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Introduction of hematoxylin as an electroactive label for DNA biosensors and its employment in detection of target DNA sequence and single-base mismatch in human papilloma virus corresponding to oligonucleotide

Navid Nasirizadeh[a], Hamid R. Zare[a,*], Mohammad Hossein Pournaghi-Azar[b], Mohammad Saeid Hejazi[c,d,**]

[a] Department of Chemistry, Yazd University, P.O. Box 89195-741, Yazd, Iran
[b] Electroanalytical Chemistry Laboratory, Faculty of Chemistry, University of Tabriz, Tabriz, Iran
[c] Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center & Pharmaceutical Nanotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

For the detection of DNA hybridization, a new electrochemical biosensor was developed on the basis of the interaction of hematoxylin with 20-mer deoxyoligonucleotides (from human papilloma virus, HPV). The study was performed based on the interaction of hematoxylin with an alkanethiol DNA probe self-assembled gold electrode (ss-DNA/AuE) and its hybridization form (ds-DNA/AuE). The optimum conditions were found for the immobilization of HPV probe on the gold electrode (AuE) surface and its hybridization with the target DNA. Electrochemical detection of the self-assembled DNA and the hybridization process were performed by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) over the potential range where the accumulated hematoxylin at the modified electrode was electroactive. Observing a remarkable difference between the voltammetric signals of the hematoxylin obtained from different hybridization samples (non-complementary, mismatch and complementary DNAs), we confirmed the potential of the developed biosensor in detecting and discriminating the target complementary DNA from non-complementary and mismatch oligonucleotides. Under optimum conditions, the electrochemical signal had a linear relationship with the concentration of the target DNA ranging from 12.5 nM to 350.0 nM, and the detection limit was 3.8 nM.

1. Introduction

DNA is the largest known molecule with a biological activity. Therefore, it is important to evaluate the interaction of DNA with various molecules (Pozo et al., 2005) and develop new strategies for sequence-specific detection of DNA samples. Different methods have been used to study such interactions, including fluorescence techniques (Zhang et al., 2010), mass spectroscopy (Sauer, 2006), quartz-crystal microbalance (Willner et al., 2002; Patolsky et al., 2000; Chen et al., 2007), surface plasmon resonance spectroscopy (Liu et al., 2005; Wang et al., 2004; Su et al., 2008), UV–vis spectroscopy (Ni et al., 2005; Shahabadi et al., 2008), Raman spectroscopy (Jiang et al., 2006; Coates et al., 1997; Xie et al., 2008), X-ray crystallography (Barton, 1985; Maciejewska et al., 2006), and electrochemical methods (White and Plaxco, 2010; Kumamoto et al., 2008; Gorodetsky et al., 2007, 2008; Buzzeo and Barton, 2008; Zhang et al., 2008; Wang et al., 2008; Pournaghi-Azar et al., 2006). However, the electrochemical methods have attracted more attention than other methods because they are highly sensitive, easy to use, inexpensive, portable, and compatible with micro fabrication technologies (Pournaghi-Azar et al., 2007; Hejazi et al., 2007). In this way, many groups of researchers have focused their studies on DNA hybridization as an important method for DNA detection (Hejazi et al., 2008).

DNA electrochemical biosensors based on monitoring the variations between single-stranded DNA (ss-DNA) probes and hybridized DNA (ds-DNA) have attracted great interest (Pournaghi-Azar et al., 2008, 2009; Wei et al., 2008; Drummond et al., 2003). DNA hybridization methods are mainly classified into two direct and indirect protocols. The principle of DNA hybridization detection in the direct strategies is based on signal transduction induced directly from oxidation of guanine or adenine moieties in DNA strands. However, indirect DNA hybridization detection methods are based on the incorporation of electroactive labels and monitoring its interaction with DNA molecules (Pournaghi-Azar et al., 2008). In this sense, evaluation of DNA interactions with other

* Corresponding author. Tel.: +98 351 8122669; fax: +98 351 8210991.
** Corresponding author. Tel.: +98(411)3372256; fax: +98(411)3344798.
E-mail addresses: hrzare@yazduni.ac.ir, hrzare@yahoo.com (H.R. Zare), saeidhejazi@tbzmed.ac.ir, msaeidhejazi@yahoo.com (M.S. Hejazi).

