Electrochemical DNA Biosensors for Bioterrorism Prevention

Hafsa Korri-Youssoufi, Anna Miodek and Wadih Ghattas

Abstract In the wake of letters containing anthrax spores terrifying the USA and other letters containing unidentified white powders circulating all over the world, the threat of bioterrorism attracts the attention of the general public as well as scientist. Therefore, it is urgent to develop rapid, sensitive, and high-throughput diagnostic methods able to counter attacks of bioterrorism by elucidating the suitable actions that should be implemented to prevent serious epidemic diseases. Numerous such methods are in development but Nucleic Acid Detection is the standard employed for identifying most biological agents that are used in bioterrorism. This method is based on PCR assays *via* the classical techniques of amplification and fluorescent detection. On the other hand, electrochemical biosensors are promising platforms that could achieve rapid highly sensitive and selective onsite detection of such agents. This chapter will present the recent developments in electrochemical biosensors for preparing DNA detection platforms that could be used to prevent attacks of bioterrorism.

Keywords Bioterrorism · Bio-agents · DNA · Biosensors · Electrochemical

1 Introduction

Biological agents (bio-agents) were used as weapons of war for many centuries but more recently the threat of bioterrorism has attracted great attention because of the letters containing anthrax spores, which terrified the USA and the other letters containing unidentified white powders, which circulated all over the world [1]. The Center for Disease Control and Prevention (CDC) classifies bio-agents based on their potential risk and those that can be used in attacks of bioterrorism are found mainly in three classes (A, B and C). More than 160 species of microorganisms

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have been recognized as pathogenic of which, thirty could be used in bioweapons. Examples of such bioagents include *Bacillus anthracis*, *Yersinia pestis*, *Brucella spp.*, *Francisella tularensis*, *Burkholderia pseudomallei* and *Clostridium botulinum*. More importantly, some of these possess the characteristics that make them ideal candidates for preparing attracts of bioterrorism. These characteristics are the eased availability, production, storage and dissemination as well as the high virulence, infectivity and lethality. Additionally, bio-agents that can infect *via* the respiratory route by inhalation of aerosols are favored for bioterrorism but other possible route of infection, such as digestive contaminations (ingestion of contaminated water or food) and percutaneous contaminations could also be exploited.

The rapid detection and identification of the threatening bio-agents are crucial to counter attacks of bioterrorism by elucidating the suitable actions that should be implemented to disinfect pollutants and cure infected individuals. Therefore, it is urgent to develop rapid, sensitive, and high-throughput diagnostic methods able to tackle bioterrorism and prevent serious epidemic diseases. Furthermore, the upmost advantage is to develop a portable and user-friendly instrument capable of onsite simultaneous identification of multiple bio-agents. Numerous methods have been used for the detection and identification of bio-agents but DNA detection is the hallmark method of accurate identification of most specimens. It is based on PCR assays through classical techniques of amplification and fluorescence detection. New advanced DNA sensing technologies are developed by using a recognition system paired with a transducer that transforms the recognition into an analyzable signal. Electrochemical biosensors are promising platforms that achieve highly sensitive and selective detection of bio-agent. This chapter will present the recent developments on electrochemical biosensors for DNA detection platforms that combine a biological recognition system with artificial transducers.

2 Bioterrorism Agents

2.1 Brief History of Bioterrorism

The use of biological agents as weapons of war has marked the history of international conflicts. Historians agree that in 1346 the agent of plague *Yersinia pestis* has been unintentionally employed by the Genoese Tatars during the siege of the city of Caffa. The Tatars smuggled the bodies of their contaminated dead comrades inside the city walls, hopping to further grime the life of the besieged citizens and accelerate their surrender. Their act resulted in a plague-ridden city, which was abandoned by survivors most of whom traveled across Europe spreading plague and causing one of the origins of Black Death that killed 20 to 30 million Europeans [2]. In the 20th century, the field of biological weapons was significantly advanced by modern microbiology and by the knowledge gathered from multiple applications that took place in international and civil wars. For example, during the First World

War, German agents used anthrax to infect the animals of the allied powers and in the Second World War as well as in the Cold War several countries had secret programs for biological weapons development.

In 1972, the Biological Weapons Convention prohibited the development, production and stockpiling of biological weapons. 173 countries have ratified this convention but unfortunately this did not stop isolated attacks of bioterrorism. Examples such as the intentional contamination by *Salmonella typhimurium* of salad bars in Oregon restaurants by some of the Rajneeshee cult followers in 1984 [3] and the release of nerve gas sarin in a Tokyo subway by some of the Aum Shinrikyo cult followers in 1995 [4] illustrate the feasibility of using bio-agents by terrorists either for political, religious, or other purposes. Biohazards may also be inflicted accidentally similarly to the incident that took place in April 1979 in Sverdlovsk (USSR). Information about the incident remains classified but it is suspected that aerosolized spores of *Bacillus anthracis* were accidentally leaked from a military laboratory infecting 79 people of whom 68 died [5]. Such examples demonstrate the potential high lethality of bio-agents and emphasize the need for developing efficient counter measures.

