by applying a positive potential (1.8 V for 2 min) and probe immobilization under potentiostatic conditions could increase biosensor efficiency.[70–72] Therefore as an effective factor, the influence of the probe immobilization method on the hybridization results was studied. For this purpose, the probe was immobilized on the GO modified PGE surface using three procedures: (a) applying a positive potential (0.5 V) to the pre-activated electrode, (b) applying a positive potential (0.5 V) without electrode activation and (c) simple soaking in the probe solution, and then the prepared electrodes were used for hybridization with ds-DNA. The results presented in Fig. 6a indicate that the probe immobilization method has a major effect on the hybridization. Based on the obtained results, probe detachment from the electrode surface takes place only when the probe is immobilized by simple soaking, and for other methods in which the probe is immobilize by potential application, hybridization doesn't induce probe release from the electrode surface. Therefore, simple soaking of the GO modified electrode in the probe solution is selected for further experiments.

Effect of hybridization solution pH

As an important factor, the influence of the pH value on hybrid formation was investigated and is illustrated in Fig. 6b. By increasing the pH, hybridization efficiency decreased and pH 5 was selected as the optimized pH for hybridization detection.

Effect of hybridization time

The effect of hybridization time as one of the effective factors on the hybridization efficiency was studied and the results (Fig. 7a) showed that increasing the hybridization time up to 30 minutes results in more decrease in the DPV signal. This may be related to the better hybridization of the complementary sequences by increasing the interaction time. Indeed, it should be noted that the formed hybrids cannot be completely released from the electrode surface and may be present in the "free-standing" mode in the electrolyte solution. Better removal of the free-standing hybrids from the electrode surface can occur by

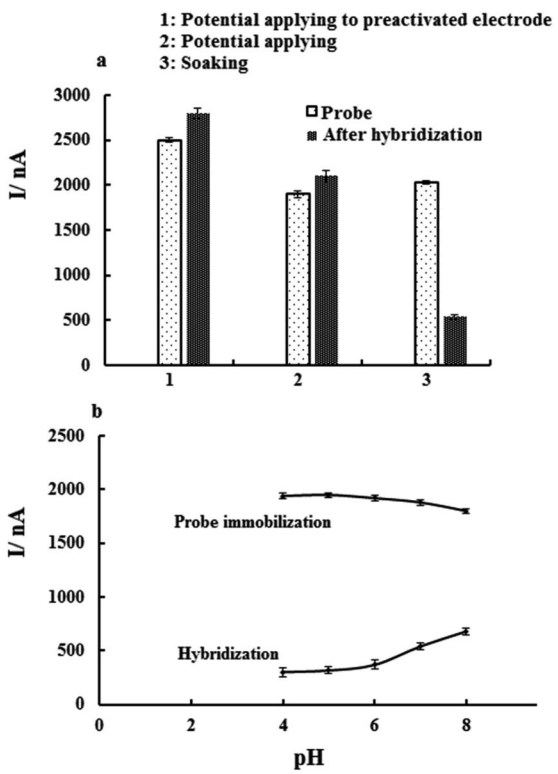


Fig. 6 Effect of (a) the probe immobilization method and (b) pH on the hybridization results.

