Chapter

Nucleic Acids for Electrochemical Biosensor Technology

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Abstract

Biosensor technology has developed extremely rapidly in recent years. This technology brings along precise measurements as well as specific measurements. Thanks to its ability to be miniaturized and be easily accessible to the end user, it is one-step ahead of other similar methods. The selectivity of biological molecules and the sensitivity of electrochemical methods enable the continuous evolvement of these new technologies. In this chapter, the use of nucleic acids as both recognition agents and target molecules, the way they are used in biosensor technology and their electrical properties are explained in detail with examples. Aptamers, which are synthetic nucleic acids, and their use in electrochemical biosensor systems with different electrochemical and immobilization methods have been compared extensively.

Keywords: biosensor, DNA, RNA, aptamer, electrochemistry, sensor, nanomaterials, impedance, differential pulse voltammetry, cyclic voltammetry, DNA tetrahedron, mediator

1. Introduction

Biosensor technology is a promising field where many outputs are continuously produced thanks to physicochemical techniques and biological materials developed every day. In this technology, a biological molecule interacts with the analyte on the biosensor and this generates a physicochemical signal which is detected by the transducer. Biosensor systems are divided into two classes in terms of biological molecules used, either catalytic-based which transforms the analyte or affinity-based which binds the analyte directly [1]. In terms of physicochemical signal transmitter, it can be designed as electrochemical, optic or piezoelectric. Systems that are combined within also continue to be open to development today. For example, both electrochemistry and optical measurements can be made in spectro-electrochemical techniques. Among these techniques, electrochemical systems [2], which are produced at low-costs without being interfered by the properties of the analyte solution, are used mostly.

The most important element of the biosensor is the biorecognition agent which shows the affinity for the analyte. These agents are of biological origin and are biomolecules with specific substrates such as proteins [3], enzymes [4], nucleic acids [5], antibodies [6], cells [7], cell surface channels. Biosensors can be designed by immobilizing these biomolecules on a suitable signal transmitter based on the

working principle. When selecting the biorecognition agent, the specimen containing the analyte to be measured and the characteristics of the reaction that will take place should be considered. It is desired that the biorecognition agent has a maximum activity and that it has a low degree of denaturation.

The living organism's structural and functional strategy is hidden in its genes. The gene structure is based on the bases that form nucleic acids, which are divided into two main groups according to their functions, are deoxyribonucleic acid (DNA) and ribonucleic acids (RNA) [8]. DNA carries the genetic information in the double helix structure that includes adenine (A), guanine (G), cytosine (C) and thymine (T) bases. RNA has two main differences when compared to DNA, which are the inclusion of uracil (U) instead of T and the single stranded nucleic acid structure different from DNA. DNA's double helix structure is formed with hydrogen bonds between the DNA bases of the complementary chains. In the same chain, ester bases between the 5 'OH group on the pentose sugar of a nucleotide and the 3' OH group of the other nucleotide sugar form the single chain structure (**Figure 1**).

Thereby, U, A, G, C and T can be ordered in countless combinations to form genetic sequences. These sequences are copied and then transcribed in the ribosomes to take part in the synthesis of peptides and proteins by encoding one amino acid corresponding to all three bases.

Although they have different structures in different living organisms, the basic function of base sequences does not change. Erwin Chargaff showed that A-T and G-C base pairs in DNA sequences are mutually paired with hydrogen bond [9]. The important point here is that the hydrogen bonds formed between DNA helixes directly affect the physical properties of DNA, since there are three hydrogen bonds between G-C, while there are two hydrogen bonds between A-T. If the G-C ratio is high, it requires more energetic power to separate the double helix. This is a considerable structural characteristic for the electrochemical techniques.

On the other hand, RNA is a single chain nucleic acid produced by using DNA sequences. Besides the three main types of RNA in protein encoding (mRNA, tRNA and rRNA), there are also other types of RNAs that serve in post-translational modifications in DNA replication or as regulators. For example, small nuclear RNA (snRNA) involved in RNA cleavage, guide RNA (gRNA) involved in CRISPR-Cas9 system, micro RNA (miRNA), small interfering RNA (siRNA) and viral RNA. These different nucleic acids are worth to identify their function and structure [10].