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molecules is required. As it was mentioned earlier, there is also a great deal of interest in the development of biosensors capable of detecting a known sequence of DNA. Human papilloma virus (HPV) is a common malignant wart virus causing various kinds of warts as well as cervical dysplasia, vaginal dysplasia, and cervical cancer. For this reason, a DNA biosensor based on methylene blue and pencil graphite electrode was developed for detection of HPV DNA (Sabzi et al., 2008).

Hematoxylin is widely used as a biological stain in biomedical research laboratories and diagnostic procedures (Sioi et al., 2006). Hematoxylin has been normally developed as a pivotal agent in the histopathology of the prostate cancer (Lynn and Sansone, 1994). We recently studied the electrochemical properties of hematoxylin modified electrodes and its application as a sensor for detecting of NADH (Zare et al., 2006) and hydrazine (Zare and Nasirizadeh, 2007). Also, simultaneous determination of some biological compounds has been reported at a hematoxylin modified electrode surface (Nasirizadeh and Zare, 2009; Zare and Nasirizadeh, 2009, 2010). In this research, a new electrochemical biosensor has been introduced based on interaction of hematoxylin with 20-mer deoxyoligonucleotides of HPV for the detection of DNA hybridization. By the self-assembled monolayer (SAM) technique, the recognition interface is fabricated using an alkanethiol single strand DNA (ss-DNA). The electrochemical methods of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) have been used to study the interaction of ss-DNA with hematoxylin as well as the detection of complementary, non-complementary and mismatch target DNA chains in the hybridization process.

2. Experimental

2.1. Materials

6-Mercapto-1-hexanol (MCH) was purchased from Aldrich Company. Hematoxylin and other chemical reagents including K_2HPO_4, KH_2PO_4, NaCl, KCl, NaOH, HCl, and absolute ethanol were purchased from Merck Company. The solutions were prepared with double distilled water. All the chemicals were used as received without further purification. DNA oligonucleotides were supplied (as lyophilized powder) from Eurofins MWG Operon with the following sequences.

Thiolated DNA probe (HSHPV or ss-DNA): 5′-HS $(CH_2)_6$ GTA TCT ACC ACA GTA ACA AA-3′

This oligonucleotide that corresponds to the sense strand of the major capsid protein L1 gene of human papilloma virus (HPV) was used as DNA probe.

*Complementary target DNA (HPVc): 5′-TTT GTT ACT G**T**G GTA GAT AC-3′*

This oligonucleotide that corresponds to the antisense strand of the major capsid protein L1 gene of HPV was used as complementary target DNA.

*Single base mismatch (SBM) DNA: 5′-TTT GTT ACT G**G**G GTA GAT AC-3′*

This oligonucleotide was identical to complementary DNA with a substitution (T/G) at position 11 shown with highlighted and underlined letters, leading to a single base mismatch with the probe.

Non-complementary DNA: 5′-GAA TAT GAT TTA CAG TTT ATT TTT-3′

The stock solutions of the oligonucleotides (100.0 μM) were prepared with a 10.0 mM Tris–HCl buffer (pH 8.0) containing 1.0 mM of EDTA. The stock solutions were kept frozen at −20 °C. The immobilization and hybridization solutions were a 1.0 M phosphate buffer (pH 4.5) and a 0.05 M phosphate buffer (pH 7.0) containing 0.3 M of NaCl. The washing solution was also a 0.05 M phosphate buffer (pH 7.0) containing 0.3 M of NaCl. A stock solution of 1.0 mM of hematoxylin was prepared using a 0.1 M phosphate buffer solution with pH 7.0. The pH was adjusted with either NaOH or H_3PO_4 solutions. The double distilled water and all the buffers were sterilized using autoclave.

2.2. Instrumentations

Electrochemical measurements were performed with an Autolab potentiostat/galvanostat model PGSTAT 30 (EcoChemic, Utrecht, Netherlands) and a GPES 4.9 software at laboratory temperature (25 ± 1 °C). The utilized three-electrode system was composed of a gold electrode (surface area of 0.0314 cm^2) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum electrode as the auxiliary electrode. All the potential in the text were reported with respect to this reference electrode. The pH measurements were done with a Metrohm model 691 pH/mV meter.