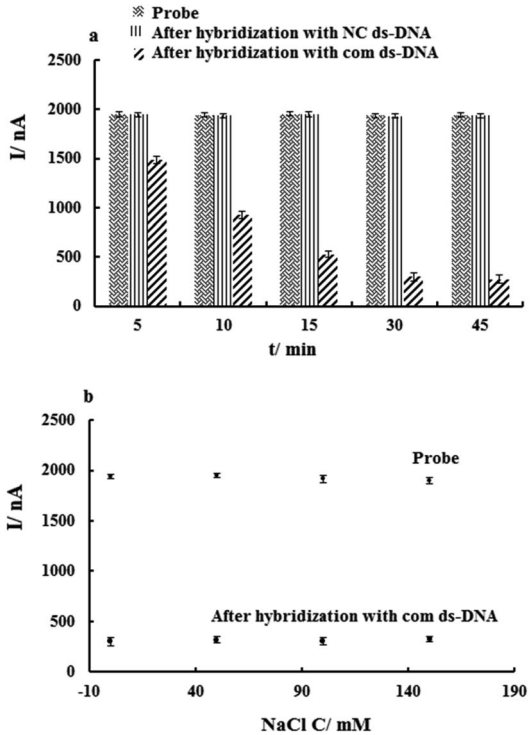


Fig. 7 Effect of (a) hybridization time and (b) NaCl concentration on the hybridization results. Probe immobilization conditions: 15 min in 1.5 μM PNA solution with pH 5; hybridization conditions: 1 nM ds-DNA with pH 5.

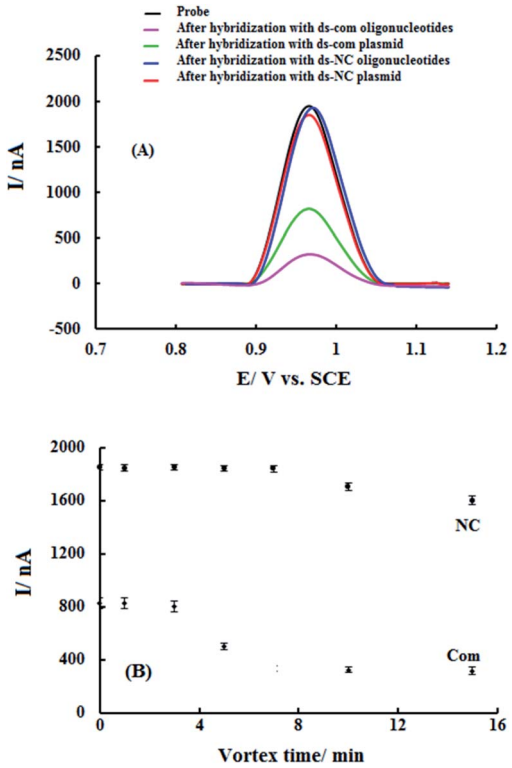


Fig. 8 (A) DPV responses of PNA-GO/PGE before and after interaction with com and NC double stranded DNAs; (B) variation of DPV signals of PNA-GO/PGE after hybridization with 1 ng μL^{-1} ds-Pl vortexed for different periods of time. Hybridization conditions: 1 nM ds-DNA with pH 5 for 30 min.

increasing the interaction time. As a result, for selective and sensitive detection of complementary sequences, hybridization has been carried out in an optimum time of 30 minutes.

Effect of NaCl concentration

To study the effect of NaCl concentration on the hybridization of the PNA probe with ds-DNA, hybridization was performed in 10 mM phosphate buffer (pH 5) with various amounts of NaCl. The experimental results (Fig. 7b) showed that when changing the NaCl concentration, no noticeable difference was observed in the results which confirms that NaCl concentration does not affect hybridization efficacy. This result may be related to the neutral backbone of PNA which leads to the absence of electrostatic repulsion between PNA and the target DNA. Indeed, similar experiments were carried out in 10 mM acetate buffer (pH 5) with various NaCl concentrations containing com-ds-DNA and the obtained results confirmed this conclusion (results not shown).

Double-stranded plasmid DNA detection

After successful detection of the target ds-DNA sequence in ds-oligonucleotides, the applicability of the proposed biosensor for the direct detection of com-ds-Pl was examined and the selectively of the proposed biosensor was investigated by experiments carried out with non-complementary sequences. For this purpose, complementary and non-complementary plasmid samples were subjected to the probe modified electrode and the results (Fig. 8A) showed that non-complementary sequences did not lead to a significant decrease in the guanine oxidation signal due to the absence of significant hybridization between the probe and non-complementary plasmid sequences. But after interaction of PNA-GO/PGE with com-ds-Pl, the guanine oxidation signal significantly changed. These results indicate