Today, DNA or RNA analysis can be done quite precisely. Therefore, genetic sequences have a central role in diagnosis and treatment processes and they guide the scientists for development of new techniques. RNA and DNA sequences can be easily illuminated with polymerase chain reactions (PCR) and next-generation sequencing analyzers [11]. Like every method, these methods have limitations. For PCR, it is necessary to use consumables for reproduction and analysis of the nucleic acid sequences. This increases analysis costs and affects the analysis time. The disadvantages of the methods naturally make it inevitable to develop new and efficient methods. Although there are different nucleic acid analysis methods, simple and precise methods are needed. Bioelectronics systems are preferable as they are in the front line with their low cost, fast analysis time, minimum consumable requirement and lower margin of errors. Efficient properties of biological molecules with physicochemical transducer sensitivity increase the preferability. These basic mini analyzers are able to provide fast, low cost and precise analysis, based on the immobilizing biological molecules on a physicochemical transducer. The term biosensor is developed by immobilizing a biomolecule of biological origin that provides the biochemical reaction on a transducer. In terms of classification, it can be divided into different areas according to both the working principle of the biomolecule and the working principle of the transducer.

Figure 1.
Representation of the hydrogen bonds between DNA chains.

In electrochemical nucleic acid biosensor systems, the electrical signal occurs because of the interaction between the biorecognition agent and the analyte. As a result of the biochemical reaction, if an electron is formed, amperometric measurement can be performed. If a molecule is formed it can be detected by potentiometric

or direct affinity-based binding can be measured as impedimetric/capacitive. When there is no molecule being exposed, only affinity-based biosensor systems can be designed amperometrically. In this design, when the interaction between biomolecules occurs, a secondary molecule (label) can generate an electrochemical signal. Measurements can be performed through the electrochemical activity of the label used here. Electrical conductivity is an extremely important parameter, especially in biosensor systems. Nucleic acids, on the other hand, can be considered as ideal transistors, because of their structure, they show conductive nanocable characteristics and are very efficient in use in electrochemical biosensors in order to be found in many different conformations. The DNA helix has between 3.4-angstrom base pairs, and the aromatic ring structure facilitates electron flow. This structure, that is the closeness between the bases, is similar to the Z-directional space of graphite and provides conductivity. Moreover, the π electrons on DNA also help electrical conductivity [10]. They help electrochemical conductivity due to structural variability.

On the other hand, beside the electroactivity of DNA, the electrochemical measurement method is important in the design of nucleic acid biosensors. Some electroactive secondary molecules can be used for the electrochemical detection. Mediators, which are frequently used in biosensor systems, can also be used to measure molecules that interact with DNA. Mediator is an intermediary molecule that facilitates electron exchange in an electrochemical reaction and lowers the reduction/oxidation potential of the detection system and also has regeneration potential [1]. The most important feature of a mediator is that it can signal in a low and narrow potential ranges. This feature increases the sensitivity of the biosensor since signaling of other electroactive species can be prevented in lower potentials.

Electroactive species can be determined using techniques such as differential pulse voltammetry (DPV) and cyclic voltammetry (CV) in studies that require the measurement of electroactive species, and the measurement of the amount of DNA through the reduction or oxidation of these species. The basic principle of the measurements is the presence of electroactive species on DNA (guanine base) or the formation of signals by binding some mediators or indicators to DNA. Indicators such as ruthenium complexes, ferrocene and methylene blue are often used for nucleic acid based biosensors. Measurement can be performed by chemically marking the DNA or RNA at the end or forming a complex with DNA helix. Measuring current generated by electroactive species in these electrochemical techniques is extremely important, but can also limit the effectiveness of measurement systems. In other electrochemical measurement systems where mediators are not used, different electrochemical techniques that can measure nucleic acid binding or conformational changes may be used. These methods are performed in redox probe solutions to characterize electrode surface. Here, CV is capable of measuring physical changes on the surface with the help of a redox probe. When the sensitivity of CV in affinity based non-electroactive detections is insufficient, electrochemical impedance spectroscopy (EIS) or capacitance (C) measurement can also be used in affinity-based nucleic acid biosensors. These two methods are really sensitive methods to detect biomolecules, charge transfers and mass transfers with reaction kinetics and also are affected by electrode surface charges [12]. Therefore, nucleic acid length, base composition, and conformational changes after target molecule binding had to be considered before study design. Moreover, the negatively charged redox probe usage reduces the interference of the redox probe interaction between nucleic acid in the measurement.

Another advantage of nucleic acids is that there is no denaturation or loss of activity like proteins. The most important point to consider here is to take measures to reduce the effectiveness of DNase and RNase enzymes that break down nucleic

acids. Some examples of methods that can be used when designing a biosensor system with nucleic acids are given in the following pages.

2. Nucleic acids as biorecognition receptors and target

With 5 different base sequences on nucleic acids, the potential for an almost unlimited sequence is theoretically available. Their use as both a biorecognition agent and a target molecule to be analyzed is among its advantages. By its ability to form a complementary structure even within itself, it shows the feature of an analyte-biorecognition agent.

