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A Nucleic Acid Biosensor for Detection of Hepatitis C Virus Genotype 1a Using Poly(L-Glutamic Acid)-Modified Electrode

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Abstract An electrochemical nucleic acid biosensor based on label-free DNA detection method was prepared for the first time by using electropolymerized poly(L-glutamic acid)-modified pencil graphite electrode (PGA/PGE) for detection of hepatitis C virus genotype 1a (HCV1a). Inosine-substituted 20-mer probes related to the HCV1a were immobilized onto PGA/PGE surface by covalent linking with the formation of amide bonds. Square wave voltammetry (SWV) was used to monitor the oxidation signal of guanine in the hybridization events, which gave an oxidation peak at +1.05 V. An increase in the oxidation signal of guanine was showed by hybridization of the probe with the complementary DNA. Noncomplementary oligonucleotides were also used to investigate the selectivity of the biosensor. The proposed nucleic acid biosensor was linear in the range of 50 nM to 1.0 μM, exhibiting a limit of detection of 40.6 nM. Finally, single-stranded synthetic PCR product analogues of HCV1a were performed in optimal condition. This PGA-modified nucleic acid sensor is cost-effective and disposable, and besides, it has superior electrocatalytic effect on the oxidation of guanine.

Keywords Nucleic acid biosensor · Hepatitis C virus · Poly(L-glutamic acid) · Inosine · Square wave voltammetry

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Introduction

Recently, the detection of specific oligonucleotide sequences has fascinated great attention of scientists due to its potential applications in forensic identification, epidemic prevention, and disease diagnosis [1]. To detect specific DNA sequences, different techniques were offered including electrochemistry [2], fluorescence [3], radiochemical assays [4], surface plasmon resonance spectroscopy [5], and quartz crystal microbalance [6]. Due to advantages such as being simple, inexpensive, reliable, sensitive, and selective, electrochemical detection is an attractive method for genetic detection [1]. Since it provides both base pair recognition of DNA probes and minimized sensing platform at the same time, studying on electrochemical DNA biosensors has attracted tremendous interests. Principle of methods for immobilizing probe oligonucleotides to the transducer is availability of functional groups at the transducer surface [7]. The performance of the biosensors depends on the immobilization of the probe DNA onto the transducer surface, and surface modification method must be suitable with the related sensing procedure [8]. Several methods have been offered to immobilize the probe DNA to the transducer surface of biosensors: biotinylated DNA probes attached through biotin-avidin interaction on electrode surface [9], the self-assembling monolayer (SAM) on gold electrodes [10], or electropolymerization that produces probes of different length [11].

Poly-amino acid films have a good alternative to manufacture electrochemical sensors due to the presence of some functional groups. In addition, they have some advantages such as rapid preparation and simple, reproducibility, and good stability [12]. Among them, poly(L-glutamic acid) (PGA) rarely used to develop electrochemical sensors, although structure of PGA can be very suitable as a biopolymer model. PGA has biodegradable and controllable size chains which are inherently forming L-glutamic acid linked together by means of amide bonds. Deposition of repetitive units formed between the α-amino and δ-carboxylic acid functional groups and free protonated carboxylic groups (pKa=4.07) onto electrode surface is an easy procedure. Electrooxidation of L-glutamic acid generates PGA film which displays excellent property in electrochemical application [12–14].

Viral hepatitis is an inflammation of the liver that can be caused by different viruses including hepatitis A, B, C, D, and E (HAV, HBV, HCV, HDV, and HEV). The infection activates immune response against infected cells that causes to the destruction of hepatic cells [15, 16]. According to the World Health Organization, hepatitis C virus (HCV) infection currently affects approximately 170 million people worldwide [17]. Some causes of HCV infection are renal dialysis, blood transfusions, organ transplantation, and intravenous drug use [17]. The observation of HCV RNA in plasma or serum is important for both diagnosing or confirming active viral infection and evaluating patient response to therapy [18, 19]. Furthermore, HCV genotyping is also important for predicting HCV treatment response and period. Various procedures for genotyping HCV have been introduced, such as type-specific PCR, direct DNA sequencing, line probe assays, restriction fragment length polymorphism, heteroduplex mobility analysis by temperature gradient capillary electrophoresis, primer-specific and mispair extension analysis, and denaturing high-performance liquid chromatography. To identify different genotypes of HCV, the analysis of nucleotide sequence is the reference method. However, since this method has some disadvantages such as being time-consuming, expensive, and requiring special equipment for sequencing, it has been restricted for large-scale clinical studies [7].