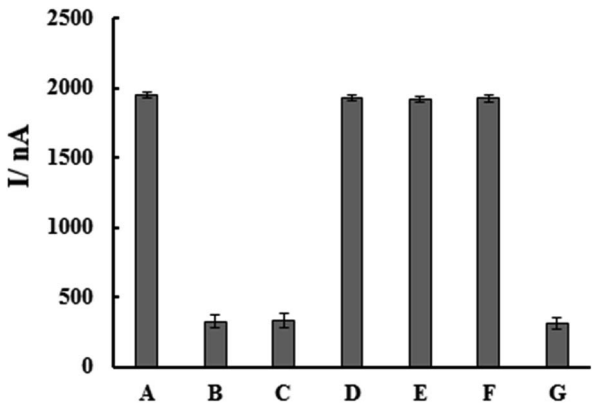


Fig. 9 Histogram showing the variation of DPV signals of PNA-GO/PGE (A) before and (B–G): after hybridization with B: 1 nM com-ds-oligonucleotide, C: 1 ng μL^{-1} com-ds-Pl, D, E, F: various concentrations (1, 10, and 100 nM) of NC-ds-DNA, and G: a mixture of 1 nM ds-com DNA and 100 nM NC-ds-DNA.

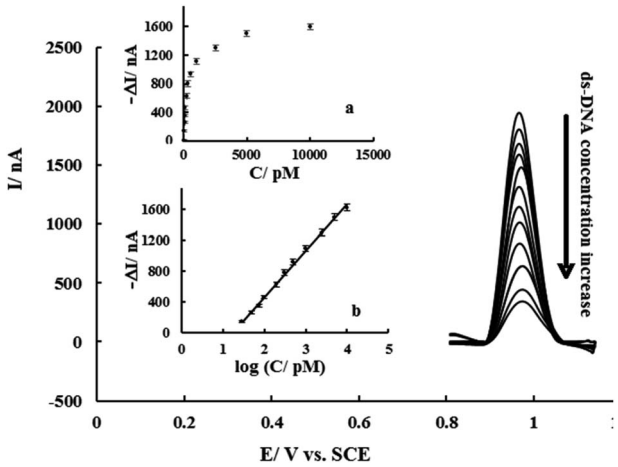


Fig. 10 DPV responses of the probe modified GO-PGE after hybridization with various concentrations of ds-com DNAs. Inset (a) variation of DPV signals *versus* ds-com oligonucleotide concentration; inset (b) calibration curve in the concentration range of 30 pM to 10 nM.

the applicability of the prepared biosensor for the detection of complementary ds-sequences in plasmid samples. Based on the obtained results (Fig. 8A), hybridization with ds-oligonucleotides results in a larger signal decrease than that with ds-Pl. This result may be related to the greater size of the plasmid compared to that of the short sequence oligonucleotides which leads to high steric hindrances and decreased hybridization efficiency. The previous study reported by Alipour *et al.*[73] showed that in the case of genomic DNA detection, the best results were obtained after DNA breakup by vortex generated shearing forces. Herein, the influence of plasmid DNA breakup on the obtained results was studied. For this reason, vigorous vortexing in different time periods was used for plasmid sample treatment to achieve fragmentation of the large DNA molecules, and the resultant vortexing sheered complementary and non-complementary plasmid DNAs used for hybridization with the PNA probe.

Based on the obtained results (Fig. 8B), an increased vortex time up to 7 min resulted in a signal decrease of com-ds-Pl without a noticeable decrease in the non-com-Pl signal; but at higher vortex times greater than 10 min, the non-complementary signal decreases too, which decreases the selectivity of the biosensor. Therefore 7 minutes was selected as the optimized time for the plasmid sample vortexing before hybridization.

The selectivity of the biosensor was examined in a mixed solution of complementary and non-complementary plasmid samples (Fig. 4S†) and the results (Fig. 9) showed that PNA-GO/PGE could detect complementary sequences in the presence of a 100-fold concentration of non-complementary ds-DNAs. This proves the sequence-specific and selective response of the biosensor to ds-DNA.