2.1 DNA and RNA usage in biosensor technology

In studies where DNA is used as a biorecognition agent, biosensor systems can be designed by measuring molecules that interact directly or indirectly with DNA. Especially in genetic analysis, DNA determinations have been performed based on complementary base pairings (**Figure 2**).

Chen et al. [13] developed a DNA biosensor whose signal was increased in triplicate for the determination of transgenic soybeans. Signal enhancement was performed as rolling circle amplification (RCA). In the biosensor system Chronocoulometry was used to detect DNAs via electrode surface charges. Firstly, Fe_3O_4@Au magnetic nanoparticles were produced and the electrode was modified with SH modified DNAs to capture the target DNA sequence. After this immobilization on carbon electrode and incubated by dropping target DNA, complementary DNA forms a double structure with the target DNA. Afterwards, the double helix was cut and removed with ExoIII and 4 single-stranded ssDNA with a gold nanoparticle cube in the center was added to the free cutted ends, and binding was achieved with the help of Phi29 DNA polymerase and T4. $[Ru(NH_3)_6]^{3+}$, which forms a complex with anionic phosphates, is used as a complex mediator specifically bound on this quaternary structure. The amount of DNA was determined chronocoulometrically over the electrochemical signals generated by the ruthenium complex. A linear calibration curve was obtained between 10^{-16} and 10^{-7} M, and LOD was calculated as 4.5×10^{-17} M. Target DNA analysis took 2 hours. By the help of this complex DNA analysis, the authors managed to develop an ultra-sensitive system. Sensitivity is increased with the DNA immobilization steps used here. However, since the study requires a repetitive enzyme treatment, it seems costly and time consuming in developmental process.

Nucleic acid biosensors can also be developed by using enzymes used in DNA analysis. In recent years, studies using the CRISPR-Cas9 system are used in biotechnological studies as a genetic editing molecule. Uygun et al. [14] developed an impedimetric graphene oxide electrode modified with CRISPR-dCas9 for the determination of the target circulating tumor DNA molecules containing the PIK3CA exon 9 mutation, which is used as a biomarker in breast cancer and is referred to as a liquid biopsy biomarker. In this study, deactivated Cas9 (dCas9) proteins without exonuclease activity, were used as biorecognition receptor by modifying them with a sgRNA that would recognize the target ctDNA sequence. Graphene oxide electrodes were modified with dCas9 and sgRNA, consequently. In this way, the modification for ctDNA analysis is completed. The short analysis time (40 seconds) needed was a great advantage. The impedimetric measurement method with a 1.92 nM LOD in the range of 2–20 nM ctDNA concentration has been developed.