2.2 Description of Bioterrorism Agents

The NATO glossary defines a biological agent as "A microorganism that causes disease in personnel, plants, or animals or causes the deterioration of materiel". This includes toxins which are substances naturally produced by bio-agents. In this case, the limit between biological warfare agents and chemical agents is blurred. Bio-agents possess unique properties that enhance their attractiveness to individuals or groups wanting to inflict high morbidity and mortality on a human population. As mentioned earlier they are classified in three categories. Category A agents pose the greatest threat because of their relative ease of transmission, infliction of high rate of mortality as well as their ease of production, transport and dissemination. Category B agents are moderately transmissible and inflict morbidity with low rate of mortality. Finally, Category C agents refer to emerging pathogens and are potential risks for the future. Table 1 sites some of the main biological agents that may be used for bioterrorism along with their most notable characteristics [6].

2.3 Approach of Detection

There are currently several methods in use and in development for the identification of biological agents [7]. Some detection systems are based on metabolomics by following for example the consumption patterns of characteristic substrates or by detecting characteristic fatty acid profiles. Other more specific methods include immunological detection and protein imprint identification using proteomics.

Table 1 Characteristic of the main biological agents that could be used in bioterrorism

Agents	Diseases	Biological threat level	Lethality
Bacillus anthracis	Anthrax	A	+++
Yersinia pestis	Plague	A	+++
Clostridium botulinum	Botulism	A	+++
Francisella tularensis	Tularemia	A	+++
Variola Major	Small pox	A	+++
Listeria monocytogenes	Listeriosis	B	++
Brucella melitensis	Ovine Brucellosis	B	+
Escherichia coli O157: H7	Hemolytic Uremic Syndrome	B	+
Salmonella genus	Typhoid fever, paratyphoid fever	**B**	+

Nonetheless, the most commonly used technic is still nucleic acid detection via quantitative PCR. It is based on PCR assays through classical techniques of amplification and fluorescent detection. This method is reliable and specific able to detect variants and genetically modified strains. Nevertheless quantitative PCR is relatively time consuming and cannot be miniaturized to portable devices that are essential for taking fast actions in case of attacks. On the other hand, electrochemical biosensors are promising platforms that could achieve rapid highly sensitive and selective onsite detection of such agents.

3 Electrochemical DNA (E-DNA) Biosensor

3.1 Definition of Biosensor

According to The IUPAC definition, a biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immune-systems, tissues, organelles, DNA or whole cells to recognize targeted chemical or biological compounds. The effect of the recognition process is transformed by a composite called transducer into an observable and measurable electrical signal. The modification of the signal is directly proportional to the concentration of the target in the sample and can be measured by different techniques such as electrochemical [8], optical [9], piezoelectric [10], conductimertic [11], spectrophotometric [12] or calorimetric [13]. Biosensors are classified according either to the nature of the biomolecules immobilized on their surface and are responsible for the recognition or to the type of interaction they engage with the target. DNA biosensors are devices in which oligonucleotides are attached to the transducers and in which the detection is owed to the formation of double-strand (ds) DNA via the hybridization between the single-strand (ss) DNA acting as a probe linked to the transducer and the target ssDNA comprising the complementary sequence of nucleotides specific to the probe.

The important parameters that characterize biosensors are the dynamic range and the linear range of detection, the limit of detection, the sensitivity and the

selectivity. The dynamic range represents the concentration range of target up to the highest that provides an observable response signal. The linear range represents the concentration range of target that provides a linear response signal. The slope of the linear range corresponds to the sensitivity of the biosensor. The lowest concentration that can be measured corresponds to the limit of detection (LOD) and by extrapolation of the dynamic range curve the detection limit (DL) can be calculated according to various methods described in analytical techniques. The most commonly used methods in biosensor devices are those that take into account signal to noise ratio of 3, where DL is obtained by the equation:

$$x_{DL} = \frac{\alpha_0 + 3s_{bl}}{\alpha_1}$$

Where s_{bl} is the standard deviation, α_o the result of the measurement obtained with a blank test and α_1 is the sensitivity.

Electrochemical biosensors are attractive devices for the identification of biomolecules due to the possibility of their miniaturization, their low manufacturing cost and their ability to directly measure the electrical signal derived from the detection of targets. The choice of the transducer has a significant impact on the characteristics of the biosensor. Transducers that are commonly reported for the construction of electrochemical biosensors are conductive organic polymers (polypyrrole [14], polythiophene [15], polyaniline [16]), carbon nanotubes [17], graphene [18], metal nanoparticles (gold nanoparticles [19]), and gold electrodes modified with self-assembled monolayers [20]. To enhance the electrochemical signal, transducers can be additionally associated with a redox marker as for example ferrocene [21], quinone [22] and metalloporphyrins [23]. Different methods are described for the attachment of biomolecules to transducers such as, physical adsorption, electrostatic interactions, chemical cross-linking, covalent grafting, immobilization through affinity systems like biotin/streptavidin [24] or adamantane/β-cyclodextrin [25], entrapment in polymers [26] or sol-gels [27].