2.3. Preparation of probe-modified electrode and DNA hybridization

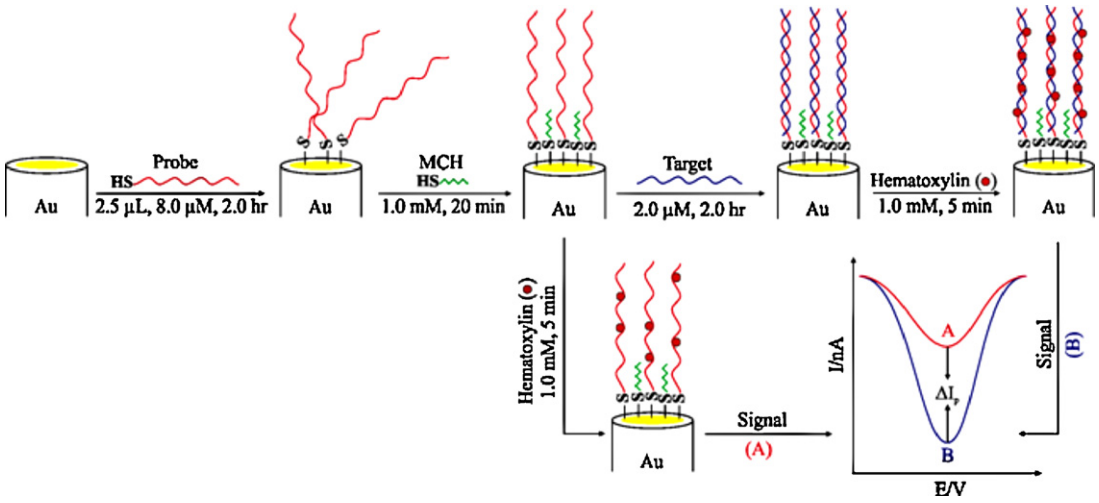
Prior to each modification, the surface of an AuE was polished with a 1.0 μm and 0.05 μm alumina–water slurry on a smooth polishing cloth. Then the electrode was sonicated for 10 min and rinsed thoroughly with distilled water. Finally, the electrode was electrochemically cleaned by electrochemical cycling between −0.3 V and +1.5 V in a 0.1 M H_2SO_4 solution at a scan rate of 100 mV s^{-1} until reproducible cyclic voltammograms were obtained. The electrode was rinsed with distilled water and dried under a nitrogen stream. Then, a 2.5 μL immobilization buffer solution containing 8.0 μmol L^{-1} HSHPV probe (ss-DNA) was dropped on the gold electrode surface for the probe self-assembly. The self-assembly of the probe was performed by incubating the electrode at room temperature (25 ± 1 °C) for 2.0 h in a high-humidity container to prevent evaporation. Finally, HSHPV self-assembled electrode (ss-DNA/AuE) was washed with a washing solution and was incubated in distilled water containing 1.0 mM of MCH for 20 min. Then, the electrode was rinsed with 80:20 (v/v) ethanol:water and distilled water respectively. The hybridization was carried out by immersing the ss-DNA/AuE into the hybridization buffer solution (pH 7.0) containing 2.0 μM of the target oligonucleotide (complementary, mismatch or noncomplementary) without stirring at room temperature (25 ± 1 °C) for 2.0 h (Scheme 1). Regeneration of the bare AuE was performed by immersion of the modified electrode in 20 mM NaOH solution for 5 min and then the electrode was rinsed with distilled water.

2.4. Hematoxylin accumulation on the modified electrode

Hematoxylin was accumulated on the ssDNA/AuE by immersing the modified electrode into the 0.1 mM phosphate buffer solution (pH 7.0) containing 1.0 mM of hematoxylin with 100 rpm stirring without applying any potential to the electrode for 5 min (Scheme 1). Once hematoxylin was accumulated, the electrode was rinsed with a washing solution for 10 s. A similar procedure was applied to the accumulation of hematoxylin on a cleaned AuE electrode modified with the probe which is hybridized with DNA samples.

2.5. Voltammetric measurements

In the cyclic voltammetric experiments, the desired modified electrode was immersed in a 0.1 M phosphate buffer solution (pH 7.0), and the electrode potential was scanned with a sweep rate of 20.0 mV s^{-1} from −0.025 to 0.375 V. The electrochemical investigation was carried out by differential pulse voltammetry (DPV),



Scheme 1. Schematic diagrams illustrating the fabrication steps of ss-DNA/AuE and ds-DNA/AuE as well as their interaction with hematoxylin label.

with an amplitude of 25 mV, a modulation time of 0.05 s, and a step potential of 50 mV in a 0.1 M phosphate buffer solution (pH 7.0). The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction using a 'peak width' of 0.01.