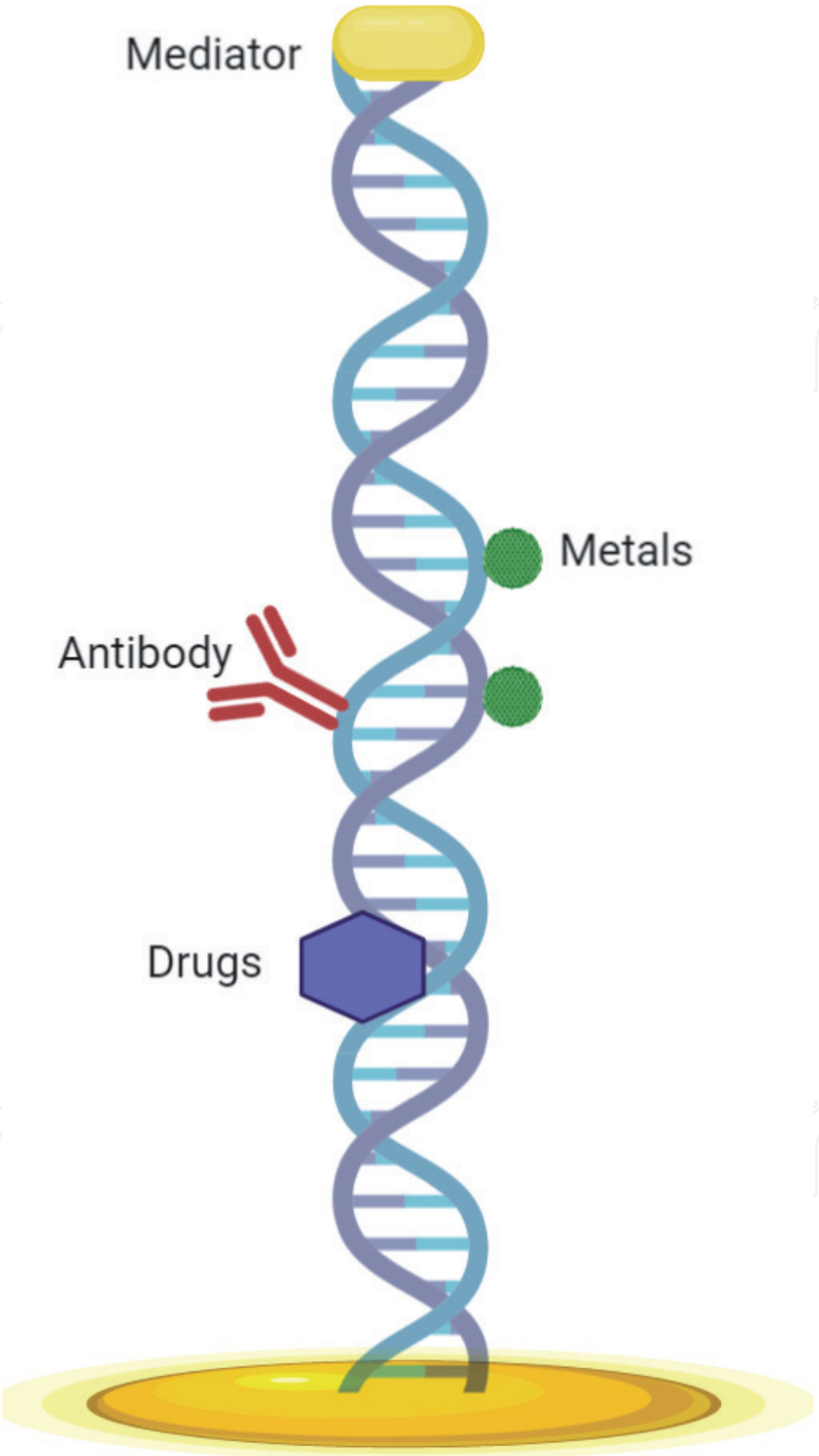

Figure 2.
Schematic representation of the DNA as biorecognition receptor.

Because of the charges on the DNA, analysis of cations, where the bases show selectivity, can be detected. In another biosensor study, due to its negatively charged structure, the ability of chelating properties of DNAs for metal ions

is used. Zhang et al. [15] used DNA molecules for the determination of mercury ions. DNA probes modified with the DNA sulfur group formed a self-assembly monolayer on the gold electrode. Afterwards, the electrode modified with the reporter DNA, which has a gold nanoparticle in the center, was measured by binding mercury ions. Both impedance and CV were used in the study. The linear measuring range was set as 1–200 nM and the LOD was calculated as 0.05 nM. By this study, thanks to the use of DNA molecules, the mercury molecule was determined with a relative standard deviation of 3% within real samples.

To observe the complementary base pairing and post translational modifications, secondary conjugates such as antibody or enzyme can be used in the DNA based biosensor studies. Huang et al. [16] developed a biosensor to determine DNA methylation levels. In this study, the methylation level was determined with the help of a bioconjugate modified by a secondary antibody. This conjugate is formed on graphene oxide layers that target CpG and have horseradish peroxidase (HRP)-labeled anti-5-methylcytosine antibodies. The signal generated by HRP is directly proportional to the presence of methyl groups, and DPV was used in this enzymatic reaction-based study. Hydroquinone as a mediator, i.e. electron transmitter, also reduced the reaction voltage and made it more selective. Then, the measurement was carried out on the complex of the bioconjugate with cytosine containing methyl groups. While the biosensor reaches 1 fM LOD value, it can measure in the linear range between 1 fM and 10 nM. The preparation process of the biosensor took approximately 5 hours. In this study, since DNA methylation is performed by measuring the enzyme activity on the bioconjugate, the measurement efficiency is based on the performance of the antibody, enzyme and the mediator.

Jahandari et al. [17] developed a biosensor modified with gold nanoparticles for DNA-based Temodal (anticancer drug) determination. They also modified dsDNA molecules electrochemically on the gold nanoparticles deposited on the pencil graphite electrode. The binding of the Temodal to the DNA on the biosensor was measured by DPV. Here, the main measurement is not the Temodal measurement directly. As the intercalator agent binds to the DNA, the reduction potential of the guanine base on the DNA decreases due to the amount of Temodal that is bound to DNA. Temodal performed intercalation by the interaction of the minor groove on the DNA. In this system with a maximum measurement time of 8 minutes, a maximum recovery deviation of 5% was observed in real samples. Linearly, it showed 1 nM LOD with measurement performance between 5 nM and 45 μM. The performance and sensitivity of this study are directly proportional to the presence of guanine bases. The base ratio on DNA is important in the design of such studies.