Various articles have been released on electrochemical nucleic acid sensor for HBV in the literature [20, 21], but there are few articles for detection and quantification of HCV based on simple and reliable electrochemical methods [22, 23].

In the present work, a simple label-free nucleic acid sensor for hepatitis C virus genotype 1a (HCV1a) detection using an inosine-substituted probe was developed, and electrochemically synthesized PGA films was used for the first time as nucleic acid biosensor. First, the PGA film was investigated onto pencil graphite electrode (PGE). Then, the probes with modified amino groups were immobilized onto a PGA film surface by covalent. After probe immobilization, the hybridization with complementary sequences was performed, and optimal conditions were determined for biosensor. Square wave voltammetry (SWV) was also used as monitoring method. The application of biosensor for synthetic PCR analogue of HCV1a was performed in optimal conditions.

Experimental

Reagents and Instrumentation

Electrochemical measurements were achieved by using a CHI 1020B electrochemical analyzer with a three-electrode cell. Reference and auxiliary electrodes were an Ag/AgCl and Pt wire electrode (3 M KCl), respectively. Working electrode was a disposable pencil graphite electrode (surface area of 0.095 cm^2). The pencil graphite was purchased as pencil lead from a local bookstore, which was obtained Tombow of type HB. Orion Model 720A pH/ion meter was used in order to measure pH values of buffer solutions. Temperature control was carried out by a Grant W14 thermostat. Scanning electron microscopy of the Au–Pd-coated compounds was done by using a JEOL JEM 100 CX II scanning electron microscope (JEOL, Peabody, MA) equipped with a link analytical system. Used electron energy was 20 keV. L-Glutamic acid, *N*-hydroxysulfosuccinimide (NHS), and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochlorides (EDC) were purchased from Sigma. All other chemicals were also obtained from Sigma. Double-distilled water was used to prepare all the solution.

In this work, the inosine-substituted 20-mer oligonucleotide called pHCV1a complementary to the consensus sequence of HCV1a core protein gene was used as the probe, and its complementary DNA oligonucleotide (cHCVa1) related to the consensus sequence of HCV1a was used as target DNA. 3mm-HCV1a and ncHCV3a oligonucleotides correspond to three-base mismatch target of HCV1a sequence and universal sequence of HCV3a core/E1 region, respectively.

All of the oligonucleotides were obtained as lyophilized powder from Ella Biotech Company (Germany) (Table 1). The stock solutions of the oligonucleotides (500 μg/mL) were prepared with ultra-pure distilled water and kept frozen. More diluted solutions of capture probes were prepared using 0.50 M acetate buffer (by mixing of CH_3COOH and CH_3COONa and adjusting the pH with NaOH, pH 4.80) solution containing 20 mM NaCl. More dilute solutions of targets were prepared using 2x SSC buffer solution (30 mM sodium citrate, 0.3 M NaCl, pH 7.0). A fresh PGE surface was used for each measurement consisted of the immobilization/detection cycle. Experiments were carried out at room temperature in an electrochemical cell.

Table 1 Oligonucleotide sequences employed

Oligonucleotide name	Length (mer)	Sequence
Prob HCV1a (pHCV1a)	20	5′NH_2-$(CH_2)_6$-CCCACCCICAICCCTCATTA-3′ (I=inosine)
Complementary DNA target (cHCV1a)	20	5′-TAATGAGGGCTGCGGGTGGG-3′
Noncomplementary DNA target (ncHCV3a)	17	5′-GTGGGTGATATGTGTGG-3′
Three-base mismatch of target HCV1a (3mm-HCV1a)	20	5′-**C**AATGAGGGC**G**GCGGGTGG**A**-3′
Synthetic HCV1a PCR analogue (RS-HCV1a)	50	5′TAATGAGGGCTGCGGGTGGGCGGGAT GGCTCCTGTCCCCCCGTGGCTCTC-3′
Synthetic HCV3a PCR analogue (RS-HCV3a)	50	5′GTGGGTGATATGTGTGGGGCCGTCTTC CTCGTGGGACAAGCCTTCACGTT-3′

Mismatches are indicated by bold and underlined characters

Preparation of PGA-Modified Pencil Graphite Electrode

Electrical contact with the lead was achieved by using copper drill chuck (diameter of the inner hole is 0.3 mm). The pencil lead was fixed vertically and immersed in the solution as working electrode. All leads had a total length of 50 mm and a diameter of 0.3 mm. A total of 5 mm of lead was immersed in solution per measurement. The surface was polished on a weighing paper to smooth before each use.