The detection by DNA biosensors has been achieved using both indirect and direct methods. Indirect methods require further steps after DNA hybridization to accomplish the measurement, while direct electrochemical DNA sensing approaches are capable of directly measuring DNA hybridization without any further step.

3.2 *DNA Detection Based on Indirect Strategy*

Indirect methods of detection rely on sensors in which two different DNA probes are used. The primary probe DNA is attached to the transducer and the secondary probe DNA is labeled either with an enzyme, a redox marker or nanoparticles. The hybridization reaction is then monitored either *via* the redox signal of the product of the enzymatic reaction, the redox signal of the electroactive markers or the enhancement of redox signal by the presence of conducting nanoparticles,

respectively. Other indirect strategies rely on using redox DNA intercalators that possess an affinity for dsDNA. In this case, the detection of DNA hybridization is observed *via* the increase in the intensity of the redox signal of the intercalator, which is proportional to the formed dsDNA. Some examples of indirect methods often used for DNA detection are presented in this section.

3.2.1 DNA Labelling with Enzymes

DNA sensing approaches based on enzymatic reactions are often based on sandwich structures for signal amplification. After the hybridization reaction between the primary probe DNA and the target DNA, an additional step is added. This step involves the hybridization of a different section of the target DNA with labeled secondary probe DNA. The secondary DNA is comprised of a redox enzyme such as horseradish peroxidase (HRP) [28, 29] alkaline phosphatase (ALP) [30, 31] or glucose oxidase [32]. The hybridization reaction may be followed through the catalytic properties of the enzymes in the presence of their substrates. The immobilized enzymes on hybridized DNA lead to the production near the electrode surface of electroactive products, which produce a current related to the amount of hybridized target DNA (Fig. 1).The detection of various bio-agent such as *Salmonella* and *Listeria* has been demonstrated by using this method [33].

3.2.2 DNA Labelling with Nanoparticles

Probe DNA can be labeled by tagging with nanoparticles like gold nanoparticles (AuNPs), [34] carbon nanotubes (CNTs) or dendrimers [35]. AuNPs and CNTs can greatly intensify the electrochemical responses signal because of their high conductivities. This fact is demonstrated in assays such as those based on the sandwich structure used in combination with CNTs conjugates encapsulating Cadmium Sulfide (CdS) nanoparticles [36]. The conjugates could be attached to a secondary DNA probe via the biotin/streptavidin system that served as tags. When compared with the conventional single-particle stripping hybridization assays a substantial (~ 500-fold)

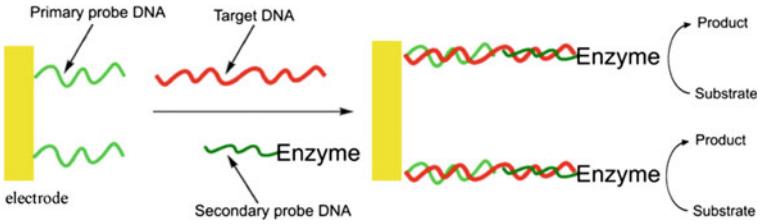

Fig. 1 Schematic representation of the sandwich structure for the detection by enzyme labeled probe DNA

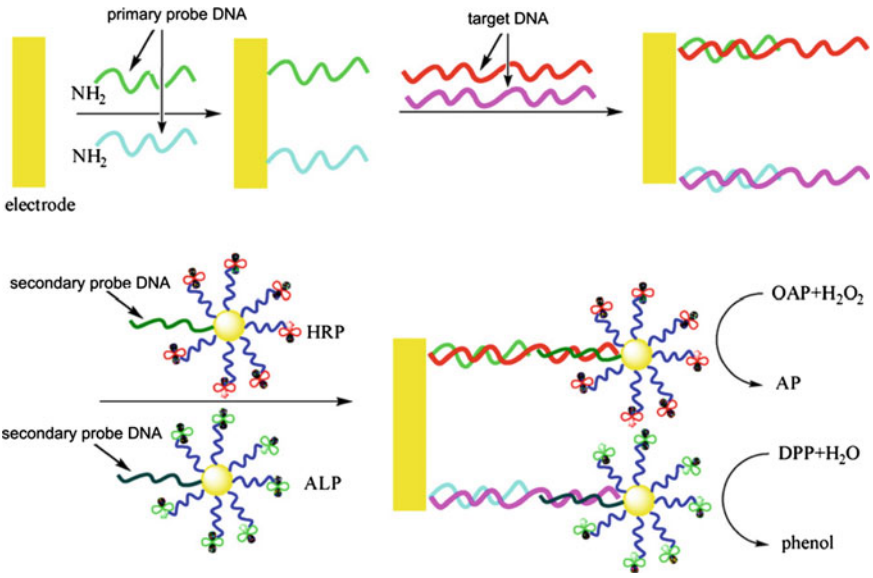

Fig. 2 Specific and highly sensitive dual target biosensor designed by Li et al. [40]

