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Electrochemical detection of the *MT-ND6* gene and its enzymatic digestion: Application in human genomic sample

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ABSTRACT

A simple electrochemical biosensor was developed for the detection of the mitochondrial NADH dehydrogenase 6 gene (*MT-ND6*) and its enzymatic digestion by BamHI enzyme. This biosensor was fabricated by modification of a glassy carbon electrode with gold nanoparticles (AuNPs/GCE) and a probe oligonucleotide (ssDNA/AuNPs/GCE). The probe, which is a thiolated segment of the *MT-ND6* gene, was deposited by self-assembling immobilization on AuNPs/GCE. Two indicators including methylene blue (MB) and neutral red (NR) were used as the electroactive indicators and the electrochemical response of the modified electrode was measured by differential pulse voltammetry. The proposed biosensor can detect the complementary sequences of the *MT-ND6* gene. Also the modified electrode was used for the detection of an enzymatic digestion process by BamHI enzyme. The electrochemical biosensor can detect the *MT-ND6* gene and its enzymatic digestion in polymerase chain reaction (PCR)-amplified DNA extracted from human blood. Also the biosensor was used directly for detection of the *MT-ND6* gene in all of the human genome.

Deoxyribonucleic acid (DNA)[1] is continuously exposed to miscellaneous genotoxic substances, environmental mutagens, carcinogens, industrial pollutants, and their metabolically active products that may cause chemical changes in it. As DNA has an essential role in the determination of hereditary characteristics and storing the genetic information necessary for the replication of living organisms, detection of DNA damage is of great importance for human health protection [1,2]. Also detection of specific sequences in viral and bacterial nucleic acids is becoming increasingly important in the diagnosis of disease [3]. Therefore, there is an increasing demand for sensitive methods of DNA detection for various applications including the genetics of disease and medical diagnosis [4].

Generally, the detection of specific DNA sequences is performed by using gel electrophoresis of DNA fragments amplified by the polymerase chain reaction (PCR). But in this method ethidium bromide, which is commonly used as a staining agent, is a carcinogenic chemical [5]. Also the sequence of the amplified target DNA may be different from that intended. On the other hand, electrochemical methods, which are simple, inexpensive, accurate, and sensitive [6–8], have attracted particular attention for detection of DNA hybridization [9–11]. DNA biosensors can detect the presence of genes or mutant genes associated with inherited human diseases or infectious diseases.

In a DNA electrochemical biosensor generally the recognition layer is constructed by immobilization of an oligonucleotide with specific sequence (probe DNA) on the electrode surface by different methods including adsorption, cross-linking, encapsulation, avidin-biotin complexation, and covalent attachment [12]. Then the target DNA is captured at the recognition layer and the resulting hybridization signal is transduced into a usable electronic signal [9]. DNA hybridization is based on the ability of single-stranded DNA (ssDNA) to recognize its counterpart strand with a complementary nucleotide sequence. The hybridization event, i.e., formation of the double-stranded (dsDNA) from probe DNA and target DNA, can be detected by use of electroactive indicators binding preferentially to ssDNA or dsDNA. Electroactive indicators were mainly composed of polymers [13], metal complexes [14], and organic dyes such as methylene blue (MB) [10,15] and neutral red (NR) [16].

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[1] *Abbreviations used:* AuNPs/GCE, glassy carbon electrode with gold nanoparticles; DNA, deoxyribonucleic acid; DPV, differential pulse voltammetry; MB, methylene blue; NR, neutral red; PCR, polymerase chain reaction.

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As nanomaterials have many applications in different electrochemical sensors [17,18], they can also be used in DNA biosensors [19]. Gold nanoparticles (AuNPs) were used largely in DNA biosensors due to their unique chemical, optical, and electronic properties [20,21]. Because of the strong affinity of biological compounds and thiolated molecules to AuNPs, modified electrodes with these nanoparticles provide a good platform for coating surfaces with bioactive substances [22].