PGE was immersed in a 5.0 mL solution of 0.02 M L-glutamic acid and pH 7.0 phosphate buffer solutions (PBS) (0.1 M NaH_2PO_4–Na_2HPO_4, and then adjusting the mixture solution's pH with NaOH). The electropolymerization of L-glutamic acid was performed upon the electrode surface by the cyclic voltammetric scans between −0.8 and +2.0 V at a scan rate of 100 mV/s (vs Ag/AgCl electrode (3 M KCl)) and 50 cycles. After electropolymerization, PGA film was rinsed with deionized water to remove the unreacted glutamic acid monomer.

Prob Immobilization

Synthetic oligonucleotides, having a terminal amino group and a six carbon arm spacer, were covalently immobilized onto PGA-modified PGE (ssDNA/PGA/PGE). Carboxylic groups that existed in poly(L-glutamic acid) were activated by interaction with an aqueous solution containing 8.0 mM EDC and 5.0 mM NHS for 60 min. The probe sequence was subsequently immobilized onto PGA/PGE surface by covalently attachment from 5′$NH_2(CH_2)_6$ link. The immobilization proceeded through the formation of amide bonds between free carboxylic group of PGA/PGE onto the electrode surface and the amino-terminal end of the oligonucleotides.

Hybridization

The probe-immobilized nucleic acid sensor was immersed into the hybridization solution (containing the fully complementary or noncomplementary sequences) for 30 min. After hybridization, the biosensor were immersed in washing buffer containing 2xSSC+0.1 % SDS (30 mM sodium citrate, 0.3 M NaCl, 0.1 % sodium dodecyl sulfate, pH 7.0) for 5 min to keep from the unspecific bounding [24]. Since the highest ratio of complementary signal to

noncomplementary signal (c/nc) was obtained with 1x SSC solution (0.15 M NaCl, 15 mM sodium citrate, pH 7.0), it was chosen as type of hybridization buffer (see Supporting Information, Fig S1a). In addition, the ionic strength of SSC buffer was evaluated and 2x SSC was selected as hybridization buffer because of the highest c/nc ratio (see Supporting Information, Fig S1b). For optimization study, 1.0 μM of target solution was used.

Electrochemical Measurement

The nucleic acid sensor was then dipped into the acetate buffer solution (ABS) (pH 4.80) for voltammetric measurement. The guanine oxidation signal at +1.05 V was measured by using SWV technique with following experimental conditions: potential range between +0.75 V and +1.25 V, frequency of 25 Hz, step potential of 0.01 V, and amplitude of 0.025 V. The study procedure steps were illustrated in Fig. 1.

Stability Studies

To determine the long-term dry storage, the biosensor (ssDNA/PGA/PGE) was dipped to buffer solutions (1x SSC buffer or 1x SSC containing 2.5 % (*w/v*) of glucose) for 5 min., dried under room temperature, and stored in a refrigerator at +4 °C. Electrochemical measurements were carried out after 1, 2, 3, 4, 5, and 15 days. Before each measurement, the electrodes were rinsed with deionized water and incubated in 1x SSC for 30 min. Results were expressed as percentage of signal decreases according to freshly prepared biosensor response.

