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ZNA probe immobilized single-use electrodes for impedimetric detection of nucleic acid hybridization related to single nucleotide mutation

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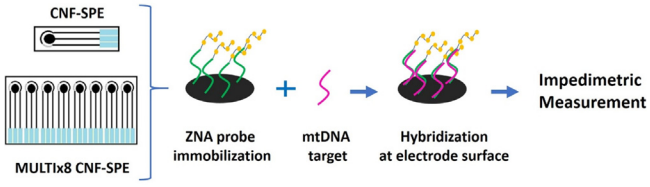
HIGHLIGHTS

- ZNA probe modified electrode was developed for impedimetric detection of single nucleotide mutation contrast to DNA probe.
- ZNA biosensor was also developed in combination with multi-channel array system.
- Impedimetric detection of single nucleotide mutation related to 'FV Leiden' was performed.
- The selectivity of ZNA probe to DNA sequences with different single mutations was tested.
- The applicability of ZNA biosensor was explored to detect a single base mutation in the synthetic PCR samples.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of a low-cost and disposable biosensing technologies has received a great interest of healthcare for the sensitive and reliable detection of single nucleotide mutation related to single nucleotide polymorphisms (SNPs). In the present study, an impedimetric biosensing platform based on zip nucleic acids (ZNA) was developed for the sensitive detection of Factor V Leiden (FV Leiden) mutation. After optimization of experimental parameters, the sequence selective hybridization between ZNA probe and target related to FV Leiden mutation was evaluated via electrochemical impedance spectroscopy technique (EIS) by measuring changes at the charge transfer resistance, $R_{ct.}$ Sensitive and selective impedimetric analysis was performed using carbon nanofiber (CNF) modified screen printed electrodes (SPE) and multi-channel screen printed array of electrodes (MULTIx8 CNF-SPE) resulting in a relatively shorter time in comparison to conventional methods. The selectivity of ZNA probe to mutation-free DNA sequences was also investigated. The applicability of single-use ZNA biosensor was also tested in synthetic PCR samples containing a single base mutation.

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1. Introduction

Zip nucleic acids (ZNA) exhibit a high affinity against to target oligonucleotide sequence and have ability to discriminate between a perfect match and a single base-pair-mismatched complementary sequence [1–4]. The Factor V Leiden (FV Leiden) with the most common inherited prothrombotic conditions has been received a great attention and studied by the researchers [5]. The diagnosis of FV Leiden requires the activated Protein C (APC) resistance assay, a coagulation screening test, or DNA analysis of F5 the gene encoding Factor V, to identify the Leiden mutation, a specific G-to-A substitution at nucleotide 1691 that predicts a single-amino acid replacement (R506Q) [5,6].

The diagnosis of FV Leiden thrombophilia is established in a proband by identification of a 1691G > A variant coupled with coagulation tests such as the APC resistance assay. It was reported that there was considerable overlap in APC ratios with a normal Factor V genotype and heterozygotes for FV Leiden. The authors concluded that the APC resistance assay in its present form is not a useful screening test for FV Leiden heterozygotes [7]. Until the performance of this assay is improved, patients should have molecular diagnostic testing performed to determine their FV Leiden status. Under this aim, different techniques for SNP genotyping have been reported in recent years [8] including, DNA sequencing [9], mass spectroscopy [10] polymerase chain reaction (PCR) [11,12] techniques. These approaches could specifically detect SNP, but the intrinsic drawbacks (e.g, low throughput and specificity) limit their applications. Alternatively, DNA microarray and denaturing high performance liquid chromatography have been considered as fast and efficient techniques for SNP analysis [13,14]. However, these methods require expensive facilities and radioactive/fluorescent tags as well as require complex procedures; because of this reason, they are not suitable techniques for the development of simple and low-cost analysis methods for point-of-care (PoC) devices. Thus, there is an urgent need to develop selective, sensitive, fast and easy to use platforms for detection of FV Leiden mutation. There are several biosensor studies including immuno-reaction based optic biosensors [15,16] voltammetric biosensors [17,18] for FV Leiden mutation analysis.

No report on the development of single-use biosensor has been available yet in the literature detection of FV Leiden mutation via electrochemical impedance spectroscopy (EIS) technique. EIS is a powerful method of analysing the complex electrical resistance of the system. It is sensitive to surface phenomena and a valuable method in electrochemical research. In the field of biosensors, it is well-suited to the detection of binding events on the transducer surface [19]. Thus, the impedimetric techniques have been developed in order to characterize the fabricated biosensors and to monitor the catalytic reactions of biomolecules such as enzymes, proteins, nucleic acids, whole cells, antibodies etc. [20,21].