The *MT-ND6* gene (mitochondrially encoded NADH dehydrogenase 6) provides instructions for making a protein called NADH dehydrogenase 6. This protein is part of a large enzyme complex known as complex I, which is one of several enzyme complexes necessary for oxidative phosphorylation within mitochondria. Several mutations in the *MT-ND6* gene have been identified in people with Leber hereditary optic neuropathy (LHON) [23–25]. A mutation in the *MT-ND6* gene also has been identified in a small number of people with Leigh syndrome [26].

In this paper an electrochemical biosensor is proposed for detecting the *MT-ND6* gene by a hybridization process with probe DNA on a gold nanoparticle-modified GCE (ssDNA/AuNPs/GCE). Also the enzymatic digestion process of this gene by BamHI restriction enzyme is investigated at this biosensor. Based on our studies, this is the first report for electrochemical detection of the *MT-ND6* gene and its enzymatic digestion. Applications of the biosensor in real samples are also studied.

Experimental

Reagents

Tris-base buffer (10 mmol L^{-1} + 0.1 mol L^{-1} NaCl, pH 6.0 and 8.0) was used as buffer solution. $HAuCl_4$, MB, NR, and all other chemicals were analytical reagent grade and obtained from Merck. The AuNPs were synthesized in our laboratory by the citrate reduction method, as described by Lee and Meisel [27]. All solutions were prepared by doubly distilled water.

The restriction enzyme BamHI was purchased from Roche Diagnostics (Mannheim, Germany) with its special buffer. The probe oligonucleotide sequence (SH-ssDNA) and complementary sequence are as below and were prepared from Takapozist (Tehran, Iran):probe sequence, 5′-(SH)-ATA**GGATCC**TCCCGAATCAACCCTGAC-3′; complementary sequence, 5′-GTCAGGGTTGATTCGGGA**GGATCC**TAT-3′; noncomplementary sequence, denaturized PCR product of the mitochondrial ATPase 6 gene

Instruments

Electrochemical measurements were performed with a potentiostat/galvanostat (SAMA 500 Electroanalyzer System, I.R. Iran). The utilized three-electrode system was composed of a glassy carbon electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire electrode as the auxiliary electrode. All potential in this paper was reported with respect to SCE. The pH measurements were done with Metrohm Model 691 pH/mV meters. Differential pulse voltammetry (DPV) was recorded at scan rates of 100 mV s^{-1} and with pulse height of 5.0 mV and pulse width of 0.05 s.

Electrode modification

GCE were polished before the experiments by 0.05 μm alumina/water slurry on a polishing cloth and then washed with ethanol and water via ultrasonication. Then 5.0 μL of synthesized AuNP solution was dropped onto the GCE surface and dried in air. This electrode is named as AuNPs/GCE.

Immobilization and electroactivation of probe DNA at AuNPs-GCE

The AuNPs/GCE was washed with doubly distilled water. The self-assembled monolayer of SH-ssDNA was formed by dipping AuNPs/GCE in the SH–ssDNA store solution for 14 h. Then this electrode was immersed in water for 1 h to remove physically adsorbed SH–ssDNA (ssDNA/AuNPs/GCE). The electroactivation of the electrode was done by two indicators including MB and NR. For this purpose ssDNA/AuNPs/GCE was immersed in the Tris–HCl buffer solution containing 20 μM MB or 150 μM NR (pH 8.0 for MB and pH 6.0 for NR) for 10 min. Then the prepared electrodes were washed with copious amounts of water and Tris–HCl buffer. These electrodes are named as MB or NR/ssDNA/AuNPs/GCE.

Hybridization

The hybridization procedure was carried out by immersing the ssDNA/AuNPs/GCE in a Tris–HCl buffer solution (pH 8.0) containing a certain concentration of complementary DNA sequence and then incubating at 40 °C for 1 h. After that, the electrode was washed with copious amounts of water and Tris–HCl buffer to remove the unhybridized ssDNA.

Enzyme digestion

For enzyme digestion reaction, modified electrode with double-stranded DNA (dsDNA/AuNPs/GCE) was immersed in buffered solution of BamHI enzyme for 1.5 h at 37 °C. This enzyme has the ability to detect a specific sequence of GGATCC at dsDNA. Then this electrode was washed with copious amounts of water and Tris–HCl buffer.