Ebrahimi et al. [18] determined cadmium ions using ethyl green (EG) on a simple DNA-based biosensor system. EG can be used as a hybridization indicator in DNA studies. Cadmium is known as a toxic heavy metal and this heavy metal measurement is especially important for biological samples. Cadmium destabilizes the double helix by forming a bond through the N7 atom of the guanine base on DNA. Similarly, in this study, measurement was performed using differential pulse voltammetry signals of EG. The signal generated by DPV is the oxidation signal that occurs as the result of the release of EG by destabilizing double helix. In other words, EG among the DNA helix is released as the result of the binding of cadmium to DNA, and it forms a destabilized DNA signal with cadmium. Measurement of cadmium ions showed 0.3 pM LOD with a linear measurement between 1 pM and 1 nM and between 10 nM and 1 μM. The limitation of the study is the accumulation performance of EG on DNA. The degree of this accumulation determines the sensitivity. It is difficult to determine the low amounts of cadmium in drinking water with a relative standard deviation of 8%.

In biosensors developed with DNA, the properties of DNA hybrids can be used. Yang et al. [19] have developed an electrochemical biosensor for the determination of MCF-7 cells, which are breast cancer cells. In this biosensor, DNA molecules were used for modification. The system provided linear measurement between 100 and 1 million cells in 1 mL and the LOD was found to be 80 cells/mL. The biosensor system was developed as a sandwich-type and the signal was made more sensitive with nanomaterials on antibodies labeled with DNA. With the 3D nanomaterials used in the design of the system, the modification steps are quite highly complex and difficult in terms of workload. In the study, the electrode was first modified with 3D-graphenes and then was modified with gold nanocages with antibody on the carbon nanotubes and immobilized on the electrode. DNA fragments labeled with a secondary antibody bonded on MCF-7 bound on these antibodies, and the measurement was performed with DPV. Here, MCF-7 measurement was performed by DNA hybridization, that is, by measuring the degree of binding of the complement bound to the ssDNA labeled with the last bound antibody.

Saeedfar et al. [20] developed a biosensor using multi-walled carbon nanotubes modified with gold nanoparticles to determine the sex of Arowana fish. The process of determining the sex of the Arowana fish before maturation is quite difficult. Arowana is an ancient and very expensive fish. This biosensor was developed as it is a very advantageous approach for the fish farming industry to distinguish the gender of the fish in time. For this, a hybridization-based approach has been adopted. Carbon nanotubes were used as a hybridization agent by complexing with ruthenium (III) chloride hex ammoniate in this study. DNA determination was performed between 10^{-21} M and 10^{-9} M and 1.55×10^{-21} M was the lowest detection limit.

Apart from DNA and proteins used in DNA determination, uncharged DNA variant known as Peptide Nucleic Acid (PNA) is also used. Unlike DNA, instead of a phosphate backbone, this molecule contains a backbone made of neutral amino acids. PNA of this nature can be used as potential probes for both DNA and RNA determination. The advantage of PNA is that it provides a great advantage in reducing the determination time. Tian et al. [21] used graphene field-effected transistors in their PNA-modified electrochemical biosensor study. In the DNA analysis study by measuring the gate voltage, it reached the level of 0.1 aM LOD and performed between 0.1 aM and 1 pM.

Due to their structural features, RNA biosensors can also be used in biosensor development, just like DNA biosensors. The structural differences of RNA with DNA were mentioned above. Apart from their metabolic functions, RNA and DNA are similar except for the difference in oxidation in ribose sugar. Studies have also been carried out which DNA and RNA can be used together in biosensor studies, especially using complementary DNAs for RNA analysis. In this section, RNA-based biosensors and types of studies are summarized.

Luo et al. [22] have developed an electrochemical DNA biosensor for exosomal miRNA analysis. The study was generally modified by adding a DNA probe modified with methylene blue (MB) over amino groups after the lysine amino acid was electrochemically coated on the glassy carbon electrode. Complementary to this probe, a ferrocene-modified secondary probe is attached. The biosensor was prepared for sample measurement by exosome extraction that releases miRNA to hybridize them to complementary chain and detected by labeled with Ferrocene (Fc). The variation of the Fc signal with the MB folded on itself indicates the miRNA and probe measurement, consequently. It shows performance at 2.3 fM level LOD and linear measurement range between 10 and 70 fM.

As a result, DNA and RNA have been used both as a recognition agent and as a target molecule with successful results. In some of these studies, the determination

was carried out by measuring a secondary molecule on the basis of DNA hybridization. Besides, DNA analysis with the use of Cas proteins and DNA analysis with PNA were performed by using different methods and extremely low detection limits were reached.