lowering of the detection limit was observed. AuNPs were also conjugated either with thiolated ferrocene, [37, 38] thionine [39] or enzymes. The specific electrochemical detection of two DNA targets sequences in one sample was performed by Li et al. using enzyme functionalized AuNPs as catalytic labels (Fig. 2) [40]. This DNA sensor was constructed by using the sandwich-assay detection strategy in which, two different primary probes DNA were immobilized on the surface of the transducer. Each primary probe DNA hybridized specifically with its complimentary DNA target. The two secondary DNA probes were also each specific for one of the targets but both were associated with AuNPs. Finally, one secondary DNA probe was linked with HRP and the other with ALP. Consequently, the electrochemical signal was generated from the products of the enzymatic catalysis of phenol by HRP and/or of alkaline phosphate by ALP. In addition, enhanced detection sensitivity was obtained because the AuNPs carriers increased the amount of enzyme molecules per hybridization.

3.2.3 Redox Intercalators

The monitoring of hybridization reactions can be performed using redox molecules that possess affinity for some DNA bases or some dsDNA structures by insertion into double helix DNA structures or interactions with minor or major DNA helix grooves. This affinity takes place because of interactions in well-defined binding sites via intercalation and/or electrostatic binding. Both organic compounds and cationic metal complexes can be used as specific DNA binders. The detection relies

on the high affinity of these compounds for one of the two DNA forms: ssDNA or dsDNA. During electrochemical detection, the intercalation in dsDNA increases the current and accordingly improves the sensitivity. In literature, this strategy is still one of the most used method for DNA detection. A recent example was reported by Steichen et al. for the construction of a biosensor based on electrostatic interactions between positively charged $Ru(NH_3)_6^{2+}$ and negatively charged DNA [41]. They used peptide nucleic acids (PNA) as a primary probe and the cationic ruthenium complexes did not interact electrostatically with the PNA probe due to the absence of the anionic phosphate groups. However after hybridization, $Ru(NH_3)_6^{2+}$ was adsorbed on the DNA backbone, giving a clear hybridization detection signal in alternating current voltammetry.

Intercalators are a class of DNA ligands that insert between adjacent base pairs of double-stranded DNA. Heterocyclic dyes are common intercalators such as ethidium bromide (EB) [42]. Some anticancer drugs are also strong DNA intercalotors such as anthracyclines including daunomycin [43–45] and doxorubicin [46, 47]. Antipsychotic and antihistaminic drugs such as phenothiazines and acridine derivatives including acridine orange [48] are also well known DNA intercalators (Fig. 3). The action mechanism of intercalators is based on the stacking of planar, aromatic groups between nucleic base pairs in an approximately perpendicular position to the double-helix axis. These interactions could be selective as for example daunomycin, which was found to inserts into the DNA duplex preferentially between GC base pairs.

Threading intercalators are those that carry substituents on the periphery of the intercalating moiety. When intercalated into dsDNA, these substituents rest in the major and the minor grooves simultaneously. Examples of threading intercalators include the naphthalene diimide derivative carrying ferrocenyl groups (Fig. 4), which has been demonstrated a detect limit of 10 zmol of DNA.

Other DNA binders rely on the strong electrostatic binding of the negatively charged sugar phosphate backbone of DNA. Examples include most metallic DNA stains like $Ru(NH_3)_6^{2+}$, $Ru(bpy)_3^{2+}$, $Os(bpy)_3^{3+}$, $Co(bpy)_3^{3+}$, $Co(phen)_3^{3+}$ and manganese complex of rutin MnR_2. Such stains can also show enantiomeric selectivity, for examples $Co(bpy)_3^{3+}$, $Co(phen)_3^{3+}$ [50, 51], and $Ru(bpy)_3^{2+}$ [52] are minor groove helix DNA binders while $Ru(phen)_3^{2+}$ [53] is a major grove helix DNA binder.

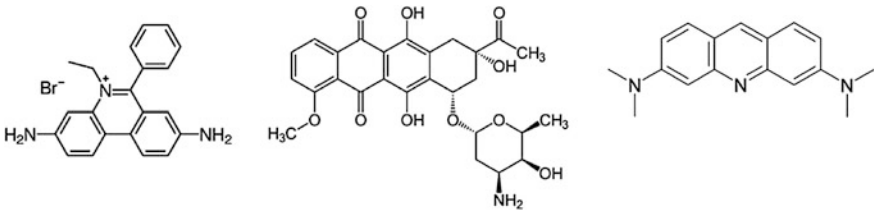

Fig. 3 Structure of different intercalators. From *left to right* ethidium bromide, doxorubicin and acridine orange

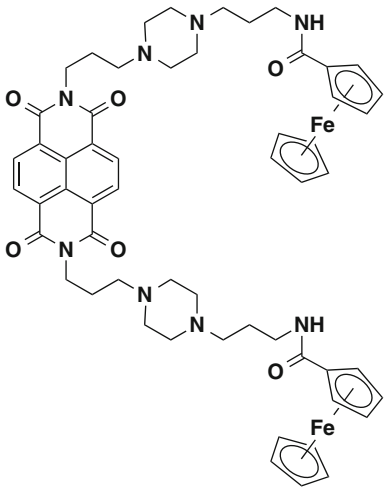

Fig. 4 Structure of naphthalene diimide carrying ferrocene [49]

DNA binders can combine an affinity for GC-rich or for AT-rich sequences within a preference for one helix structure [54]. For example, Hoechst 33258 specifically binds to dsDNA in a solution by recognizing AT-rich sequences within minor grove helixes (Fig. 5). Other DNA binders such as $Ru(bpy)_3^{2+}$ rely on electrostatic affinity and shows preference for G-rich DNA sequences [55, 56], while Methylene Blue (MB) is a DNA intercalator that has affinity for GC-rich sequences [57–59].