Preparation of real samples

Genomic DNA was extracted from peripheral blood by a DNA extraction kit (DNA fast Kit-Genfanavaran, Tehran, Iran). The *MT-ND6* gene (complementary target DNA) was PCR-amplified from genomic DNA (325 bp fragment). PCR amplification of human genomic DNA samples was carried out in 25 μL volumes, using 0.5 μM primer and 0.2 mM deoxynucleotide triphosphates with *Taq* DNA polymerase (Sinaclone, Iran), on a personal Thermocycler (Techne, England). Following target denaturation at 94 °C for 5 min, PCR was performed for 35 cycles, each cycle consisting of 94 °C for 1 min, 65 °C for 1 min, extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. All PCR fragments sizes were confirmed using a 1.0% agarose electrophoresis grade gel (Invitrogen, UK) stained with ethidium bromide and visualized under UV light.

Results and discussion

Electroactivation of ssDNA/AuNPs/GCE

By immersion of AuNPs/GCE in SH-ssDNA solution the self-assembled SH-ssDNA on the surface of AuNPs/GCE was prepared because of strong affinity of the thiol group of SH-ssDNA to AuNPs. The DPV behavior of this electrode was investigated in Tris-base buffer solution (pH 8.0). As seen from curve a of Fig. 1, the ssDNA/AuNPs/GCE has no oxidation peak in the potential region of −0.8 to −0.2 V. Therefore this electrode is nonelectroactive in this potential range and should be electroactivated by a suitable indicator.

Two indicators including MB and NR were used for electroactivation of ssDNA/AuNPs/GCE by its immersion in Tris-base buffer solution with optimum conditions for each indicator (MB with

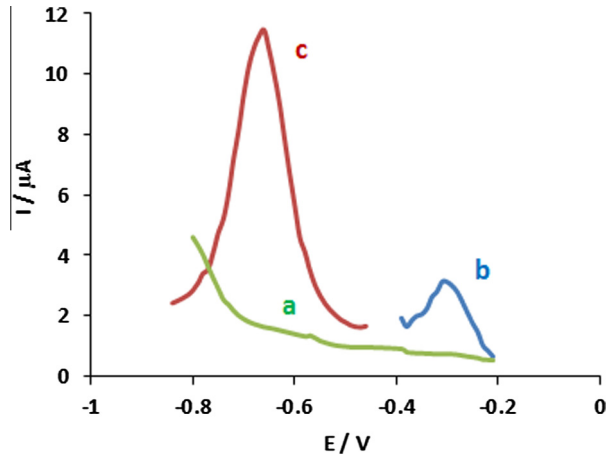
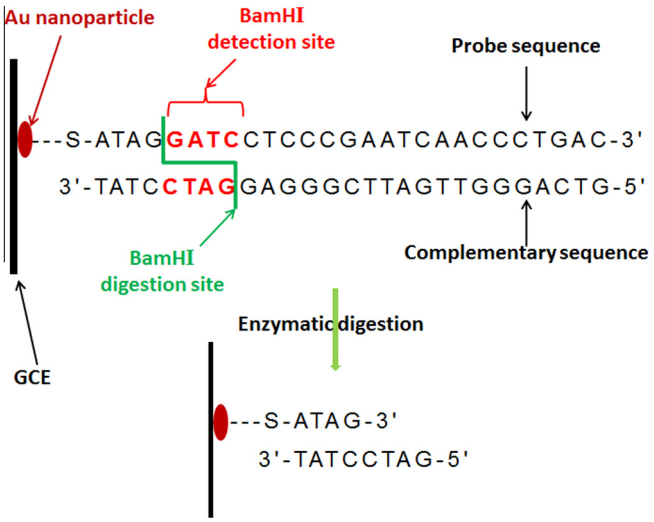


Fig.1. DP Voltammograms of different electrodes in Tris-base buffer solution (a) ssDNA/AuNPs/GCE in pH 8.0; (b) MB/ssDNA/AuNPs/GCE in pH 8.0, and (c) NR/ssDNA/AuNPs/GCE in pH 6.0.