3. Results and discussion

3.1. Factors influencing the hematoxylin accumulation on a ss-DNA/AuE

Two common probe immobilization methods were compared. In the first method, which is named droplet self-assembly, 2.5 μL droplet of HSHPV probe solution was placed onto the bare AuE. In the second method, that was called solution self-assembly, the bare AuE was immersed in the immobilization buffer containing a 8.0 μM probe solution for 2.0 h. As already brought up in Section 2.3, the electrodes prepared by both methods were soaked first in the MCH and afterwards in the hematoxylin solution. Likewise, the accumulation of hematoxylin on the hybrid DNA was done by immersing the probe self-assembled AuE in the target DNA solution and then in the hematoxylin solution. Fig. 1A depicts the differential pulse voltammograms (DPVs) of the hematoxylin accumulated on the surface of ss-DNA/AuE before hybridization (curves a and b) and after hybridization with the complementary DNA (curves c and d) using the electrodes prepared by the droplet self-

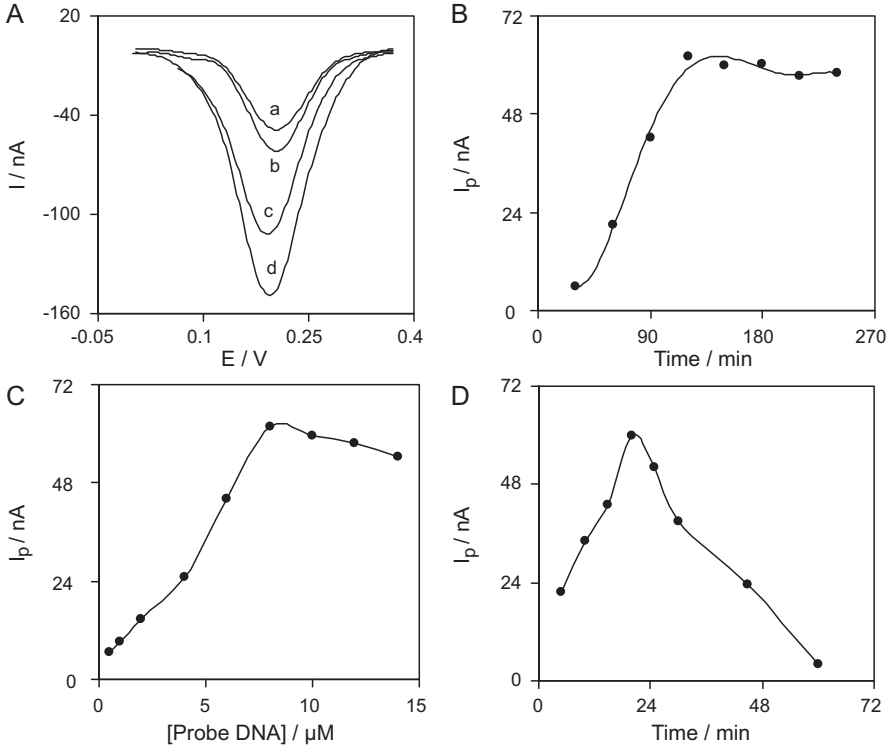


Fig. 1. (A) DPVs of the hematoxylin accumulated on the surface of ss-DNA/AuE before (voltammograms of a and b) and after hybridization with the complementary DNA (voltammograms of c and d) using the electrodes prepared with two different methods of the droplet self-assembly (voltammograms of b and d) and the solution self-assembly (voltammograms of a and c). The accumulated hematoxylin current response on ss-DNA/AuE versus (B) the consumed time for the probe self-assembly, (C) the probe concentration, and (D) the consumed time for the MCH self-assembly.

assembly (curves b and d) and the solution self-assembly (curves a and c).

As shown, the signal of the hematoxylin accumulated on ss-DNA/AuE prepared by the droplet method (curve b) is higher than the ones taken from the solution method (curve a). Moreover, the results indicate that after the hybridization process, the signal obtained from the first method (curve d) is larger than that obtained from the second method (curve c), standing to the fact that the probe molecules are better self-assembled in the droplet self-assembly method. Scheme 1 shows the schematic diagrams of fabrication steps of the ss-DNA/AuE and ds-DNA/AuE as well as their interaction with hematoxylin label.