The use of intercalators in E-DNA biosensors is now a very promising approach. For example, intercalators are used in PCR as markers for electrochemical detection instead of optical detection [60]. In the same way, detection in microsystems including PCR have been achieved with redox intercalators and lead to a detection limit lower than 10 aM [61].

3.2.4 Metal Ions

Another strategy is based on detecting DNA hybridization through the metalation of dsDNA to form metal complexes of DNA (M-DNA). The detection is based on the modulation of the conductivity of DNA since the formation of M-DNA decreases the resistance of the electronic transport of the electrode interface, which can be

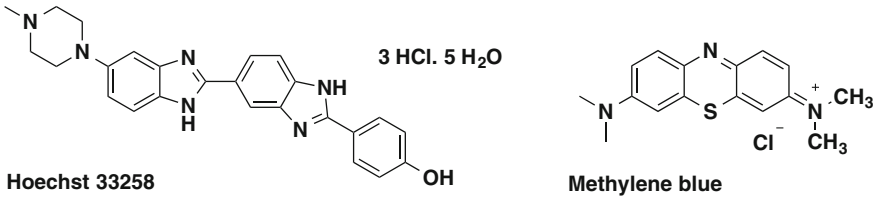

Fig. 5 Structures of Hoechst 33258 and methylene blue

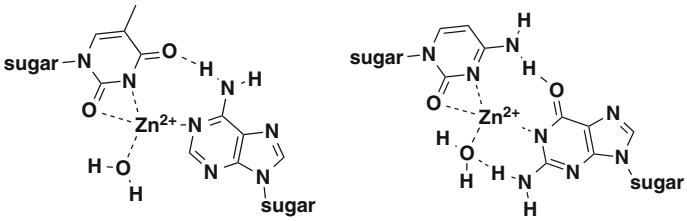

Fig. 6 Structural models of M-DNA showing that the imino protons of the T and G bases are replaced by Zn^{2+} [63]

monitored through electrochemical impedance spectroscopy. M-DNA is usually formed by dsDNA at pH above 8 in the presence of Zn^{2+}, Co^{2+} or Ni^{2+} but not Mg^{2+} or Ca^{2+} [62]. Metal ions are known to bind in the center of DNA helixes, coordinating the N3 of thymine and the N1 of guanine in every base-pair. Xu et al. demonstrated that in M-DNA double-stranded chains, Zn^{2+} is a better electrons transporter than both Co^{2+} and Ni^{2+} [63] (Fig. 6).

3.3 DNA Detection Based on Direct Strategy

The second strategy for monitoring DNA hybridization is based on direct detection. The main advantage of this method is that not require the use of labels or indicators. There are various possibilities for direct DNA detection: (1) monitoring of the redox signal of DNA bases, (2) monitoring of the conformational changes of DNA tagged with some redox marker after the hybridization with the target and (3) monitoring of the changes in the electrochemical properties of transducers due to the hybridization, which could be obtained by monitoring the decrease or the increase of the redox signal of the transducers.

3.3.1 Detection Based on Redox Properties of Guanine

The observation of the redox peaks of DNA bases due to reduction and oxidation reactions, leads to the monitoring of DNA hybridization. Guanine and adenine are the most electroactive DNA bases. Palecek et al. [64] reported the first example of direct detection of DNA hybridization through the monitoring of the redox signal of the nucleotidic bases [65]. The results demonstrated that the amount of oxidized or reduced DNA reflected the amount of hybridized DNA (Fig. 7).

This strategy was used in DNA detection and quantification. For example, Bollo et al. studied the oxidation peak of guanine at about 1 V after accumulation of DNA on the electrode surface at open circuit potential [66]. Additionally, they achieved signal amplification when the surface of the electrode was modified with carbon nanotubes entrapped in chitosan. For up to 90.0 ppm of dsDNA, a linear

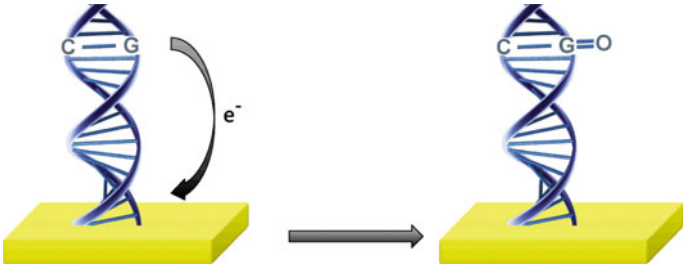

Fig. 7 Illustration of a biosensor based on the redox properties of DNA via the oxydation of guanine [63]

relationship was obtained between the amount of DNA and the measured current corresponding to the redox signal of guanine. However compulsory use of high potential for DNA oxidation is a major drawback for this method but improvements have been attempted such as the addition of redox mediators that amplify the redox signal.