Scheme 1. Schematic illustration of the dsDNA/AuNPs/GCE and its digestion by BamHI enzyme.

concentration of 20 µM in pH 8.0 and NR with concentration of 150 µM in pH 6.0). Curve b in Fig. 1 shows the DP voltammograms of MB/ssDNA/AuNPs/GCE with an oxidation peak at potential about −0.3 V. DP voltammograms of ssDNA/AuNPs/GCE with another indicator, i.e., NR/ssDNA/AuNPs/GCE, is seen from curve c of this figure. As can be seen the DPV current response of the ssDNA/AuNPs/GCE with NR as indicator is much higher than that with MB.

Detection of target DNA by hybridization

Parts A and B of Fig. 2 respectively show the DP voltammograms of MB/ssDNA/AuNPs/GCE and NR/ssDNA/AuNPs/GCE, before hybridization (curve a), after hybridization with target DNA (curve b), and after contact with noncomplementary sequence (curve c).

Comparison of curves a and b in Fig. 2A shows a decrease in the peak current of MB/ssDNA/AuNPs/GCE after hybridization with complementary target DNA. The MB has a strong affinity with the guanine residues in ssDNA. After hybridization the current signal of MB decreased markedly due to less binding of MB to dsDNA because of the inaccessibility of MB to the guanine bases [28]. On the other hand, the peak current of MB/ssDNA/AuNPs/GCE, after hybridization with a noncomplementary sequence (curve c) in Fig. 2A), is close to the current before hybridization. Because the noncomplementary sequence has no interaction with the probe ssDNA at MB/ssDNA/AuNPs/GCE, the probe ssDNA remained single strand with adsorbed MB. This experiment was repeated for five different prepared sensors and the DP voltammograms were recorded for each of them. The obtained results are similar for all sensors. Also the difference between curves a and b was investigated statistically and it was found that these two DP voltammograms are significantly different. As can be seen the prepared electrochemical biosensor can be used for detecting the desired target DNA, *MT-ND6* gene, from a noncomplementary sequence.

These experiments also were done with NR/ssDNA/AuNPs/GCE (Fig. 2B). As can be seen the peak current of the biosensor after hybridization with complementary target DNA (curve b) is increased in comparison with the peak current before hybridization (curve a). This is due to more affinity of NR to dsDNA than ssDNA. The NR can bind to dsDNA through an intercalative model, but ssDNA do not have a double-stranded structure and NR cannot intercalate to it. However the backbone of ssDNA is in negative charge and can easily attract positive charged NR molecules.

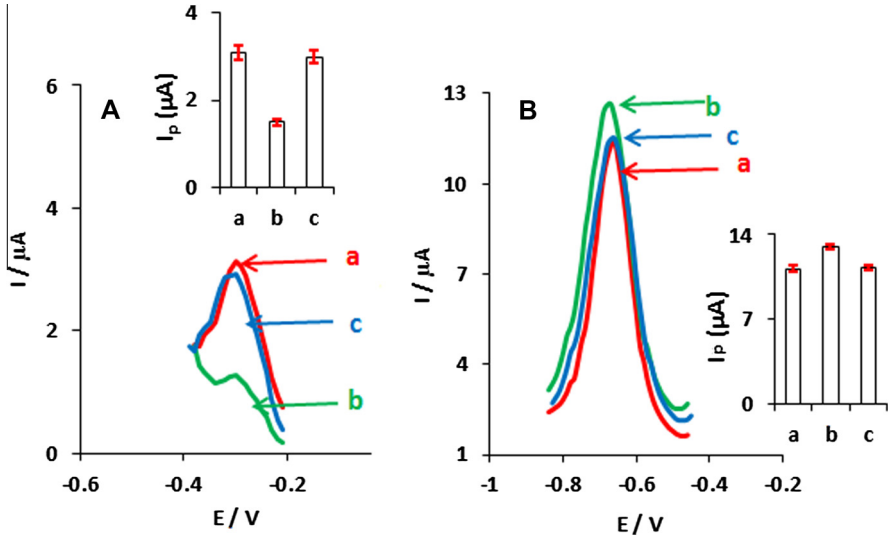


Fig.2. DP voltammograms of (A) MB/ssDNA/AuNPs/GCE in Tris-base buffer pH 8.0 and (B) NR/ssDNA/AuNPs/GCE in Tris-base buffer, pH 6.0; curve a, before hybridization; curve b, after hybridization with target DNA; and curve c, after hybridization with noncomplementary sequence. Insets show the error bars of peak current for five repeated experiments with five different prepared sensors.