Fig. 1B shows the accumulated hematoxylin current response on ss-DNA/AuE versus the consumed time for the probe self-assembly. As it can be seen, the accumulated hematoxylin current response increases with the increase of the probe immobilization time up to about 120 min and starts to level off after 120 min. This may be due to the full coverage of the gold electrode surface by the DNA probe after 120 min. A probe immobilization time of 120 min was, therefore, selected in subsequent studies.

The effect of the thiolated DNA immobilization concentration on the bare AuE was also investigated. The experiments were performed as described in Section 2.3. Fig. 1C shows the results obtained from DPV measurements related to the hematoxylin accumulated on ss-DNA/AuE versus the concentration of the probe. The accumulated hematoxylin current response increased to about 8.0 μM and then slightly decreased with the increase of the probe concentration. This decrease may be attributed to the massive accumulation of the probe on the electrode leading to less availability of hematoxylin to DNA. This suggestion can be confirmed by a similar behavior that was observed in an experimental investigation concerning the effect of probe self-assembly time. Therefore, 8.0 μM of thiolated DNA was suggested as an optimum concentration for the immobilization of the probe on the bare AuE.

The effect of MCH on the ss-DNA/AuE was also studied (Fig. 1D). The DPV results revealed that the signal of the hematoxylin accumulated on ss-DNA/AuE elevated as the MCH self-assembly time increased to about 20 min and rapidly decreased with the increase of the time. The increase in the signal of the accumulated hematoxylin to 20 min could be attributed to ordering the thiolated probe molecules on the surface of AuE. The decrease of the accumulated hematoxylin signal after 20 min is probably due to removing some probe molecules from the AuE surface.

3.2. Optimization of the hybridization process

Having optimized the electrode preparation factors, the researchers employed three DNA hybridization methods and compared them together. In the first method, which is named drop hybridization method, the hybridization process was performed by depositing 2.5 μL of the complementary solution onto the ss-DNA/AuE, followed by incubation in high-humidity container to prevent evaporation at room temperature ($25 \pm 1\,^{\circ}C$) for 2.0 h. Then, the electrode was rinsed thoroughly in a washing solution with 100-rpm stirring, and hematoxylin was accumulated on the electrode surface. Fig. 2A, curve a, shows the DPV of the accumulated hematoxylin on this electrode surface. In the second method that is called preheated solution hybridization method, the ss-DNA/AuE was immersed in the hybridization solution with the temperature of 85 °C for 3 min while the solution was stirred. Then, the solution was left at room temperature in order to cool down gradually. Afterwards, the electrode was rinsed with a washing solution and hematoxylin was accumulated on the electrode surface. The DPV of the accumulated hematoxylin in the preheated solution hybridization method is presented in curve b of Fig. 2A. Finally, in the tertiary method, which is named solution hybridization method, the hybridization process was carried out as described in Section 2.3 and shown in Scheme 1. After hybridization, the electrode was immersed in the hematoxylin solution. Fig. 2A, curve c, shows the accumulated hematoxylin current response of this method.

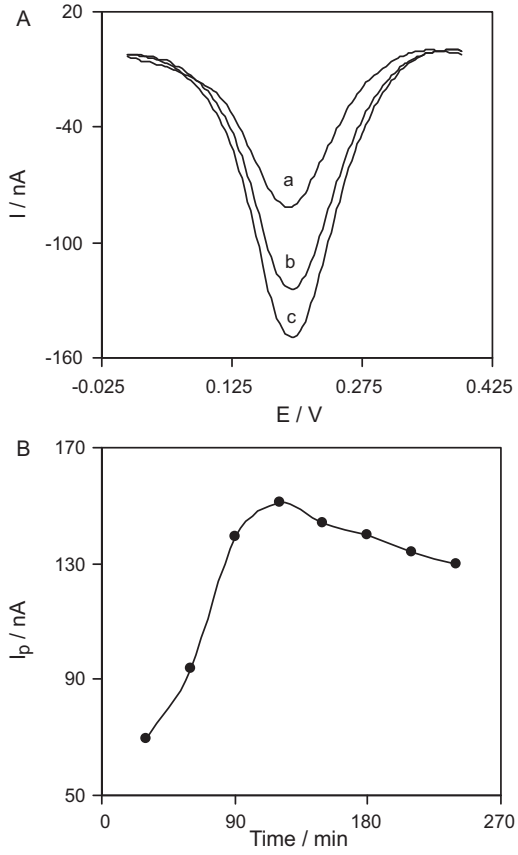


Fig. 2. (A) DPVs of the hematoxylin accumulated on the surface of ds-DNA/SAM that were prepared with different hybridization methods of (a) the drop hybridization, (b) the preheated solution hybridization and (c) the solution hybridization. (B) The accumulated hematoxylin current response on ds-DNA/AuE versus the hybridization time in the solution hybridization method.