2.2 Aptamers as synthetic biorecognition receptors

Aptamers have caught a very rapid trend after their discovery. These artificial nucleic acids are produced by the systematic evolution of ligands by exponential enrichment (SELEX) method [5]. By being artificially produced, they show affinity for a wide range of target molecules. They achieve this affinity with easy physical changes. Aptamer sequences designed in the correct sequence for the target molecule work by binding to the target site by undergoing conformational change when the target molecule approaches. The conformational change can be measured by using a label or surface resistance, shown in **Figures 3** and **4**, respectively.

Ohno et al. [23] developed a label-free immunosensor-based aptamer-modified graphene FET. The researchers, who modified the G-FET with IgE aptamers, determined IgG by measuring the gate voltage on the FET. With a protein concentration measurement between 0.29 and 340 nM, a LOD value of 0.29 nM was reached. 47 nM isotherm value was observed as the Kd value. Since nonspecific binding of different proteins except this molecule was not observed.

In another study, Chen et al., [24] developed an aptamer-based biosensor for the electrochemical determination of Human epidermal growth factor (HER2). Differential pulse voltammetry was chosen as a measurement technique. In the study, after modifying the gold nanorod nanomaterials with palladium, they formed a bioconjugate for analysis by modifying it with an aptamer containing SH group and horse radish peroxidase (HRP). Later, they modified the gold electrode with DNA tetrahedrons. DNA tetrahedron is a nanoscale structure designed by using the complementary base pairing reactions of nucleic acids. They modified the single-stranded DNA aptamer from one corner of the DNA tetrahedron structure.

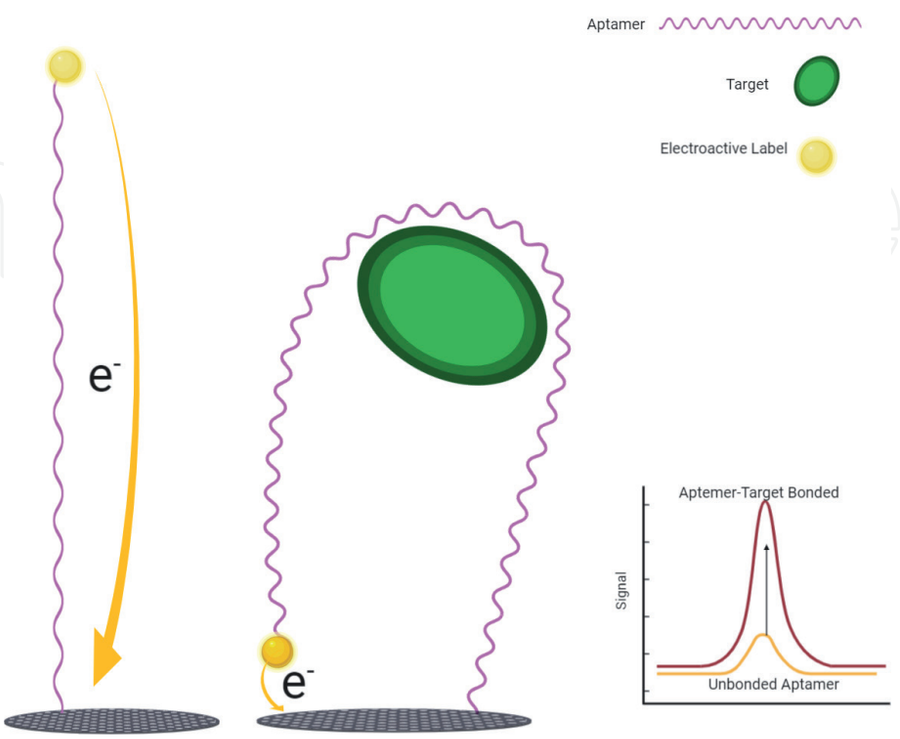

Figure 3.
Labeled-aptamer based electrochemical biosensor technology.