Nowadays, electrochemical DNA sensing strategies based on nanotechnology have become one of the most exciting forefronts area due to the challenging advances of nanomaterials, e.g., magnetic particles [22–24] carbon nanomaterials [25–28] by using different electrochemical transducers [29,30].

Carbon nanofibers are easily massproduced at a lower cost, better mechanical stability, and a larger surface-active groups-to-volume ratio than carbon nanotubes. Due to their physical and chemical properties, carbon nanofibers possess a lot of edge sites on the outer wall, leading to high tensile strength, elasticity, high thermal and electric conductivity, which facilitate electron-transfer reactions of analytes [31]. Therefore, there is numerous study which implements carbon nanofibers modification onto the electrode surface. There is a great attention recently to use of CNFs in the development of biosensors due to their unique properties. For example, Baker et al. [32] reported that DNA molecules have been covalently immobilized on vertically aligned carbon nanofibers (VACNFs). According to the results, it was reported that carbon nanofiber samples were efficiently functionalized with DNA molecules with excellent biomolecular recognition properties, according to the hybridization measurements. Thus, it was concluded that VACNFs have shown a higher binding specificity for complementary DNA in contrast to the one of unmodified electrode.

To our best knowledge, we introduce a new generation nucleic acid “ZNA” probe based biosensor for the first time in this present work. It was developed for the detection of single nucleotide mutation related to FV Leiden in combination with multi-channel electrochemical array system. This novel assay involves ZNA probe immobilization onto the surface of carbon nanofiber modified screen printed electrodes (CNF-SPE) or multi-channel screen printed array of electrodes (MULTIx8 CNF-SPE). After hybridization occurred between ZNA probe and mtDNA target onto the surface of electrode, the impedimetric measurement was performed and accordingly, the R_{ct} values were recorded as a response of hybridization related to FV Leiden detection. The selectivity of ZNA probe was tested in the presence of mutation-free DNA sequences (G > C or G > T) as well as synthetic PCR samples (143 nt and 220 nt) containing a single base mutation (G > A).

2. Experimental

2.1. Apparatus

Electrochemical measurements were performed by AUTOLAB-30 and AUTOLAB-302 PGSTAT electrochemical analysis system with NOVA (version 1.1.2 EcoChemie, The Netherlands) or GPES 4.9.007 software package (Eco Chemie, The Netherlands) using electrochemical impedance (EIS) technique. The impedimetric measurements with CNF-SPEs was done in a Faraday cage (EcoChemie, The Netherlands).

2.2. Chemicals

The amino linked from 5′ end of ZNA probe and the other oligonucleotides; complementary mutant type DNA target, wild type DNA oligonucleotide, the oligonucleotides having different types of single base mutation, noncomplementary DNA sequences and PCR products were purchased from (as lyophilized powder) TIB Molbiol (Germany). The sequences of oligonucleotides and PCR products are given in Supporting information.

ZNA probe stock solution as 472 μg mL^{-1} was prepared in Dulbecco's modified Phosphate Buffer Solution (pH 7.40) and kept frozen. The stock solutions of other oligonucleotides were prepared in ultrapure water (i.e, RNase/DNase free). The diluted solutions of ZNA probe, DNA probe, wtODN, C-mtDNA ODN, T-mtDNA ODN, NC-1, NC-2 and PCR products were prepared in 50 mM phosphate buffer solution containing 20 mM NaCl (PBS, pH 7.40). The diluted solutions of mtDNA target was prepared in PBS (pH 7.40), acetate buffer solution (ABS, pH 4.80), or carbonate buffer (CBS, pH 9.50), that was preferentially used according to the protocol followed herein.

Other chemicals were in analytical reagent grade and supplied from Sigma-Aldrich and Merck.

The detailed information about carbon nanofibers enriched screen printed electrodes (CNF-SPEs) and multi-channel screen printed array of electrodes (MULTIx8 CNF-SPEs) is given in Supporting information.

2.3. Procedure

The procedure included ***(i)*** immobilization of ZNA probe onto the surface of CNF-SPE or MULTIx8 CNF-SPE ***(ii)*** hybridization of ZNA probe with complementary mtDNA target, or other oligonucleotides; wtDNA oligonucleotide, noncomplementary ODNs (C-mtDNA ODN, T-mtDNA ODN, NC-1, NC-2) as well as complementary mutant type PCR-1,2 and wild type PCR-1,2 onto the electrode surface, ***(iii)*** impedimetric measurement.