As illustrated, the highest current response was obtained in the tertiary method. The best result for the tertiary method may be due to that in the solution hybridization method, the target DNA can easily be selected according to its best orientation which is necessary for the hybridization. Based on these observations, the solution hybridization method was chosen as an optimal procedure for the hybridization process. In addition, the effect of hybridization time, in the solution hybridization method, on the current response of the accumulated hematoxylin was investigated. Based on the results given in Fig. 2B, the hybridization time of 120 min was found as optimal and to be used in all the subsequent experiments of this research.

3.3. Analytical performance of the DNA biosensor

Supplementary Fig. S1(A) demonstrates the cyclic voltammetric responses of hematoxylin on the bare AuE (curve a) and MCH modified AuE (curve b). As seen, there is no significant difference between the signals of hematoxylin interacting with bare and MCH modified electrodes. This observation indicated that hematoxylin does not interact with MCH. The signals of hematoxylin before and after hybridization of the ss-DNA/AuE with complementary DNA are shown in Supplementary Fig. S1(A), curves c and d, respectively. As it can be seen, the interaction of hematoxylin

with ss-DNA/AuE resulted in a significant increase in the hematoxylin signal, which is attributed to the interaction of hematoxylin with the self-assembled probe molecules. A comparison of curves c and d represents a significant increase in the hematoxylin signal following hybridization of the probe with the complementary DNA. For more investigation, the bare AuE, MCH modified AuE, ss-DNA/AuE and ds-DNA//AuE were immersed in a 1.0 mM hematoxylin solution (pH 7.0) for 5 min with stirring of 100 rpm. Then, the electrodes were rinsed with a washing solution. Curves a–d of Supplementary Fig. S1(B) show the cyclic voltammograms of these modified electrodes in a 0.1 M phosphate buffer (pH 7.0) respectively. As it can be seen, there are small currents for the accumulated hematoxylin responses at the bare AuE (curve a) and the MCH modified AuE (curve b), while a redox couple with a significant current was observed at ss-DNA/AuE (curve c) and ds-DNA/AuE (curve d). In addition, the signal of the hematoxylin accumulated on ds-DNA/AuE is larger than that accumulated on ss-DNA/AuE. These results indicate that the interaction hematoxylin with double-stranded DNA (ds-DNA) is stronger as compared with single-stranded DNA (ss-DNA). The interaction of hematoxylin with ss-DNA could rely on its interaction with nucleobases. In addition, intercalation of hematoxylin into DNA duplex (ds-DNA/AuE) can be considered as another interaction. Having observed the affinity of hematoxylin to DNA molecules and considering the variation in the affinity of hematoxylin to ss-DNA and ds-DNA, the researchers proposed employing hematoxylin for development of DNA biosensors. To test this idea, the probe modified electrode was hybridized with non-complementary DNA. Fig. 3 shows the DPVs of hematoxylin accumulated on the MCH modified AuE (curve a), ss-DNA/AuE before (curve b) and after hybridization with 2.0 μM solution of non-complementary (curve c), mismatch (curve d) and complementary (curve e) oligonucleotides.