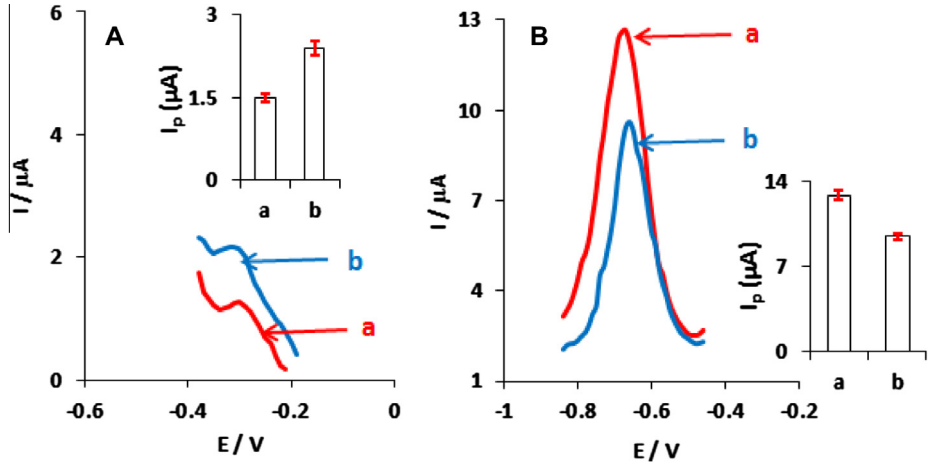


Fig.3. DP voltammograms of (A) MB/dsDNA/AuNPs/GCE in Tris-base buffer, pH 8.0, and (B) NR/dsDNA/AuNPs/GCE in Tris-base buffer, pH 6.0; curve a before and curve b after enzymatic digestion. Insets show the error bars of peak current for five repeated experiments with five different prepared sensors.

Therefore, the bonding constant of NR with dsDNA is higher than ssDNA [29]. Also the peak current is almost constant for NR/ssDNA/AuNPs/GCE before and after hybridization with a noncomplementary sequence (curves a and c in Fig. 2B). Therefore NR/ssDNA/AuNPs/GCE can detect the safe *MT-ND6* gene similar to MB/ssDNA/AuNPs/GCE. Of course, the difference of (a) and (b) voltammograms was confirmed by statistical analysis for five different prepared sensors.

Electrochemical detection of enzymatic digestion

The restriction enzymes, which cut DNA at defined sites, represent one of the more important groups of enzymes for the manipulation of DNA [30]. In this work the enzymatic digestion process of BamHI enzyme on target DNA was detected by the DPV technique. For this purpose, dsDNA/AuNPs/GCE was immersed in a buffered solution of BamHI enzyme. BamHI binds at the recognition sequence of 5′-GGATCC-3′, and cleaves these sequences just after the 5′-guanine on each strand (Scheme 1). After enzymatic digestion the electrode was immersed in MB or NR solutions for electroactivation.

Fig. 3 shows the effect of enzymatic digestion on DP voltammograms of (MB or NR)/dsDNA/AuNPs/GCE. Fig. 3A indicates the increase of current signal of MB after enzymatic digestion (curve b). The increase of the current signal of MB is due to the cleavage of double strands in DNA and accessibility of one more guanine residue for MB (see Scheme 1). On the other hand, Fig. 3B shows a decrease in current signal of NR after enzymatic digestion (curve b). The length of the dsDNA strand is shortened after cutting by enzymatic digestion and therefore a lesser amount of NR can be intercalated to dsDNA (see Scheme 1). Consequently the current signal of NR is decreased after digestion of dsDNA/AuNPs/GCE by BamHI enzyme. The enzymatic digestion process was repeated for five times by five different prepared sensors (for two indicators, NR and MB). DP voltammograms were recorded before and after digestion and the peak current difference between them was obtained each time. Statistical analysis showed that this difference is significant. Accordingly, the prepared electrochemical DNA biosensor in this work can detect the enzymatic digestion process of BamHI enzymes on a specific target DNA by a simple electrochemical method.