Diagnostic performance

Biosensing was performed under the optimized conditions and assessed by monitoring the changes in the guanine oxidation signal of the probe modified GO/PGE after interaction with different concentrations of ds-complementary DNA (Fig. 10).

As displayed in inset (a) of Fig. 10, the oxidation current decreased significantly when increasing the target concentration. The results (inset b of Fig. 10) showed that there was a good linear relationship between ΔI and target concentration in the range of 30 pM to 10 nM for ds-oligonucleotides with a detection limit of 1.3 pM ($3S$/slope). The obtained linear regression equation was $\Delta I = -730.89 + 598.88 \log C$ (C: pM, $R^2 = 0.995$).

The analytical parameters of some ds-DNA detection electrochemical biosensors are summarized in Table 1. It can be seen that in spite of the fast, simple and label-free preparation and operation, the analytical parameters of the proposed biosensor are comparable with other electrochemical reports. In addition, this work is comparable to the fluorometric articles reported for the detection of ds-DNA with LODs of 260 pM and 1.4 nM.[41,42]

Reproducibility and stability of the biosensor

For fast and accurate detection, the reusability of the sensor surface is very important. To examine this matter, after each

Table 1 Comparison of the analytical parameters of this ds-DNA biosensor with those of others[a]

Electrode (signal amplification or indicator)	Linear range (nM)	LOD	Ref.
AuE* (MB*)	0.01–0.1	1.8 pM	32
AuE (MB)	0.01–0.1	4.97 pM	33
AuE (MB)	10–300 pg mL^{-1}	9.5 pg mL^{-1}	34
AuE (MB)	5 and 200 ppm	1.58 ppm	35
AuE (MB)	0.01–10	4.15 pM	36
AuE (HCR* + AuNPs*)	0.015–1.0	2.6 pM	37
AuE	0.35–25	275 pM	38
AuE (SI-eATRP*)	10^{-6} to 1.0	0.47 fM	39
AuE (EIS*)	1–100	0.1 nM	40
PGE*	0.03–10	1.3 pM	This work

[a] AuE: gold electrode; MB: methylene blue; HCR: hybridization chain reaction; AuNPS: Au nanoparticles; SI-eATRP: surface-initiated electrochemically mediated atom transfer radical polymerization; EIS: electrochemical impedance spectroscopy; PGE: pencil graphite electrode.

hybridization detection, the electrode surface was thoroughly washed and reused for probe immobilization without the need for pre-treatment and GO modification of the PGE. The results showed that by using this procedure, the probe signal recovered and this could be performed up to nine times (RSD < 1.9%). Also, the regenerated electrodes were used for hybridization detection with the RSD of sensitivity lower than 3.3%. These results prove the reusability of the sensor.

The stability of the electrode was assessed by guanine oxidation current determination after different time intervals and the results showed that there was no significant change (lower than 5.5%) in the current intensity of the probe-modified electrode after 12 days of storage at 4 °C, which indicated the fine stability of the biosensor. In a parallel study, 9 electrodes were prepared similarly and used for hybridization detection of 50, 100 and 1000 pM ds-DNA after 12 days. The obtained results with an RSD lower than 6.1% in the current intensity of these electrodes after hybridization indicated the fine stability of the biosensor.

Conclusions

In conclusion, we have developed a novel label-free electrochemical biosensor using GO as the electrode modifier and PNA as a detection probe. The change in the guanine oxidation signal of the probe modified electrode before and after hybridization with double-stranded DNA was used for the detection of complementary strands. In this biosensor, the detection mechanism is based on the detachment of the probe from the electrode surface after hybridization with complementary ds-DNA. The proposed biosensor presented a detection range from 30 pM to 10 nM, and a low detection limit of 1.3 pM was achieved. The proposed method could be considered as a platform for the recognition of specific ds-DNA sequences in genomic DNA samples.

Conflicts of interest

The authors declare there are no conflicts of interest.

Acknowledgements

We gratefully acknowledge financial support from the Research Council of Urmia University.

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