2.3.1. Immobilization of ZNA probe onto the surface of electrodes

ZNA probe was prepared in PBS (pH, 7.40) and immobilized onto the surface of working electrode; CNF-SPE/MULTIx8 CNF-SPE via passive adsorption during 30 min as reported in our previous work [33]. Same experimental procedure was followed in the presence of DNA probe selective to mtDNA target as well as control experiments performed spermine alone. Then, the electrode was washed with PBS (pH 7.40) in order to eliminate unspecific binding.

2.3.2. Hybridization between ZNA probe/DNA probe and its complementary mtDNA target, or other ODNs

The required amount of mtDNA target or other ODNs prepared in PBS (pH 7.40) was dropped onto the probe immobilized working electrode of CNF-SPE/MULTIx8 CNF-SPE during 20 min. Then, the electrode was washed with PBS (pH 7.40) in order to eliminate unspecific binding.

2.3.3. Impedimetric measurements

Impedimetric measurements were performed in the presence of 2.5 mM $K_3[Fe(CN)_6]$ and 2.5 mM $K_4[Fe(CN)_6]$ (1:1). The impedance was measured in the frequency range between 100 kHz and 0.1 Hz at a potential of +0.23 V with an amplitude of 10 mV. The frequency interval was divided into 98 logarithmically equidistant measure points. All measurements were performed by AUTOLAB-30 system and NOVA software package (version 1.1.2 Eco Chemie, The Netherlands).

The real and imaginary impedance (Z′ and -Z″) are the components of the complex impedance (Z). An equivalent circuit model (Randles circuit) was utilized for fitting of impedimetric results. The electron transfer was limited at higher frequencies and the linear section seen at lower frequencies may be attributed to the diffusion as explained earlier studies [19] According to the Randles circuit, R_s is the solution resistance. Q is the constant phase element. The charge transfer resistance (R_{ct}) is defined as the resistance related to the dielectric and insulating characteristics at the electrode/electrolyte interface. W is the Warburg impedance due to mass transfer to the electrode surface and observed at higher frequencies.

3. Results and discussion

3.1. Impedimetric detection of FV Leiden mutation using ZNA probe in combination with CNF-SPEs

In order to perform efficient ZNA-DNA hybridization, the experimental conditions, such as, the hybridization temperature, the concentration of ZNA probe, mtDNA target and the effect of Mg^{+2}, pH and hybridization time were optimized. Additionally the interference effect of spermine is tested and all results were presented in Figs. S1–S7 as well as discussed in the supporting information. Further experiments related to the detection of FV Leiden mutation (G > A) were done under the optimized conditions.

3.2. The effect of mtDNA target concentration upon the hybridization process

The nucleic acid hybridization between 0.5 μg mL^{-1} 5′ZNA probe and mtDNA target in different concentrations varying from 2 to 14 μg mL^{-1} (0.3 μM–2 μM) was carried out under optimum conditions. The changes at the R_{ct} value was evaluated and shown in Fig. 1. There was a gradual increase at the R_{ct} value after the full match hybridization of ZNA probe with its target, mtDNA up to 12 μg mL^{-1} (1.7 μM) concentration level of mtDNA, and then it levelled off (Fig. 1 and Fig. S8). The highest R_{ct} was measured as 1081.7 ± 45.3 Ohm (RSD%, 4.2%, n = 3). Thus, 12 μg mL^{-1} mtDNA target was used in our further studies performed by CNF-SPEs.

The limit of detection (LOD) was calculated according to the Miller and Miller method [34] and found to be 1.48 μg mL^{-1} (207 nM) in the linear concentration range of mtDNA target with the equation y = 47.75x + 144.90 and $R^2 = 0.98$ (Fig. S8 inset).

3.3. Selectivity of ZNA probe in contrast to DNA probe

The selectivity of the ZNA probe based biosensor was tested against to wild type DNA target (wtDNA ODN). The average R_{ct} was recorded in the case of full match hybridization between 5′ZNA probe and mtDNA target as 1081.7 ± 45.3 Ohm (RSD %, 4.2%, n = 3), whereas it was obtained as 644.5 ± 68.6 Ohm (RSD %, 10.6%, n = 3) in the case of hybridization between 5′ZNA probe and wtDNA target. On the other hand, the R_{ct} values were measured as 1061 Ohm and 1187 Ohm after the hybridization of DNA probe respectively with mtDNA, or wtDNA ODN (Fig. 2). It was concluded that the 5′ ZNA probe presented more selective behaviour to detect SNP in contrast to DNA probe.