Application in real samples

A DNA biosensor for detection of target DNA is useful when it can be used in real samples. Therefore the application of a prepared biosensor for detection of the *MT-ND6* gene was investigated in real samples. At first the ability of the biosensor was tested for detection of complementary sequences produced by PCR amplification of genomic DNA. The ssDNA/AuNPs/GCE was immersed in a solution of PCR product and then electroactivated with NR. The DPVs of the biosensor before and after hybridization with PCR complementary sequence are shown in curves a and b of Fig. 4, respectively. Also curve c of Fig. 4 shows the DPV of the dsDNA (with PCR product)/AuNPs/GCE after enzymatic digestion by BamHI enzyme. The obtained results, increase of the peak current after hybridization and its decrease after enzymatic digestion, are expected for a complementary target DNA. This experiment was repeated for four other times. The results showed that the differences among curves a, b, and c are significant statistically.

To investigate the application performance of the biosensor, it was used for detection of the *MT-ND6* gene in human genome without PCR amplification. For this purpose, ssDNA/AuNPs/GCE was immersed in a 43.0 µg mL^{-1} solution of human genome extracted from blood for hybridization of the probe ssDNA with genomic target DNA. Fig. 5 shows the DP voltammograms of the ssDNA/AuNPs/GCE before and after hybridization by genomic solution. The increase of the NR peak current after hybridizations (curve b) indicates that this genomic sample has a complementary

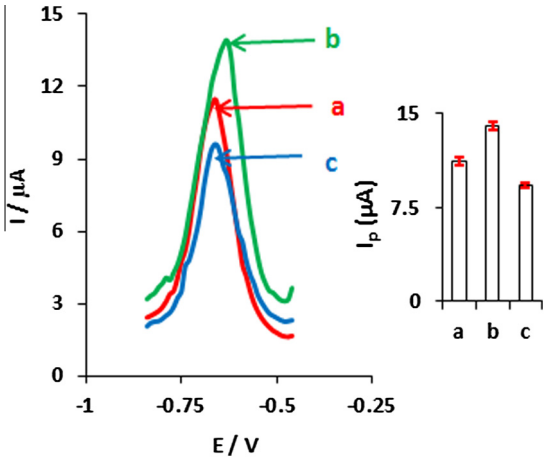


Fig.4. DP voltammograms of NR/ssDNA/AuNPs/GCE in Tris-base buffer pH 6.0, curve a before and curve b after hybridization with PCR complementary sequence; curve c is the DP voltammogram of the dsDNA(with PCR product)/AuNPs/GCE after enzymatic digestion by BamHI enzyme. Insets show the error bars of peak current for five repeated experiments with five different prepared sensors.

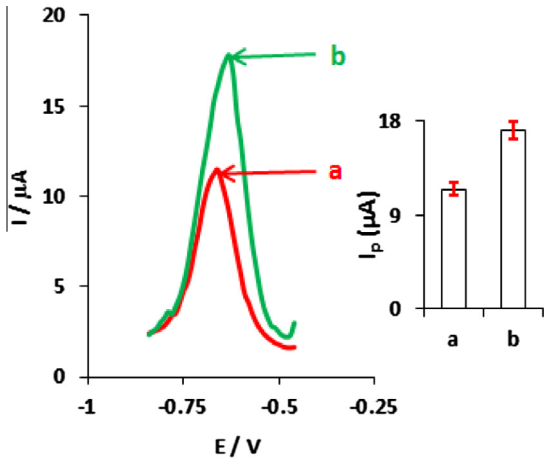


Fig.5. DP voltammograms of NR/ssDNA/AuNPs/GCE in Tris-base buffer, pH 6.0, curve a before and curve b after hybridization with genomic sample. Insets show the error bars of peak current for five repeated experiments with five different prepared sensors.

sequence for ssDNA, i.e., healthy *MT-ND6* gene, because the genome sample was extracted from a healthy person. The enzymatic digestion cannot be applied for genomic sample because in this sample the cytosine base is methylated and therefore the specific sequence cannot be detected by BamHI enzymes.