The mean values of the current responses obtained for different modified electrodes are measured and the results are shown in Supplementary Table S1. As demonstrated, the highest hematoxylin current response (151.8 ± 3.3 nA) was observed following hybridization of the probe with the complementary DNA. Also, as it can be seen, the hematoxylin responses significantly decrease when the probe hybridization carried out with the mismatch and

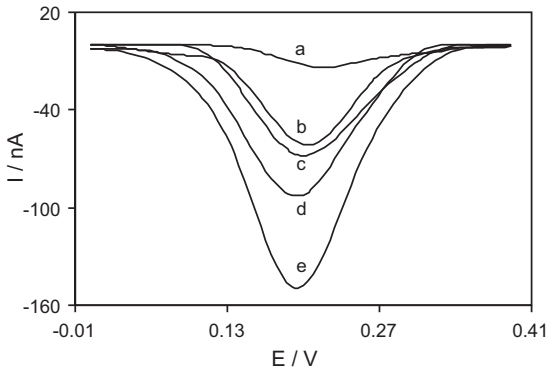


Fig. 3. DPVs of the hematoxylin accumulated on the (a) MCH modified AuE, ss-DNA/AuE before (b) and after hybridization with 2 μM oligonucleotide solution of the (c) non-complementary, (d) mismatch, and (e) complementary in 0.1 M phosphate buffer solution (pH 7.0).

non-complementary DNA. The decrease in the hematoxylin signal after hybridization of the probe with the mismatch and the non-complementary, compared to the complementary oligonucleotide could be due to lack of complete and strong pairing between the two DNA strands nucleobases in the oligonucleotide chains. Also, this result indicates that only a complementary sequence could cause a significant increase in accumulated hematoxylin signal. Above observations also clearly proved that the hematoxylin-based DNA biosensor is able to detect the single base mismatch in the oligonucleotide chain.

Fig. 4 shows the accumulated hematoxylin signal after immersing of ss-DNA/SAM in different concentrations of the complementary (target) DNA for 120 min in 0.1 M phosphate buffer solution. As presented, the accumulated hematoxylin signals are increased with the increase of the target concentration and leveled off at ca. 400 nM (inset of Fig. 4). This result indicates that at this concentration, all of the available probe strands on the electrode surface are involved in hybridization event. The plot of difference in the accumulated hematoxylin current response on ds-DNA/AuE and ss-DNA/SAM ($\Delta I = I_{\text{ds-DNA}} - I_{\text{ss-DNA}}$) versus the target concen-

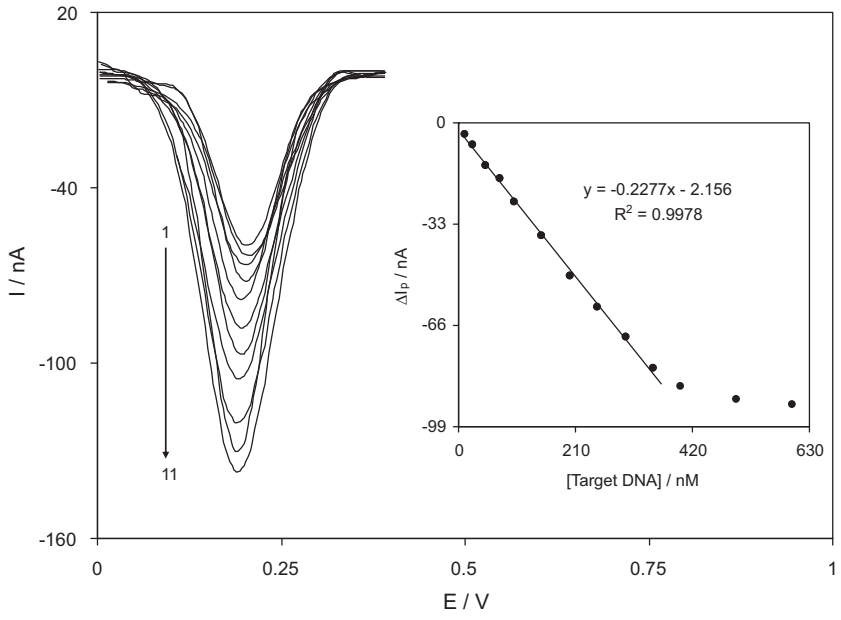


Fig. 4. DPVs of the hematoxylin accumulated on the hybridized ss-DNA/AuE with different concentrations of the complementary (target) DNA for 120 min in 0.1 M phosphate buffer solution (pH 7.0). The numbers of 1–11 correspond to 12.5–400.0 nM target DNA. Inset shows the plot of difference in the accumulated hematoxylin current response on ds-DNA/AuE and ss-DNA/SAM ($\Delta I = I_{\text{ds-DNA}} - I_{\text{ss-DNA}}$) versus the target DNA concentration in hybridization process.

Table 1
Comparison of the analytical parameters of different biosensors for DNA determination.