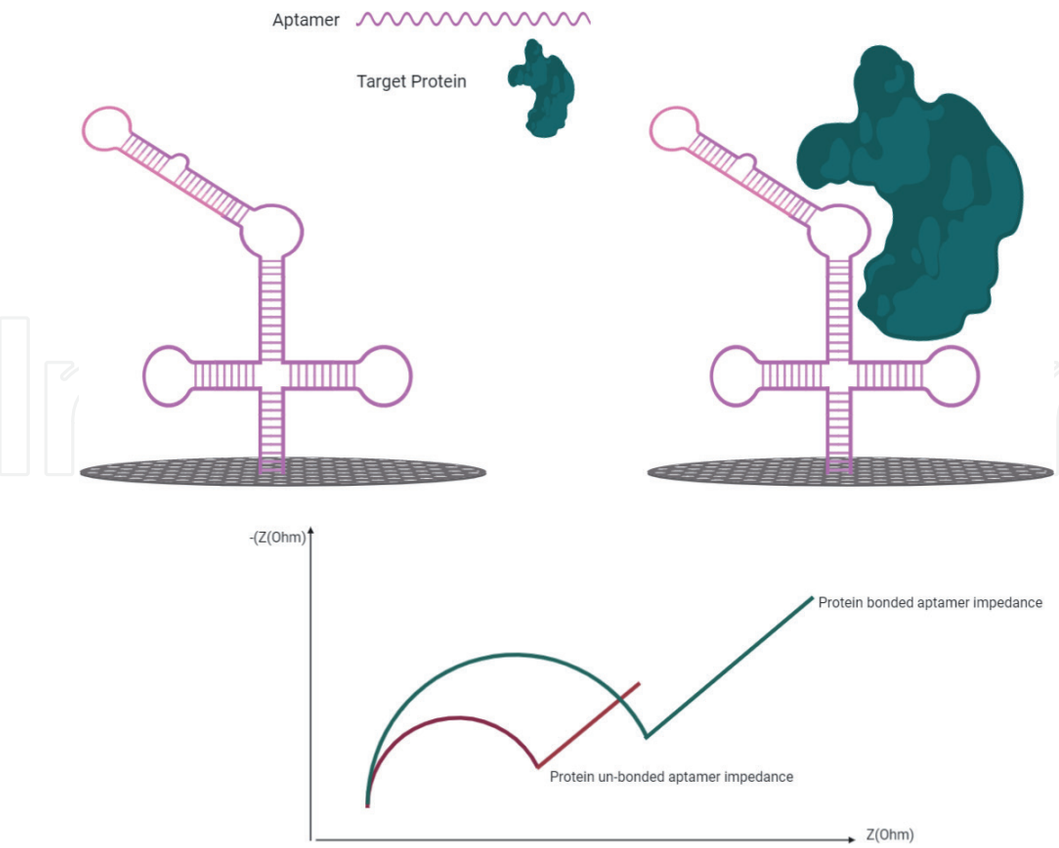

Figure 4.
Schematic representation of the impedimetric aptamer biosensor.

DNA tetrahedron is preferred because of its mechanical structural rigidity and high affinity (5000 times higher for ssDNA aptamer). The DNA was dropped onto the tetrahedron-aptamer modified gold electrode, aptamer's unbound ends were modified with BSA. After HER2 molecules were captured by the aptamer on the electrode, the bioconjugate was added on this modification and the biosensor was constructed???. HER2 measurement was performed indirectly through the conversion of HRP to hydrogen peroxide and the measurement of hydroquinone (mediator) added to the environment. In this study, researchers used the DNA tetrahedron structure and aptamer composite, thus HER2 recognition capacity was increased with this modification. The biosensor, with a working range of 10–200 ng/mL, provided the opportunity to make the analysis in 60 minutes. LOD was found to be 0.15 ng/mL.

Apart from large proteins, aptamer molecules are also used to identify small molecules. Aptamer molecules are particularly useful for the determination of molecules which are extremely difficult and demanding to identify easily. Swensen et al. [25] conducted real-time cocaine measurement in their biosensor study. This determination was carried out in approximately one minute. The microfluidic chip has been modified with cocaine-specific aptamers for cocaine binding. One end of the aptamer is modified with methylene blue. The sample, injected into the microfluidic chamber, was added to the fetal bovine serum with a flow, and the measurement was performed by binding to the aptamer. Rapid measurement is important for the rapid determination of this drug, which has a half-life of 60–90 minutes in serum. Besides the speed advantage, this system has a 20 pM LOD with a linear detection range of 10–2000 μM.

Apart from organic molecules, aptamer-based biosensors have also been developed for the determination of ions. Radi et al. [26] developed a biosensor system for the aptamer-based potassium determination. The gold electrode was made ready

for potassium ion measurement after being modified with aptamers with SH at one end and Fc at the other. The biosensor had a measurement limit between 0.1 and 1 mM and reached a value of 0.015 LOD by using electrochemical impedance spectroscopy.