The hybridization efficiency (HE %) is used as the signature of the full match hybridization effectiveness [35]. It was calculated for each hybridization occurred between DNA probe/ZNA probe with each type of target; mutant type or wild type according to equation (1).

$$\text{The hybridization efficiency (HE \%)} = [\Delta R_{ct} / R_{ct\ hybrid}] \times 100 \quad (1)$$

$$(\Delta Rct = R_{ct\ hybrid} - R_{ct\ probe})$$

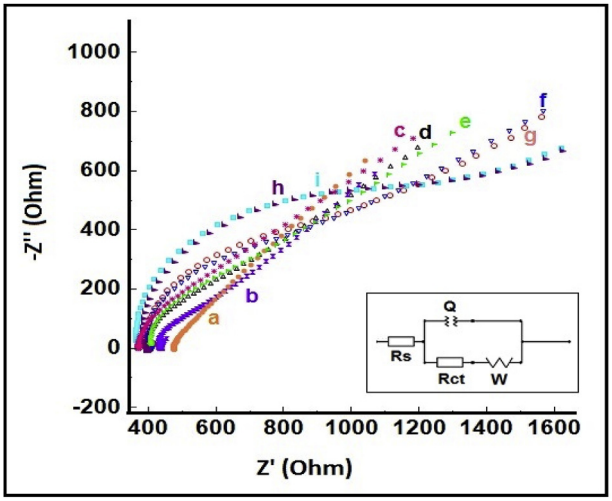

Fig. 1. Nyquist diagrams obtained by **(a)** CNF-SPE, **(b)** 0.5 μg mL^{-1} 5′ZNA probe immobilized CNF-SPE, after the hybridization between 0.5 μg mL^{-1} 5′ ZNA probe and mtDNA target in its different concentrations **(c)** 2, **(d)** 4, **(e)** 6, **(f)** 8, **(g)** 10, **(h)** 12, **(i)** 14 μg mL^{-1} by CNF-SPE. Inset was the equivalent circuit model used for fitting of the impedance data.

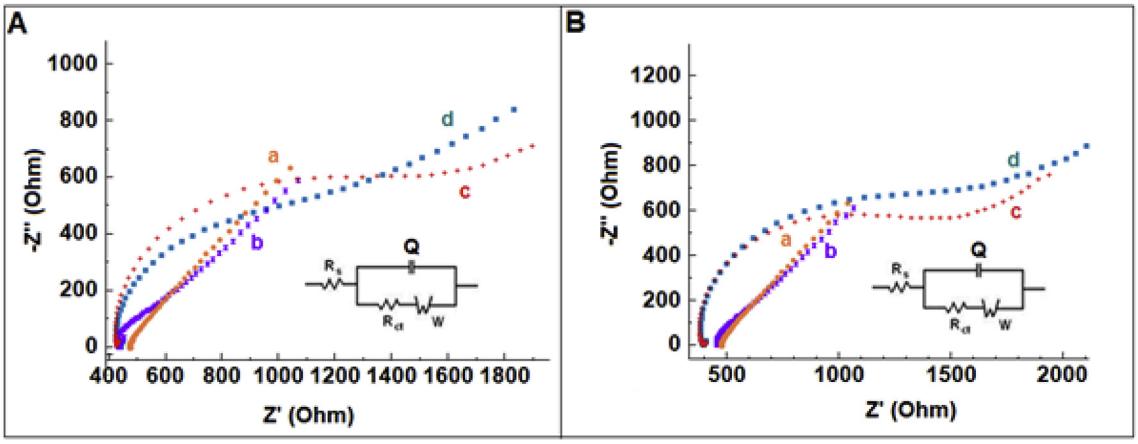

Fig. 2. The Nyquist diagrams of **(a)** CNF-SPE, **(b)** 0.5 μg mL^{-1} **(A)** 5′ ZNA probe, **(B)** DNA probe immobilized CNF-SPE, hybridization between **(A)** 5′ZNA probe or **(B)** DNA probe and 12 μg mL^{-1} **(c)** mtDNA target or **(d)** wtDNA ODN. Inset was the equivalent circuit model used for fitting of the impedance data.

HE % is accepted as 100% in the case of hybridization between ZNA probe with its complementary mutant type target. The lower HE % value is expected in the case of hybridization between ZNA probe and wild type target. The differentiation between the values of HE % that is calculated in the presence of ZNA probe should be higher in comparison to the differentiation between HE %s obtained in the presence of DNA probe. Therefore, we can consider that ZNA probe can recognize SNP more selectively than DNA probe. After the possible interaction between spermine and mtDNA, the value of HE % was calculated and found to be 60%, whereas HE % was accepted as 100% in the case of full hybridization between ZNA probe and mtDNA (Fig. S7). According to the data obtained in control experiment, it was concluded that the spermine did not show any interference effect on hybridization process.