3.3.2 DNA Labelling with Redox Markers

Monitoring of the hybridization between two complementary DNA strands was also performed by labelling of ssDNA probe with a redox marker, such as MB [67–69] or ferrocene (Fc) [70–72]. The advantage of this method is the direct measurement of the electroactive molecules on the surface of the electrode by straightforward transduction.

Various methods relying on the conformational changes in the structure of the probe DNA have been developed [73]. Due to hybridization with targets, DNA change conformation on the surface of the electrode leading to changes in the redox signal of the label molecule. For example, Anne et al. developed a biosensor in which single strand ssDNA chains were modified with Fc on the free end. This led the Fc tag to the surface of the electrode and generated an intense electrochemical signal. Hybridization with the target DNA resulted in the formation of a rigid dsDNA chain that distanced the tag from the electrode surface and led to the decrease in the electrochemical signal (Fig. 8).

Another approach has been developed by exploiting the properties of ssDNA that could form two-dimensional structures such as stem-loops. In this case ssDNA was labelled with redox marker including MB at the end of the stem-loop. Because linear ssDNA distance MB from the electrode, the employment of ssDNA with stem-loops induced a shorter distance and therefore a better electrochemical signal for the redox marker (Fig. 9) [74].

The formation of secondary structures as stem-loop has been exploited to obtain a positive response rather than a decrease in signal after the hybridization with target DNA [75]. The advantage of the increase of the current in redox signal "signal on" over its decrease "signal off" is that, the signal decreases after DNA

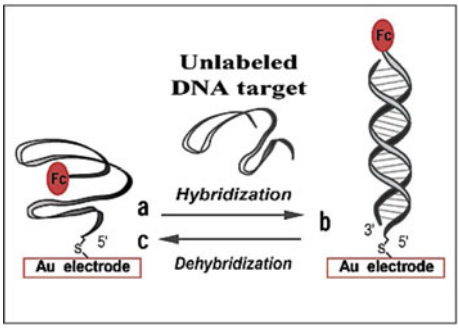

Fig. 8 Schematic representation of biosensors based on DNA probe tagged with redox markers [71]

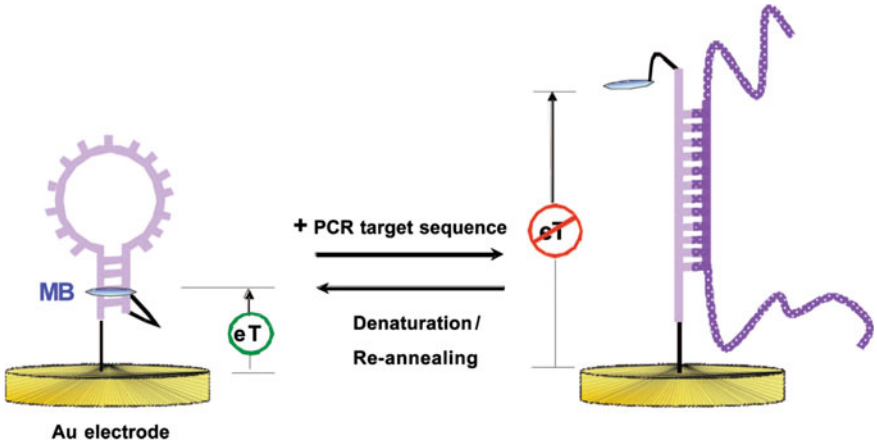

Fig. 9 Stem-loop structure of DNA probe labelled with ferrocene [74]

hybridization could unfortunately derive from false positives, e.g. resulting from degradation of the redox-labelled DNA. The signal increase in redox response "signal-on" strictly depends on the change of the architecture of the DNA structure i.e. DNA hybridization. The first example was demonstrated by Heeger et al. with a biosensor formed by ssDNA probe forming two stem-loops (pseudoknot) in which a portion of each loop formed one strand of the stem of the other loop [76]. This pseudoknot DNA was modified at its free end with redox-active MB. In the absence of DNA target, the formation of this pseudoknot structure distanced the MB tag from the electrode, reducing the redox current. Hybridization with complementary target DNA disrupted the pseudoknot DNA, liberating the flexible MB-labelled single-strand DNA. MB was then closer to the surface and led to a significant increase in the redox current (Fig. 10). The detection limit of this biosensor was determined to be 2 nM.