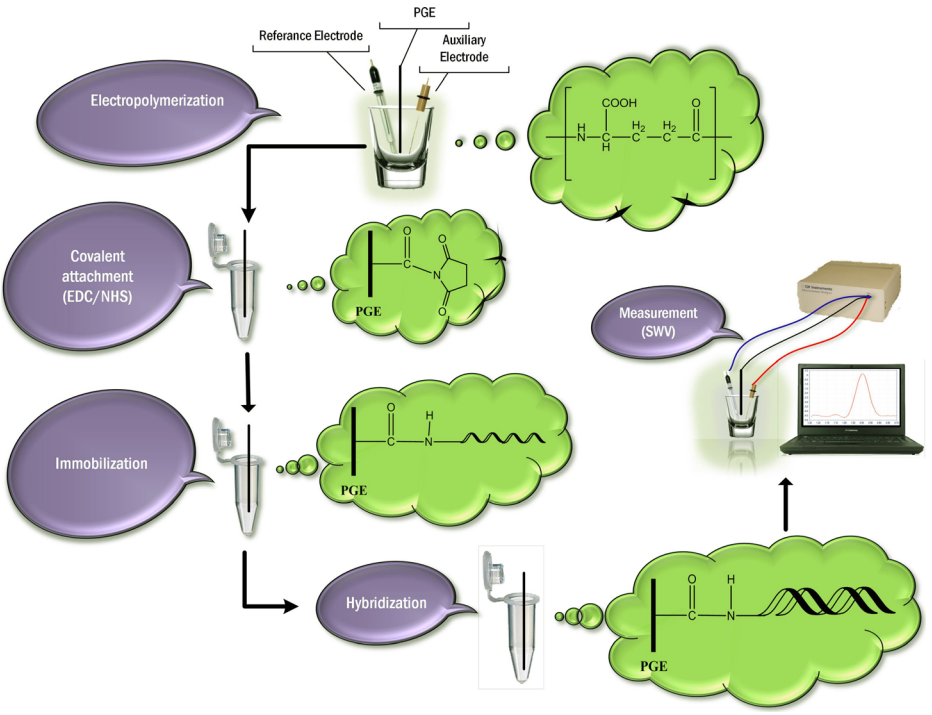

Fig. 1 Schematic illustration of experimental procedure for the detection of HCV1a

Results and Discussion

Direct label-free electrochemical detection having a triggered change of electrical signal by hybridization event has attracted researchers in the last years. Because this technique does not need the indicator addition, association, and detection steps, it simplifies the sensing protocol and provides an instant detection of the hybrid formation [25].

In this study, the label free nucleic acid sensor depends on the square wave voltammetric transduction of the hybridization reaction between capture probes and the target DNA sequences. Hybridization is determined by the presence of the guanine oxidation signal at about +1.05 V, but there is no guanine signal from inosine-substituted probes in this potential. In addition, compared to PGE, PGA-modified biosensor has an excellent electrocatalytic activity on guanine oxidation peak (Fig. 2).

The changes in the magnitude of the guanine oxidation peak were showed hybridization of the target with the immobilized probe on the surface. As a result of the hybridization to complementary target, a large electrochemical response was recorded; on the other hand, when voltammetric measurement was performed after hybridization to noncomplementary target, a much smaller response was recorded. This difference in the magnitude of the electrochemical response can prove the complementary hybridization event.

Electropolymerization of L-Glutamic Acid

An L-glutamic acid polymer film-modified electrode was prepared by electropolymerization of L-glutamic acid at the PGE. As shown in Fig. 3, an oxidation current appeared at about +1.7 V which can be attributed to a peak of free radical. At the second cycle, the sharp peak disappeared whereas a new wide peak appeared at about +1.8 V. The new peak was increased with the number of cycles which could belong to the peak of the polymer [26].

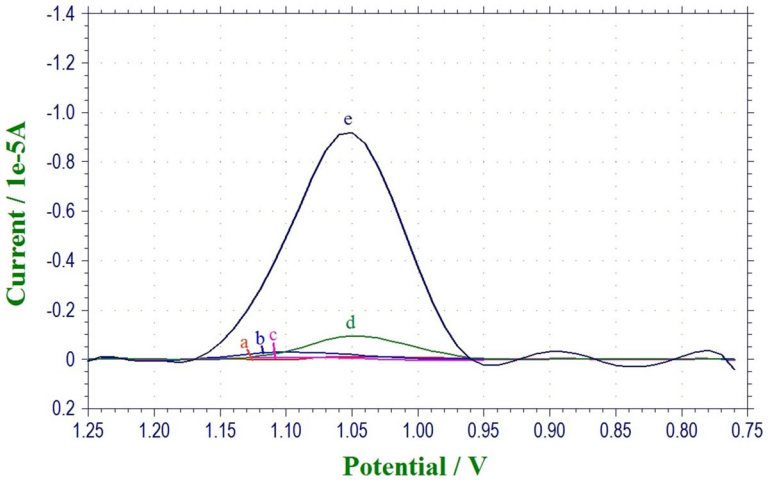

Fig. 2 Square wave voltammograms of *a* bare PGE, *b* bare PGA/PGE, *c* ssDNA (inosine-substituted)/PGA/PGE, *d* ssDNA/PGE hybridized with cHCV1a (0.5 μM), *e* ssDNA/PGA/PGE hybridized with cHCV1a (0.5 μM) in 0.5 M acetate buffer solution pH (4.8)