The selectivity of ZNA probe to FV Leiden mutation was then tested against to other oligonucleotides having different mutations (Fig. S9). In the presence of hybridization of ZNA/DNA probe with its complementary strand, the highest R_{ct} value is expected in contrast to the ones measured in the presence of other ODNs. In order to examine the selectivity of the ZNA based platform, a batch of experiment was performed in the presence of other oligonucleotides containing single-base mutation located at mtDNA target sequence, or non-complementary sequences. As expected, the highest R_{ct} value was obtained as 1081.7 ± 45.3 Ohm (RSD%, 4.2%, n = 3), after the full match hybridization of ZNA probe with mtDNA target. The average R_{ct} and HE % values that obtained after hybridization of ZNA probe with mtDNA target/C-mtDNA/T-mtDNA/NC-1/NC-2 presented in Table S1. According to HE % values, it can be concluded that ZNA probe exhibited a selective behaviour even in the presence of oligonucleotides having a different single-base mutation (G > C or G > T), or non-complementary sequences.

3.4. Detection of FV Leiden mutation in synthetic PCR products using ZNA based biosensor in contrast to DNA based biosensor

FV Leiden mutation in 143 nt length PCR products was detected using ZNA probe/DNA probe immobilized CNF-SPEs, and the results were given in Fig. 3 and Table S2. The HE % was calculated related to each experiment on possible hybridization between ZNA probe/DNA probe with mtPCR/wtPCR (Eq. (1)). After the hybridization of ZNA probe with wtPCR-1, the value of HE % was calculated as 34%, whereas HE % was accepted as 100% in the case of hybridization of ZNA probe with mtPCR-1. However, the values of HE % were found to be 79% and 58%, respectively in the case of hybridization of DNA probe with mtPCR-1, or wtPCR-1. According to the HE % values (shown in Table S2), it is obvious that a selective detection of SNP can be performed by ZNA probe, even this target sequence is a part of PCR product with the length of 143 nt.

Similarly, a batch of experiments was performed using synthetic PCR products in the length of 220 nt (named as mtPCR-2 and wtPCR-2), and the results were shown in Fig. 4 and Table S3. After the hybridization of ZNA probe with mtPCR-2, or wtPCR-2, the average R_{ct} values were measured as 895.5 ± 48.8 Ohm and 612.5 ± 36.1 Ohm with the RSDs % (n = 3) as 5.4% and 5.9%, respectively. After hybridization of DNA probe with mtPCR-2, or wtPCR-2, the average R_{ct} values were measured as 921.0 ± 241.8 Ohm and 1153.0 ± 287.1 Ohm with the RSDs % (n = 3) as 26.3% and 24.9%, respectively (Fig. 4).

According to HE % values that calculated for each synthetic PCR products in length of 220 nt, (shown in Table S3), it was concluded that DNA probe could not present any selective behaviour in hybridization process with mtPCR-2, or wtPCR-2. On the other hand, ZNA probe selectively detect the single-base mutated target, mtPCR-2 over to wtPCR-2, even the target sequence is a part of synthetic PCR product in length of 220 nt.

3.5. Impedimetric detection of FV Leiden mutation by multi-channel array of electrodes (MULTIx8 CNF-SPE)

It is aimed to implement our assay to multi-channel array of electrodes which are specially designed for the development of multiple simultaneous analysis. Under this aim, the effect of the changes at the concentration of probe, mtDNA target, hybridization temperature upon to the response measured by MULTIx8 CNF-SPE was examined. In view of that these results were presented in Figs. S10–S12 as well as discussed in the supporting information. Further experiments related the detection of FV Leiden mutation by MULTIx8 CNF-SPE were done under the optimized conditions.

After the full match hybridization between 5′ZNA probe and mtDNA target in different concentrations varying between 2 and 12 μg mL^{-1}, a gradual increase at the R_{ct} value was obtained until 10 μg mL^{-1} mtDNA target, and then it levelled off (shown in Fig. 5 and Fig. S13). The average R_{ct} value was measured as 2419 ± 216.0 Ohm with the RSD% as 8.9% (n = 3) after the hybridization of 1 μg mL^{-1} 5′ZNA probe with 10 mtDNA target. Thus, the optimum mtDNA concentration was chosen as 10 μg mL^{-1} (equals to 1.4 μM)