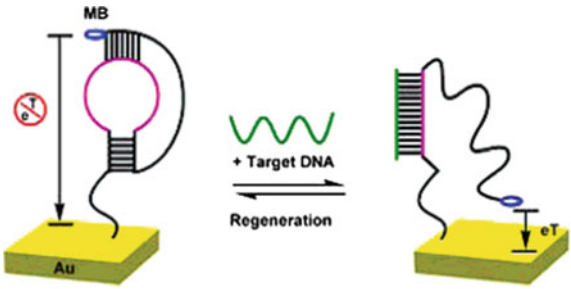

Fig. 10 Biosensor based on signal-on developed by Heeger et al [76]

3.3.3 Detection Based on Electrochemical Response of Transducers

The hybridization of the DNA target with DNA probe deposited on the electrode leads to changes in the electrical properties of the modified surface. This can be used for direct DNA detection. For example, Yang et al. used electrochemical impedance spectroscopy for the monitoring of DNA hybridization in the presence of the $[Fe(CN)_6]^{3-/4-}$ redox species [77]. After the interaction of the two complementary DNA strands an increase in the electron transfer resistance (R_{ct}) of the electrode surface was observed. It was justified by the role of electronegative phosphate skeletons of DNA which prevents negatively charged $[Fe(CN)_6]^{3-/4-}$ from reaching the electrode surface during the process of the redox reactions leading to a decrease in the ability of the electrode surface to transfer electrons. Additionally, the increase in resistance was proportional to the increase in DNA amount immobilized on the electrode surface.

Changes in the redox properties of conducting polymers as polypyrrole, polyaniline, polythiophene can also be employed for monitoring DNA hybridization. The first example was demonstrated by Korri-Youssoufi et al. [78, 79] using copolymer formed with pyrrole modified with carboxylic groups and pyrrole modified with activated ester groups. The activated ester groups were used for the bonding of DNA probe modified with amine groups. The hybridization with the complementary DNA target led to changes in the redox signal of polypyrrole with an increase in the oxidation potential and a decrease in current. This was explained by the effect of the formation of double strand DNA affects the electrical properties of polypyrrole backbone leading to the modification of their electrochemical properties.

Another approach was based on the association of conducting polymers with redox markers such as copolymer formed with polypyrrole conjugated with ferrocene. The association with Fc led to an enhancement of the sensitivity of detection [80, 81].

The association of conducting polypyrrole with Multi-Walled Carbon Nanotubes (MWCNTs) and redox dendrimers was demonstrated in the detection of the DNA of *Mycobacterium tuberculosis* [82]. By using this method specific probe genes were able to distinguish between DNA polymorphism and detected the rifampicin resistant strain. In this case, the biosensor was formed through a simple two-step

method following electrochemical patterning wherein the formation of the polypyrrole/MWCNTs and their modification with dendrimers were achieved through electrochemical deposition and the detection of the signal was followed by monitoring the redox signal of ferrocene attached within the layer (Fig. 11).

Biosensors following the "signal on" concept were also described based on conducting polymers for the direct detection of DNA. The "Signal on" results from an increase in conductivity after immobilization of the complementary DNA on the electrode surface and/or after conformational changes of the probe DNA caused by the hybridization.

Lien et al. constructed a biosensor based on polypyrrole films doped with MWCNTs for the detection of genetically modified organisms [83]. Polypyrrole was modified with the probe DNA and the hybridization with the complementary target DNA was studied using electrochemical impedance spectroscopy (EIS). An increase in the concentration of complementary target DNA resulted in a decrease in the faradic charge transfer resistance (R_{ct}), which was described as "signal on". The authors assigned this behaviour to the electrostatic effect and/or the steric effect due to the polyelectrolyte character of the DNA strands, which modified the ionic transport to and across the polymer/solution interface.

A well suited example of DNA detection with "signal on" was prepared based on a conducting polymer composed of 5-hydroxy-1,4-naphthoquinone (juglone, JUG) and its carboxylic acid derivative (JUGA) [84–87]. The electroactivity of this

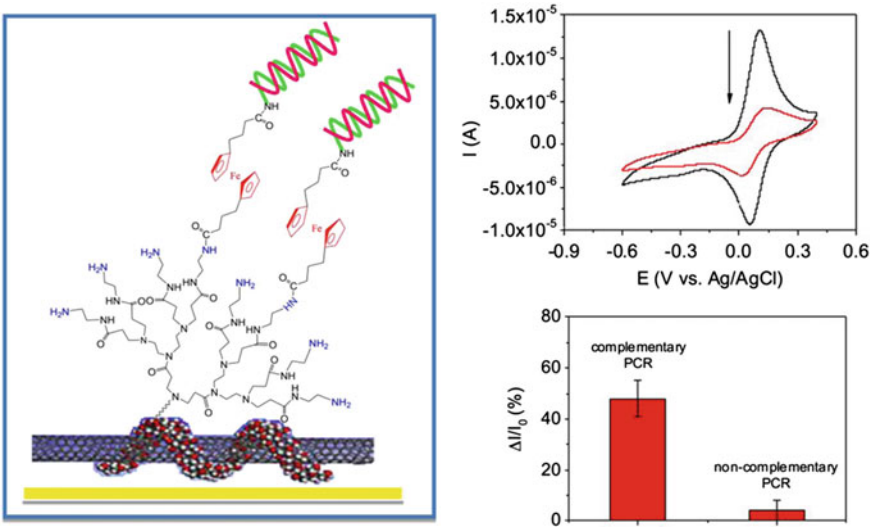

Fig. 11 *Left* Biosensor constructed using conducting polypyrrole with MWCNTs and redox dendrimers bound to ferrocene, then DNA probe was attached for the detection of *Mycobacterium tuberculosis*. *Right* Electrochemical signal variation after DNA detection [82]