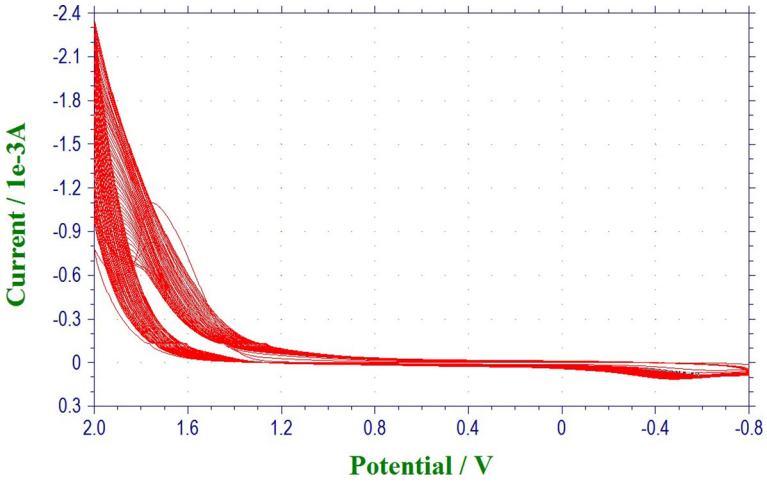

Fig. 3 The electropolymerization of glutamic acid on PGE surface was performed using cyclic scans between −0.8 and +2.0 V at a scan rate of 100 mV/s for 50 cycles in 0.1 M PBS (pH 7.0) containing 0.05 M glutamic acid

After 50 cycles, the electropolymerization of L-glutamic acid was finished, and to remove the physically adsorbed material, the polymer film electrode was washed with distilled water. After dried in air, a gray color PGA film was observed at the electrode surface clearly, showing that a polymer film was figure out on the electrode surface (see Supporting Information, Fig. S2). Some amino acids, such as lysine [27] and threonine [28], can be electropolymerized at the carbon electrode surface. Similarly, possible electropolymerization mechanism of L-glutamic acid at the PGE was illustrated in Fig. S3 (see Supporting Information). L-Glutamic acid linked together through amide bonds. Repetitive units were linked between the δ-carboxylic acid and α-amino functional groups, and free protonated carboxylic groups (pKa=4.07) were simply accumulated on an electrode surface [12, 26].

SEM Image of PGE and PGA/PGE

Morphologies of PGE and PGA/PGE were investigated by scanning electron microscopy (SEM) (Fig. 4a, c: 2000 magnitude; 4b, d: 30,000 magnitudes). The surface morphology of bare PGE (Fig. 4a–b) is completely different from than that of PGA (Fig. 4c–d). One can clearly see that PGE has almost smooth surface morphology, whereas PGA/PGE has layered particles on the surface which can be attributed the formation of PGA onto PGE (Fig. 4b, d).

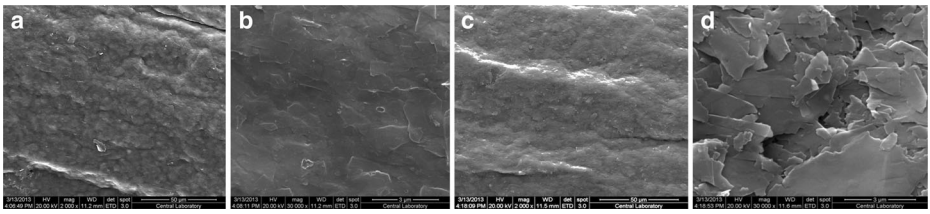

Fig. 4 SEM micrographs of the bare PGE (**a**–**b**) and PGA/PGE (**c**–**d**)

Effect of Electropolymerization Cycles

A significant factor on morphology and performance of PGA is electropolymerization cycle, which eventually affects the oxidation current of guanine [29]. The effect of electropolymerization cycles on the current response of guanine (1.0 μM cHCV1) is exhibited in Fig. S4 (see Supporting Information). It is demonstrated that when cycles shift from 5 to 100, the oxidation current increases. This increment can be explained with accumulation of more PGA films on the PGE surface. Highest guanine signals were obtained at 100 cycles. However, standard deviation in 50 cycles was smaller than 100 cycles. As it can be seen from Fig. S4 (see Supporting Information), current values are nearly same at 50 and 75 cycles. Moreover, as the number of cycles increases, the time required for the fabrication of biosensors increases as well. Therefore, 50 cycles were chosen as cycle number and the fabrication of biosensors time was also shortened.

Optimization of Conditions

Probe concentration is an important factor for hybridization efficiency. It was reported in the literature that the concentration of immobilized DNA can affect the hybridization thermodynamics and the biosensor selectivity [30]. In this work, we used a series of concentration of the probe solution so as to find an optimal probe density for hybridization efficiency and results are given in Fig 5a. Optimum probe concentration was determined by comparing c/nc ratio [31]. As seen in Fig. 5a, the highest ratio for c/nc was obtained at a probe concentration of 1.0 μM. Thus, 1.0 μM was selected as the optimal probe concentration for all subsequent experiments. Above this concentration, diminished guanine oxidation signals were observed, and c/nc ratio was remained nearly constant. This can be due to the presence of steric hindrance [32]. Finally, higher probe densities could cause steric hindrance of the target which lead to poorer affinity and decreased guanine oxidation signals [33]. In addition, compared to 0.5 μM, a high c/nc ratio was obtained by 0.1 μM probe concentration. This behavior can be explained by low probe concentration at electrode surface possessed a limited number of biorecognition sites, and hence, nonspecific adsorption of target can increase guanine oxidation signal [34].