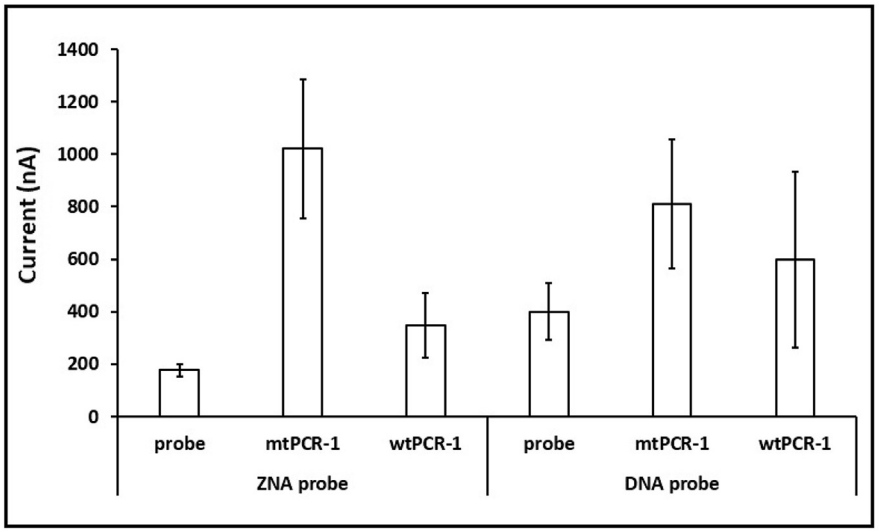

Fig. 3. Histograms representing the average R_{ct} values measured by DPV using CNF-SPEs after hybridization of 0.5 μg mL^{-1} ZNA probe or DNA probe with 12 μg mL^{-1} mtDNA target, mtPCR-1 and wtPCR-1 using CNF-SPEs (n = 3).

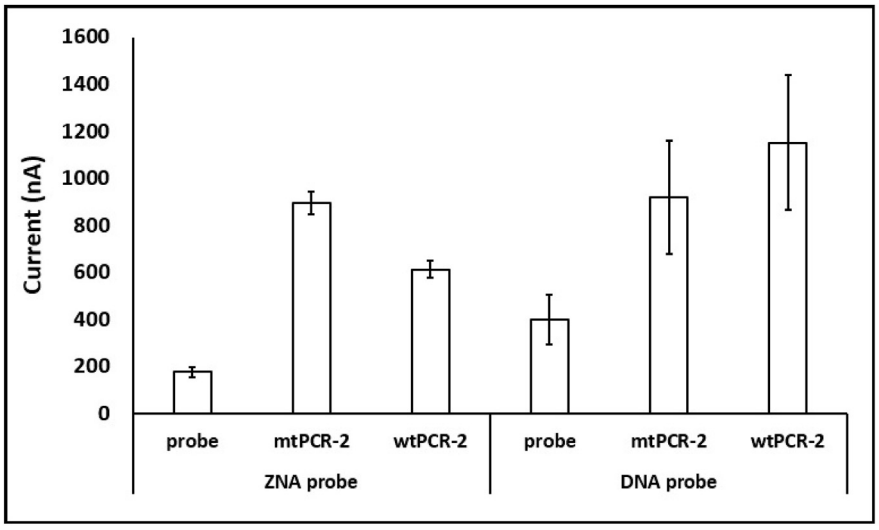

Fig. 4. Histograms representing the average R_{ct} values measured by DPV using CNF-SPEs after hybridization of 0.5 μg mL^{-1} ZNA probe or DNA probe with 12 μg mL^{-1} mtDNA target, mtPCR-2 and wtPCR-2 using CNF-SPEs (n = 3).

for further studies with MULTIx8 CNF-SPE.

The LOD was calculated according to the Miller and Miller method [34] and found to be 0.95 μg mL^{-1} (133 nM) in the linear concentration range of mtDNA target with the equation $y = 225.69x+150.35$ and $R^2 = 0.99$ (shown Fig. S13 inset).

3.6. Selectivity of ZNA probe in contrast to DNA probe by MULTIx8 CNF-SPE

The selectivity of ZNA probe to FV Leiden mutation was tested against to oligonucleotides having different mutation (G > C or G > T), where the mutant base was located at mtDNA target, or noncomplementary oligonucleotides; such as, C-mtDNA/T-mtDNA/NC-1/NC-2 (Fig. S14). The values of average R_{ct} with the calculated HE % were given in Table S4. According to the HE % values, ZNA probe exhibited a selective behaviour to its complementary target in contrast to other DNA oligonucleotides containing different single-base mutation, or noncomplementary DNA sequences.