Conclusions

A new electrochemical biosensor is developed for the detection of the *MT-ND6* gene and also for study of enzymatic digestion of this gene by BamHI restriction enzymes. This biosensor was prepared by immobilization of AuNPs on a GCE and self-assembling of probe SH-ssDNA to AuNPs/GCE. The electroactivation was performed by two indicators including MB and NR. The biosensor has been applied in a simple procedure for the detection of target DNA based on the significant difference between the voltammetric signals of MB or NR obtained for ssDNA and dsDNA. Also electrochemical study of enzymatic digestion of the target DNA indicates the ability of the sensor for detection of the enzymatic digestion by DPV technique. The obtained results in study of real samples show that the prepared biosensor can provide a simple and practical method for detection of the *MT-ND6* gene in human genomic sample without need for PCR amplification.

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References

[1] E. Palecek, F. Scheller, J. Wang, Electrochemistry of Nucleic Acids and Proteins, vol. 1, Elsevier, Amsterdam, 2005.
[2] J. Wang, Electrochemical nucleic acid biosensors, Anal. Chim. Acta 469 (2002) 63–71.
[3] P.A. Baird, T.W. Anderson, H.B. Newcombe, R.B. Lowry, Genetic disorders in children and young adults: a population study, Am. J. Hum. Genet. 42 (1988) 677–693.
[4] T.R. Golub, D.K. Slonim, P. Tamayo, C. Huard, M. Gaasenbeek, J.P. Mesirov, H. Coller, M.L. Loh, J.R. Downing, M.A. Caligiuri, C.D. Bloomfield, E.S. Lander, Molecular classification of cancer: class discovery and class prediction by gene expression monitoring, Science 286 (1999) 531–537.
[5] E. Kai, S. Sawata, K. Ikebukuro, T. Lida, T. Honda, I. Karube, Detection of PCR products in solution using surface plasmon resonance, Anal. Chem. 71 (1996) 796–800.
[6] M. Mazloum-Ardakani, M.A. Sheikh-Mohseni, B.B.F. Mirjalili, Selective and simultaneous voltammetric determination of glutathione, uric acid and penicillamine by a modified carbon nanotube paste electrode, Electroanalysis 25 (2013) 2021–2029.
[7] F. Gholamian, M.A. Sheikh-Mohseni, H. Naeimi, Simultaneous determination of phenylhydrazine and hydrazine by a nanostructured electrochemical sensor, Mater. Sci. Eng. C 32 (2012) 2344–2348.
[8] M. Mazloum-Ardakani, M.A. Sheikh-Mohseni, B.B.F. Mirjalili, L. Zamani, Simultaneous determination of captopril, acetaminophen and tryptophan at a modified electrode based on carbon nanotubes, J. Electroanal. Chem 686 (2012) 12–18.
[9] T.G. Drummond, M.G. Hill, J.K. Barton, Electrochemical DNA sensors, Nat. Biotechnol. 21 (2003) 1192–1199.
[10] M. Mazloum-Ardakani, N. Rajabzadeh, A. Benvidi, M.M. Heidari, Sex determination based on amelogenin DNA by modified electrode with gold nanoparticle, Anal. Biochem. 443 (2013) 132–138.
[11] K. Zhang, Y. Zhang, Lable-free electrochemical DNA sensor based on gold nanoparticles/poly(neutral red) modified electrode, Electroanalysis 22 (2010) 673–679.
[12] M.I. Pividori, A. Merkoci, S. Alegret, Electrochemical genosensor design: immobilisation of oligonucleotides onto transducer surfaces and detection methods, Biosens. Bioelectron. 15 (2000) 291–303.
[13] H. Peng, L. Zhang, J. Spires, C. Soeller, J. Travas-Sejdic, Synthesis of a functionalized polythiophene as an active substrate for a label-free electrochemical genosensor, Polymer 48 (2007) 3413–3419.