It is known that the hybridization time between probe and target oligonucleotide on the electrode surface affects the determination of target DNA [35]. Hybridization step was performed 1.0 μM of the target solution (containing complementary or noncomplementary strand) on the biosensor for 10, 20, 30, 60, and 90 min in 2x SSC buffer pH 7.0. In Fig. 5b, the c/nc discrimination increased with incubation period for times up to 30 min. However, diminished c/nc discrimination was observed if incubation times exceeding 30 min. Thus, 30 min was employed optimum hybridization time for the best discrimination between complementary target and noncomplementary oligonucleotide. Because of this, in all the following experiments, 30 min was used as hybridization time.

In order to explore the effect of the target solution temperature on the hybridization, probe oligonucleotide immobilized PGA/PGE was immersed for 30 min in 2x SSC buffer (pH 7.0) at different temperatures ranging from 25 to 70 °C and containing 1.0 μM complementary oligonucleotide. Then guanine oxidation signal was measured by SWV. As shown in Fig. 5c, the maximum efficiency of hybridization was obtained in 60 °C. The optimum

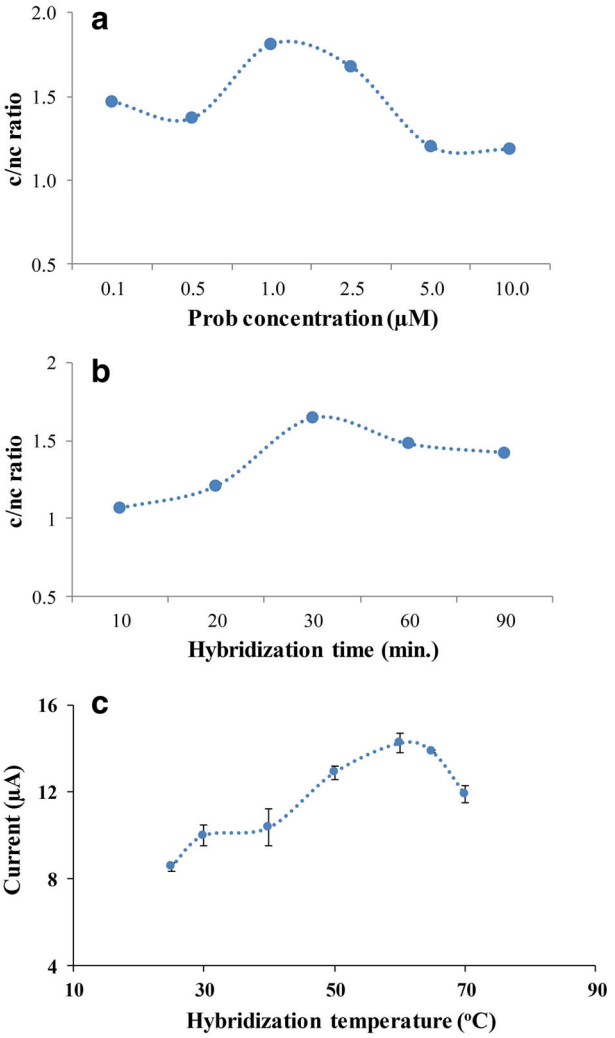

Fig. 5 **a** Probe concentration effect onto the complementary/non-complementary signal ratio (c/nc). Hybridization: 1 μM of the complementary and non-complementary sequence solutions (in 2x SSC). Measurement: SWV (frequency=25 Hz, step potential=0.01 V, amplitude=0.025 V) in an unstirred acetate buffer solution (pH 4.8), between +0.75 and +1.25 V. **b** Hybridization time effect onto the complementary/noncomplementary signal ratio (c/nc). Probe and target concentrations were 1.0 μM. Measurement: SWV (frequency=25 Hz, step potential=0.01 V, amplitude=0.025 V) in an unstirred acetate buffer solution (pH 4.8), between +0.75 and +1.25 V. Each experiment has been repeated three times. **c** Guanin oxidation signals versus hybridization temperature. *Error bars* represent the standard deviation of three independent measurements at each cHCV1a

hybridization temperature was thus selected as 60 °C. By increasing the hybridization temperature up to 60 °C, the signal of complementary target increased, and after this temperature, it decreased. This can be explained that high temperature would cause DNA destabilization and denaturation, and hence, guanine oxidation signal of target can be diminished in high temperature [36]. The current response of the biosensor is adequate for further biosensor studies even at room temperature of 25 °C.