Besides the principle of binding aptamer molecules to a molecule, a bifunctional aptamer-based biosensor for determination of adenosine and lysozyme was developed by Deng et al. [27]. In their study, they performed the determination of adenosine and lysozyme on DNA/DNA duplex. The gold electrode was first modified by a probe with SH group (adenosine selective) and then a secondary probe (lysozyme selective) attached to this probe, and the modification was completed with a DNA loaded nanomaterial with a central gold nanoparticle. Finally, ruthenium complexes were added as electroactive species on this structure. After the primary probe captures adenosine with adenosine binding, the gold nanoparticle structure together with the secondary probe is separated from the biosensor. With lysozyme binding, the secondary structure is separated from the main structure by binding lysozyme. All these bonding and separation reactions were followed by the electrochemical activity of ruthenium complexes. Electrochemical measurement

Electrochemical method	Biorecognition receptor	Target	Linear range	LOD	Reference
Coul	Phi29 DNA polymerase and T4	Transgenic soybeans DNA	10^{-16} and 10^{-7} M	4.5×10^{-17} M	[6]
EIS	CRISP-dCas9	PIK3CA	2–20 nM	1.92 nM	[7]
CV	DNA	Hg^{2+}	1–200 nM	0.05 nM	[8]
DPV	DNA-(HRP)-labeled IgG	DNA Methylation	1 fM–10 nM	1 fM	[9]
DPV	AuNPSs-DNA	Temodal	5 nM–45 μM	1 nM	[10]
DPV	DNA-ethyl green	Cd^{2+}	1 pM–1 nM	0.3 pM	[11]
DPV	DNA-antibody-graphene	MCF-7 Cells	10^2–10^6 cells/mL	80 cells/mL	[12]
DPV	DNA-ruthenium	Arowana fish gender (DNA)	10^{-21}–10^{-9} M	1.55×10^{-21} M	[13]
G-FET	DNA	PNA	0.1 aM–1 pM	0.1 aM	[14]
DPV	DNA-MB, DNA-Fc	Exosomal miRNA	10–70 fM	2.3 fM	[15]
G-FET	IgG aptamer	IgG	0.29–340 nM	0.29 nM	[17]
DPV	DNA tetrahedron – aptamer	HER2	10–200 ng/mL	0.15 ng/mL	[18]
AC voltammetry	Cocaine aptamer	Cocaine	10–2000 μM	20 pM	[19]
EIS	5′-ferrocene(Fc)–DNA–SH-3′	K^+	0.1–1 mM	0.015 mM	[20]
CV	Adenosine-lysozyme aptamers	Lysozyme Adenosine	10–60 μg/mL 0.02–40 nM	0.01 μg/mL 0.02 nM	[21]

DPV: differential pulse voltammetry, EIS: electrochemical impedance spectroscopy, CV: cyclic voltammetry, Coul: coulometry, fc: ferrocene, PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, AuNPs: gold nanoparticles, G-FET: graphene field effect transistor, MB: methylene blue, HER2: human epidermal growth factor, AC: alternative current.

Table 1.
Comparison of the nucleic acid based biosensors.

was carried out by CV. Lysozyme and adenosine bifunctional biosensor reached the LOD limits of 0.01 μg/mL and 0.02 nM, respectively. Measurements were made between 0.02 and 40 nM for adenosine and 10–60 μg/mL for lysozyme.

With aptamer nucleic acids, target biomolecules can be easily identified. Although aptamer molecules work similarly to antibodies, they can easily be modified with a secondary biomolecule. Molecules that do not have denaturation problems unlike proteins can be very useful in the development of biosensors, especially in the development of electrochemical biosensors. Electrochemical activation of electroactive species or direct aptamer binding to the target molecule can be measured impedimetrically. As a result, aptamer systems are suitable for affinity-based sensor development in biosensors (**Table 1**).

3. Conclusion

Biosensors are low cost analyzers with high potential to be miniaturized. They also provide short measurement time. Thanks to the numerous combinations of biorecognition receptor and transducers, biosensor systems can easily be adapted for the measurement of any kind of analyte. The biological activity of the biorecognition agent on the transducer is the critical element in a biosensor. Biomolecules with low probability of denaturation, adaptable to all kinds of measurements and strong electrical components should be selected for biosensor development. Nucleic acids meet the needs of biosensors at this point. They are advantageous biomolecules that can interact from metal ions to large proteins thanks to their multiple negative charges. Nucleic acids also have the potential to facilitate the determination of organic molecules, such as drugs and pesticides, which can enter between nucleic acid chains thanks to the electroactive guanine base in their structure. They can be used as simple biorecognition agents, similar to an unlimited antibody-antigen interaction, with the potential to be designed to synthetically show affinity for the target molecule. Another important feature of nucleic acids is the potential of their end portions to be modified by secondary signal transmitters without causing any deactivation. Apart from being both a biorecognition agent and an analyte molecule, their immobilization capacity also increases their usage and advantages. In conclusion, the fact that they can be used as a material which provides stability and ease of modification for analysis in biosensor systems puts nucleic acids one step further among other biorecognition agents.

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Conflict of interest

The authors declare no conflict of interest.

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