[14] X.M. Li, H.Q. Ju, L.P. Du, S.S. Zhang, A nucleic acid biosensor for the detection of a short sequence related to the hepatitis B virus using bis(benzimidazole)cadmium(II) dinitrate as an electrochemical indicator, J. Inorg. Biochem. 101 (2007) 1165–1171.
[15] M. Mazloum-Ardakani, M.M. Heidari, M. Naderi, M.A. Sheikh-Mohseni, Detection of amplified SRY gene by a novel electrochemical biosensor based on gold nanoparticles, Sci. Iran. F 19 (2012) 913–918.
[16] Y. Ni, D. Lin, S. Kokot, Synchronous fluorescence UV–visible spectrophotometric and voltammetric studies of the competitive interaction of bis(1,10-phenanthroline)copper(II) complex and neutral red with DNA, Anal. Biochem. 352 (2006) 231–242.
[17] M. Mazloum-Ardakani, M.A. Sheikh-Mohseni, M. Abdollahi-Alibeik, A. Benvidi, Electrochemical sensor for simultaneous determination of norepinephrine paracetamol and folic acid by a nanostructured mesoporous material, Sens. Actuators B 171–172 (2012) 380–386.
[18] M. Mazloum-Ardakani, M.A. Sheikh-Mohseni, M. Abdollahi-Alibeik, Fabrication of an electrochemical sensor based on nanostructured polyaniline doped with tungstophosphoric acid for simultaneous determination of low concentrations of norepinephrine acetaminophen and folic acid, J. Mol. Liq. 178 (2012) 63–69.
[19] A.A. Ensafi, M. Amini, B. Rezaei, Detection of DNA damage induced by chromium/glutathione/H_2O_2 system at MWCNTs-poly (diallyldimethylammonium chloride) modified pencil graphite electrode using methylene blue as an electroactive probe, Sens. Actuators B 177 (2013) 862–870.
[20] S. Liu, J. Liu, L. Wang, F. Zhao, Development of electrochemical DNA biosensor based on gold nanoparticle modified electrode by electroless deposition, Bioelectrochemistry 79 (2010) 37–42.
[21] M. Mazloum-Ardakani, M.M. Heidari, E. Salehpour, Digestion of restriction enzyme for the detection of single-base mismatch in DNA, Anal. Biochem. 421 (2012) 125–129.
[22] J. Kang, X. Li, G. Wu, Z. Wang, X. Lu, A new scheme of hybridization based on the Au_{nano}–DNA modified glassy carbon electrode, Anal. Biochem. 364 (2007) 165–170.
[23] R.M. Chalmers, A.H. Schapira, Clinical, biochemical and molecular genetic features of Leber's hereditary optic neuropathy, Biochim. Biophys. Acta 1410 (1999) 147–158.
[24] P. Riordan-Eva, M.D. Sanders, G.G. Govan, M.G. Sweeney, J. Da Costa, A.E. Harding, The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation, Brain 118 (1995) 319–337.
[25] D.C. Wallace, G. Singh, M.T. Lott, J.A. Hodge, T.G. Schurr, A.M. Lezza, L.J. Elsas, E.K. Nikoskelainen, Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy, Science 242 (1988) 1427–1430.
[26] C. Ugalde, R.H. Triepels, M.J. Coenen, L.P. Van Den Heuvel, R. Smeets, J. Uusimaa, P. Briones, J. Campistol, K. Majamaa, J.A.M. Smeitink, L.G. Nijtmans, Impaired complex I assembly in a Leigh syndrome patient with a novel missense mutation in the ND6 gene, Ann. Neurol. 54 (2003) 665–669.
[27] P.C. Lee, D. Meisel, Adsorption and surface-enhanced Raman of dyes on silver and gold sols, J. Phys. Chem. 86 (1982) 3391–3395.
[28] W. Yang, M. Ozasoz, D.B. Hibbert, J.J. Gooding, Evidence for the direct interaction between methylene blue and guanine bases using DNA modified carbon paste electrodes, Electroanalysis 14 (2002) 1299–1309.
[29] W. Sun, M. Yang, K. Jiao, Electrochemical behaviors of neutral red on single and double stranded DNA modified electrode, Int. J. Electrochem. Sci. 2 (2007) 93–101.
[30] D.S.T. Nicholl, An Introduction to Genetic Engineering, third ed., Cambridge University Press, Cambridge, UK, 2008.