Selectivity of the Nucleic Acid Sensor in Optimized Condition

In order to investigate the proposed nucleic acid sensor responds selectively to the target complementary oligonucleotide, a hybridization experiment with some oligonucleotides was performed. Three different target DNAs including the perfect complementary target (cHCV1a), mismatch (ncHCV3a), and three-base mismatch (3mm-HCV1a) were chosen to examine the selectivity of the sensor. In addition, suggested nucleic acid sensor was subjected to RS-HCV1a and RS HCV3a including 50 bases as synthetic PCR analogues of HCV. The current of SWV of guanine on the 1.0-μM probe-modified PGA/PGE after hybridization with ncHCV3a and 3 mm-HCV1a oligonucleotides was to −7.65±0.57 and −8.04±0.78 μA, respectively, which after hybridization with cHCV1a changed to −11.6±0.49 μA (Fig. 6a). The significant current differences of target DNA demonstrates that the proposed sensor readily discriminates between perfect-matched and mismatched or three mismatched oligonucleotides. The selectivity of the nucleic acid biosensor was also achieved in synthetic PCR analogues which are related to HCV1a and HCV3a. As depicted in Fig. 6b, proposed nucleic acid sensor successfully discriminated synthetic PCR analogues which are related to HCV1a and HCV3a. The current of SWV of guanine for RS-HCV1a and RS-HCV3a was −5.01±0.35 and −3.70±0.09 μA, respectively. Current changes of shorter synthetic DNA are higher than 50 bases synthetic PCR analogues. This can be attributed to the greater steric hindrance of these synthetic PCR sequences compared to the shorter oligonucleotides. In addition, hybridization in solution is easier compared to a solid support [37]. This can be resulted in weaker electrochemical signal for longer sequences on a solid support.

Stability of the Nucleic Acid Biosensor

The examination of dry storage stability was carried out for 15 days both with and without stabilizing agent. The stability of biosensor was limited without stabilizing agent. After 24 h, 30 % decrement of the peak current values was observed, while after 2 days, the peak current values of the biosensor decreased rapidly and lasted about same values for 15 days (Fig. 7). Similar results were reported by Li et al. In this work, it is showed that response of the biosensor was rapidly decreased at end of the third day [35]. In contrast to the performance above, Lai et al. reported that when glucose was used as stabilizing agents, the nucleic acid biosensor had a good stability with only about 9 % signal decreases after 32 days [38]. Same

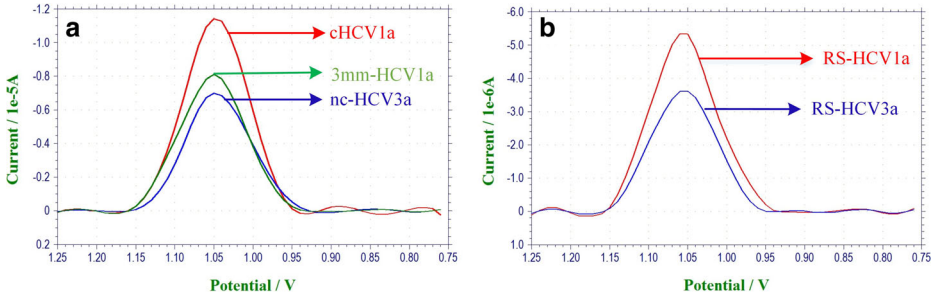

Fig. 6 Square wave voltammograms of guanine oxidation on prob-immobilized PGA/PGE. **a** cHCV1a, ncHCV3a, and 3mm-HCV1a oligonucleotide discrimination. **b** Complementary HCV1a (RS-HCV1a) and noncomplementary HCV3a (RS-HCV3a) synthetic single-stranded PCR product analogues discrimination

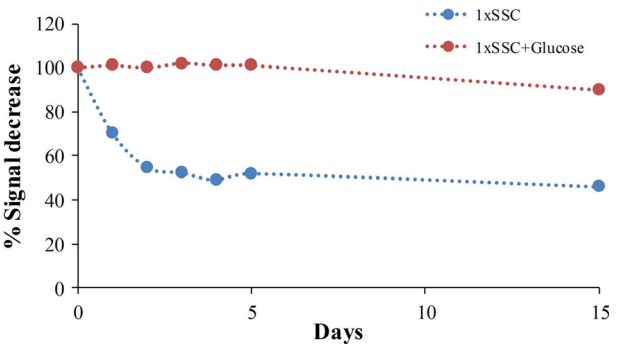

Fig. 7 Dry storage stability of nucleic acid biosensor in the presence or absence of stabilizing agents during 15 days. Measurement was performed in optimal condition (probe concentration is 1.0 μM in biosensor, hybridization solution contains 1.0 μM complementary HCV1a sequences. After hybridization, biosensor was dipped in 1x SSC buffer or 1x SSC buffer with 2.5 % (*w/v*) glucose)

results were also observed in our study. The peak current values of the biosensor were almost same values of their first measurement values when glucose was used as stabilizing agent. The %signal decrease post storage was just about 10 % of their first measurement in 15th day (Fig. 7).