3.7. Detection of FV Leiden mutation in synthetic PCR products using ZNA based multi-channel array of electrodes in contrast to DNA based electrodes

The impedimetric detection of FV Leiden mutation in the synthetic PCR products with the length of 143 nt was carried out using MULTIx8 CNF-SPE and the results were given in Fig. 6 and Table S5. After the hybridization of ZNA probe with wtPCR-1, the value of HE % was calculated as 59%, whereas HE % was accepted as 100% in the case of hybridization of ZNA probe with mtPCR-1. On the other hand, the values of HE % were found to be 57% and 93%, respectively after hybridization of DNA probe with mtPCR-1, or wtPCR-1. It can be concluded that a more selective detection was performed by ZNA probe to a single-base mutated target in PCR product with 143 nt in contrast to the result obtained by DNA probe.

Similarly, the selectivity of ZNA based biosensor was tested when the FV Leiden mutation was a part of PCR products with 220 nt; mtPCR-2 and wtPCR-2, and the results were shown in Fig. 7

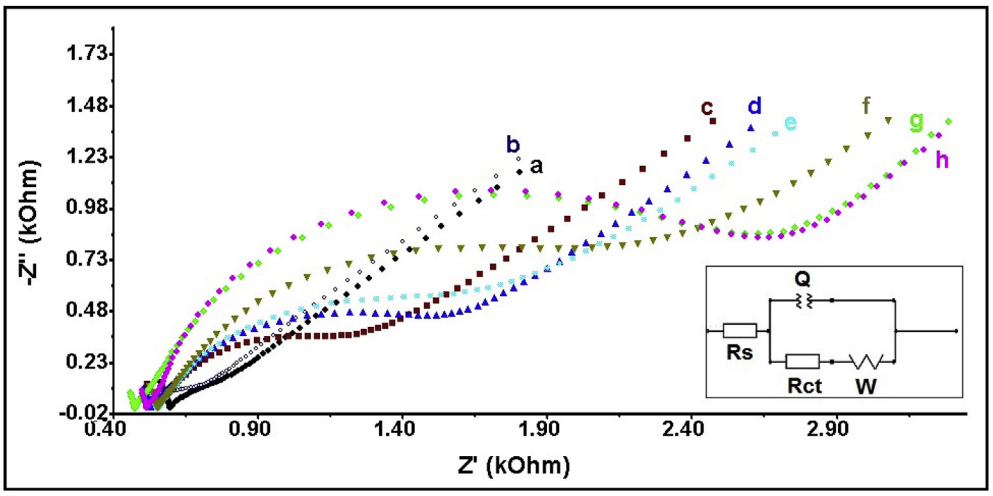

Fig. 5. Nyquist diagrams obtained by **(a)** MULTIx8 CNF-SPE, **(b)** 1 μg mL^{-1} 5′ZNA probe immobilized MULTIx8 CNF-SPE, after the hybridization between 1 μg mL^{-1} 5′ ZNA probe and mtDNA target in its different concentrations **(c)** 2, **(d)** 4, **(e)** 6, **(f)** 8, **(g)** 10, **(h)** 12 μg mL^{-1} by MULTIx8 CNF-SPE. Inset was the equivalent circuit model used for fitting of the impedance data.

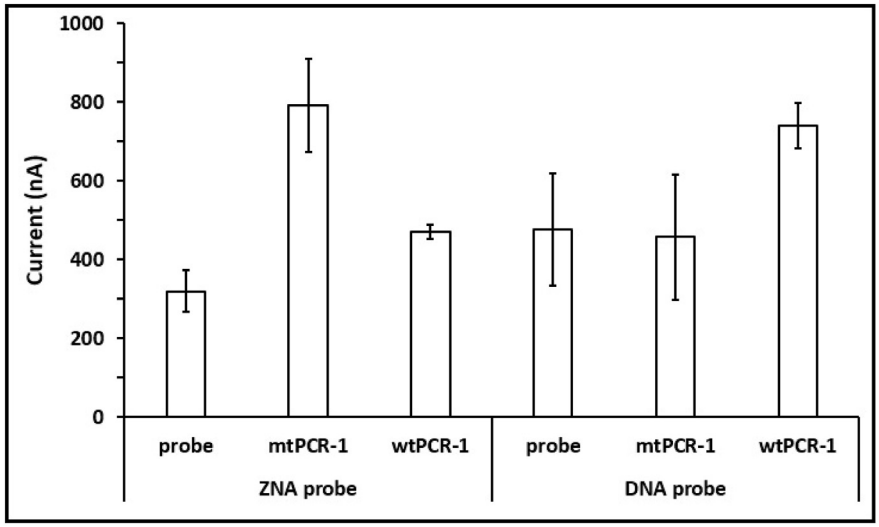

Fig. 6. Histograms representing the average R_{ct} values measured by DPV using MULTIx8 CNF-SPEs after hybridization of 1 μg mL^{-1} ZNA probe or DNA probe with 10 μg mL^{-1} mtDNA target, mtPCR-1 and wtPCR-1 (n = 2).