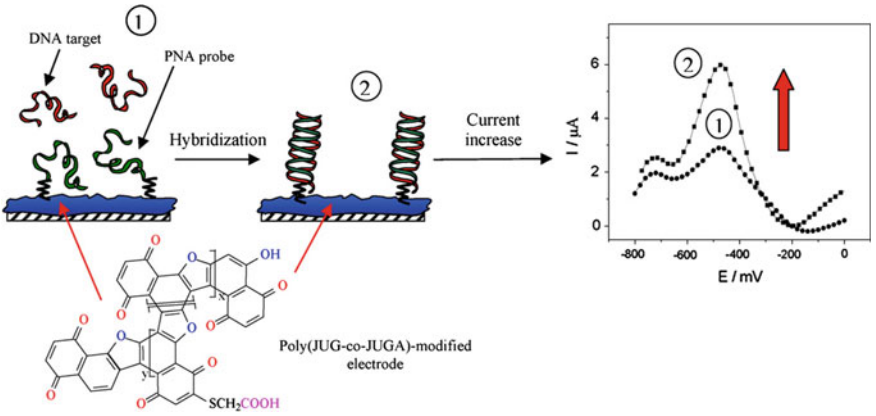

Fig. 12 Schematic illustration of "signal on" biosensor based on JUG polymer [85]

molecule comes from quinone groups, which can provide an intense redox signal. These polymers are able to form hydrogen bonds with single-strand DNA resulting in a decrease in the electroactivity of JUG. When hybridization occurred, the dissociation of hydrogen bonds and the release of dsDNA were observed. This restored the redox activity of the polymer based on juglone and consequently the signal on was obtained (Fig. 12).

3.4 Electrochemical Detection Without PCR Amplification

PCR-less target DNA amplification methods are based on the combination of novel two-component oligonucleotide-modified gold nanoparticles (NPs) and single-component oligonucleotide-modified magnetic microparticles (MMPs) followed by the detection of amplified target DNA in the form of bar-code DNA using a chip-based method. Two components oligonucleotide-modified nanoparticle probes have been designed and used in the bio-bar-code assay, which showed a sensitivity limit of 500 zmolar target DNA. Because the DNA Bar-Code Amplification (BCA) approach is a pseudo-homogeneous system with both MMPs and NPs in solution, a large concentration of the probe DNA can be used to achieve very efficiently binding to target DNA, thereby reducing the time of experiments required for highly sensitive detection (Fig. 13). Indeed, an advantage of the DNA-BCA approach over conventional microarray sandwich assays is that the entire assay can be carried out in 3–4 h, regardless of target concentration. Additionally, the system has an excellent dynamic range and is ideally set up for multiplexing [88].

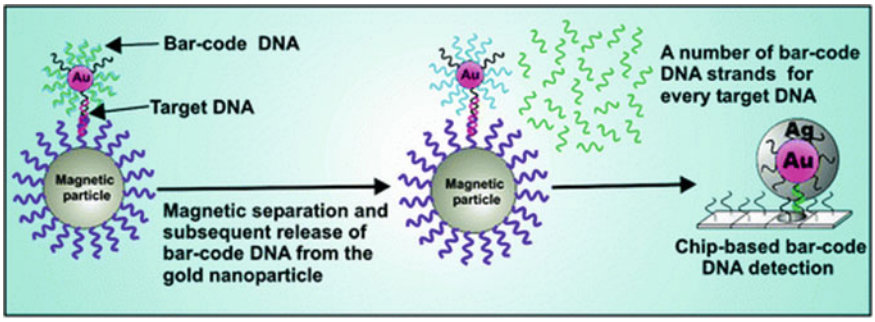

Fig. 13 Illustration of an electrochemical biosensor based on the combination NPs and MMPs followed by the detection using a chip-based BCA method

4 Conclusion

The main electrochemical methods in development for the detection of DNA are discussed and some of the most notable examples are highlighted above. The detection of pathogenic DNA is of upmost priority for tackling bioterrorism. Some E-DNA biosensors have been already reported in literature. The concern mainly class B and class C pathogens particularly *E. Coli*, *Salmonella* and *Listeria*. There are various DNA probes in literature databases that can be used for the development of E-DNA biosensors for the detection of pathogens and biothreats. While better performing platforms should be developed, many system presented in this chapter provided rapid and accurate identification of biothearts agent.

E-DNA detection is a promising application due to sensitive detection and eased implementation into miniaturized and automated devices suitable for rapid screening of multiple unprocessed samples. Because of the advantages of electrochemical biosensors, these are looming as efficient DNA identifiers to replace conventional methods for the detection of biothreats.

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