Diagnostic Performance of the Sensor

The diagnostic performance of this PGA-modified biosensor was examined by using a series of concentrations of cHCV1a. Figure 8 illustrates the SWV peak current strongly relied on the concentration of cHCV1a. As the cHCV1a concentration was increased from 50 nM to 5 μM, SWV peak increased and leveled off at 10 μM. There is a linear dependence between SWV signal and cHCV1a concentration from 50 nM to 1.0 μM with a correlation coefficient of

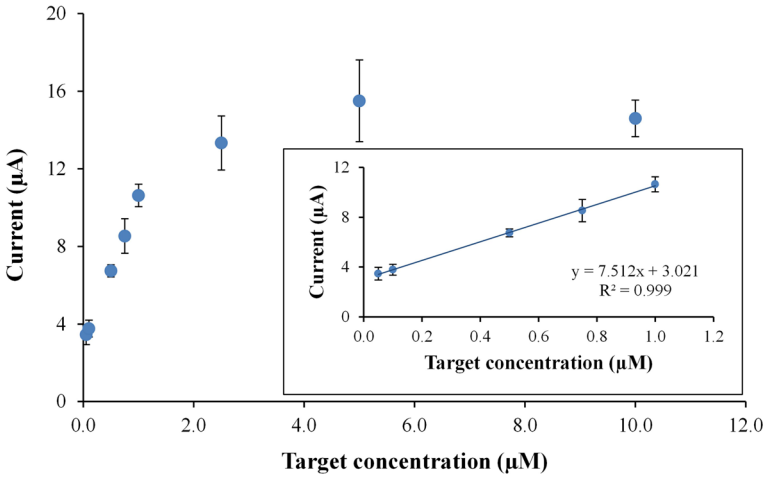

Fig. 8 Plot of the peak current vs. the concentration of cHCV1a from 50 nM to 10 μM. *Inset*: calibration graph at concentration range 50 nM–1.0 μM

0.999 (inset of Fig. 8). The limit of detection was determined by using the equation $y_{LOD}(I)=y_B+3S_{y/x}$, where $S_{y/x}$ standard deviation of blank (in this study, standard deviation of the calibration graph) and y_B is signal of the blank (in this study intercept of calibration graph) [39]. Also, regression equation was $y(I)=3.02+7.51c$ (μM). The limit of detection was calculated 40.7 nM. This limit of detection is acceptable with those reported for the other electrochemical hybridization detections [40, 41]. The relative standard deviation was measured as 6.8 % using three different ssDNA/PGA/PGE (target concentration 1 μM). This result shows that the detection method has a good reproducibility.

Conclusion

In the present study, a novel disposable and label free electrochemical nucleic acid biosensor was prepared by using PGA/PGE for the detection of HCV1a. The carboxyl group of PGA reacts with the amino group-modified probe DNA. Thus, PGA can be considered as a good candidate for the immobilization of DNA and highly useful for biosensing and bioelectrochemical applications. Although PCR step has not been completely eliminated for nucleic acid biosensors, the present biosensor has some advantages such as favorable dry storage stability, and modification was simple (one step modification). In addition, fabrication of biosensor is inexpensive due to using PGE. Furthermore, inosine-substituted probe eliminates the usage of indicators; it makes the sensing protocol easier and safer, since generally, toxic or carcinogenic label did not use to construction of it.

Experimental results showed that the PGA-modified nucleic acid biosensor exhibits excellent electrocatalytic effect on the oxidation of guanine. Prepared biosensor was highly selective. After optimizing the experimental parameters, effective discrimination against ncHCV3a and 3mm-HCV1a was obtained by ssDNA/PGA/PGE nucleic acid sensor. In addition, it successfully discriminated synthetic PCR analogues. The offered nucleic acid sensor showed wide linear detection range, good sensitivity, satisfactory reproducibility, long-term stability by stabilizing agent, low limit of detection, and relevantly short complete detection time (about 2.5 h including modification of the PGEs, hybridization, and detection).

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