and Table S6. After the hybridization of ZNA probe mtPCR-2, the average R_{ct} value was recorded as 987.0 ± 130.1 Ohm (RSD%, 13.2%, n = 3) and HE % is accepted as 100% for this case. After the hybridization of DNA probe with mtPCR-2 or wtPCR-2, the R_{ct} values were measured as 1015.5 ± 40.3 Ohm (RSD%, 4.0%, n = 3) and 875.0 ± 224.9 Ohm (RSD%, 25.7%, n = 3), respectively (Fig. 7). According to HE % values, it can be concluded that ZNA probe selectively detected the single-base mutated target of mtPCR-2 comparison to wtPCR-2, even the target sequence was a part of PCR product in length of 220 nt.

4. Conclusion

In the present study, the impedimetric detection of a single nucleotide mutation related to FV Leiden mutation was performed by using the new generation nucleic acids (ZNAs) in combination with carbon nanofiber (CNF) modified screen printed electrodes (SPE) and multi-channel screen printed array of electrodes (MULTIx8 CNF-SPE). The LODs were found to be 1.48 μg mL^{-1} (207 nM) and, 0.95 μg mL^{-1} (133 nM) for CNF-SPEs and MULTIx8 CNF-SPEs, respectively. The LODs were lower that our earlier ZNA based biosensor studies related to FV Leiden mutation [36,37]. The selectivity of ZNA probe to mutation-free DNA sequences, or synthetic PCR samples in different length; 143 and 220 nt containing a single base mutation, or without any mutant base was also examined using impedimetric ZNA biosensor.

Herein, the impedimetric analysis of FV Leiden mutation was performed in relatively shorter time (i.e 30 min) and selectively in comparison to earlier studies [17,18]. As an another advantage, detection is performed without using any metallic nanoparticles or inosine base substituted probe (metallic nanoparticles or inosine base substituted probe) which makes our assay more practical and cost effective earlier studies [16,38–40].

The remarkable hybridization properties of ZNA with respect to both its affinity and specificity make it an advanced molecule towards to research and innovation on biotechnology. Novel biosensing platforms were successfully used herein by incorporation of ZNAs with the advantages of their unique chemical and

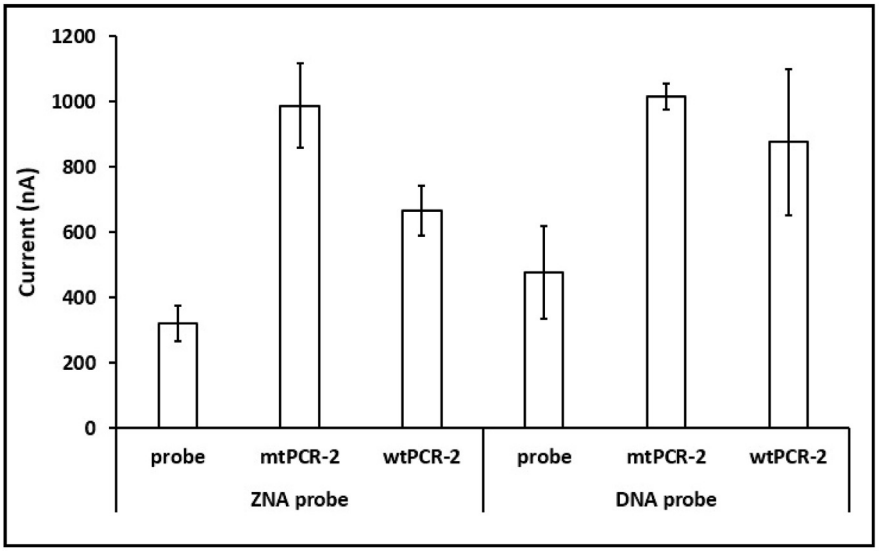

Fig. 7. Histograms representing the average R_{ct} values measured by DPV using MULTIx8 CNF-SPEs after hybridization of 1 μg mL^{-1} ZNA probe or DNA probe with 10 μg mL^{-1} mtDNA target, mtPCR-2 and wtPCR-2 (n = 3).

structural properties. Moreover, CNF modified electrodes presented some important advantages such as, being disposable, cost-effective, easy-to use, and not requiring any time consuming modification step, or any sophisticated instruments with a special training in comparison to other biosensor studies in the literature [16–18,38–40].

CRediT authorship contribution statement

Arzum Erdem: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - review & editing. **Ece Eksin:** Formal analysis, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.04.036.

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