Electroactive indicator	Linear range	Detection limit	Electrochemical method[a]	Mismatch detection	Electrode	References
Gold(I) complexes	1.0–20.0 nM	0.5 nM	CV	–	Glassy carbon	Escosura-Muniz et al. (2007)
Brilliant cresyl blue	10–5000 nM	9.00 nM	DPV	–	Carbon paste	Hejazi et al. (2010)
Methylene blue	25–100 nM	0.2 nM	CV	Single-base mismatch	Gold	Jin et al. (2007)
Methylene blue	–	–	DPV	Single-base mismatches	Gold	Kerman et al. (2002)
Poly vinylferrocenium	–	–	DPV	Single-base mismatches	Pencil graphite	Kuralay et al. (2009)
Methylene blue	125–6.75 nM	59 nM	DPV	–	Glassy carbon	Lin et al. (2007)
Daunomycin	0.1 mg ml^{-1}–0.1 ng ml^{-1}	30 pg ml^{-1}	LSV	–	Gold	Sun et al. (1998)
Methylene blue	1–20 ppm	–	DPV	–	Gold	Siddiquee et al. (2010)
$Co(phen)_3^{3+}$	100–1200 nM	4.0 nM	CP	Three-base mismatch	Carbon paste	Wang et al. (1996)
2,6-Disulfonic acid Anthraquinone	–	500 nM	SWV	Single-base mismatches	Gold	Wong and Gooding (2003)
Anthraquinone monosulfonic acid	10–500 nM	0.5 nM	SWV	Single-base mismatch	Gold	Wong and Gooding (2006)
Promethazine hydrochloride	2–500 nM	0.38 nM	CV	Single-base mismatch	Gold	Wei et al. (2008)
Carboxylic group functionalized SWCNTs	40–110 nM	20 nM	DPV	Single-base mismatches	Glassy carbon	Zhang et al. (2009)
Hematoxylin	12.5–350.0 nM	3.8 nM	DPV	Single-base mismatches	Gold	This work

[a] CV: cyclic voltammetry; DPV: differential pulse voltammetry; LSV: linear sweep voltammetry; CP: chronopotentiometry; SWV: square wave voltammetry.

tration is shown in the inset of Fig. 4. As suggested, the calibration plot was linear for a wide concentration range, 12.5–350.0 nM of the target DNA.

The lower detection limit, C_m, was obtained using the equation $C_m = 3\,s_{bl}/m$ (Skoog et al., 1998), where s_{bl} is the standard deviation of the blank response (the signal of hematoxylin at ss-DNA/AuE) and m is the slope of the calibration plot (-0.2277 nA nM^{-1}). In this experiment, eight replicate measurements were made on the blank solution, and the resulting data were then treated statistically to obtain $s_{bl} = 0.29$ nA. From the analysis of these data, we estimated the detection limit of 3.8 nM for the target DNA. The average voltammetric current and the precision estimated in terms of relative standard deviation (CV) for ten repeated measurements ($n = 10$) of 50.0 nM of the target DNA were 74.0 ± 3.1 nA and 4.2% respectively. In Table 1, some of the analytical characteristics obtained in this research are compared with those previously reported by others (Hejazi et al., 2010; Wong and Gooding, 2003, 2006; Wei et al., 2008; Kerman et al., 2002; Kuralay et al., 2009; Escosura-Muniz et al., 2007; Siddiquee et al., 2010; Jin et al., 2007; Wang et al., 1996; Zhang et al., 2009; Lin et al., 2007; Sun et al., 1998). As it can be seen, the responses of the proposed biosensor are superior in some cases, especially linear dynamic range, to those of the previously reported biosensors.

4. Conclusions

In this study, the interaction of hematoxylin as an electroactive DNA label was investigated. The difference between affinity of hematoxylin to ss-DNA and to ds-DNA is employed for the development of a DNA biosensor for the detection and discrimination of human papilloma virus (HPV) corresponding to oligonucleotide from non-complementary DNA. The experiments also confirm the potential of the hematoxylin-based DNA biosensor in the detection of single-base mismatch in target DNA. In this study, the best method for probe self-assembly is indicated to be drop self-assembly and the best hybridization method is found to be solution hybridization method. Under optimum conditions, the electrical signal has a linear relationship with the concentration of target DNA ranging from 12.5 nM to 350.0 nM, and the detection limit is 3.8 nM.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.11.026.

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