Modified Electrodes with Grafted DNA and Oligonucleotides for Detection and Quantification of Peroxynitrate

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MODIFIED ELECTRODES WITH GRAFTED DNA AND
OLIGONUCLEOTIDES FOR DETECTION AND QUANTIFICATION OF
PEROXYNITRITE

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Submitted in partial fulfillment of requirements
For the degree
MASTER OF SCIENCE IN CHEMISTRY
At the
CLEVELAND STATE UNIVERSITY
May 2016
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Peroxynitrite (ONOO$^-$) is a strong oxidizing and nitrating agent, and its formation has been correlated with many pathological conditions. It is generated \textit{in-vivo} through the diffusion-controlled reaction between nitric oxide and superoxide. Peroxynitrite has been linked to nitration of protein and DNA as well as to DNA strand breaks. Accumulation of mutations and/or other kinds of DNA damage represent a carcinogenic risk. The accurate measurement of peroxynitrite concentration has been a challenge since this analyte is very unstable and reacts with many cellular targets. Development of analytical techniques capable of rapid and sensitive detection of this fast-reacting and damaging agent is an important research target to determine the chemical damage by this oxidant both at the tissue and the cellular levels.

In this work, we develop DNA films as sensitive sensing platforms to detect and quantify ONOO$^-$ DNA damage. We have used two methods for DNA immobilization on the electrodes surfaces: (1) electrochemical grafting and (2) layer-by-layer (LBL) deposition methods. In the first method, we generate carboxylic acid groups on the electrode surface via electrochemical reduction of trans-4cinnamic acid diazonium tetrafluoroborate, followed by coupling of pre-activated carboxylic groups with amino terminated oligonucleotide. In the LBL deposition method, we construct films of alternate layers of
positively charged poly(diallyl dimethyl ammonium) and the target DNA as a negatively charged counterpart on the surface of the graphite electrode. On both platforms (grafted oligos and DNA films), we assess the effect of defined exogenous levels peroxynitrite metabolite on the electrochemical response of the DNA interface. Particularly for the grafted DNA oligonucleotides, we focused on detecting the differential response of complementary strands versus DNA helices with a single base mismatch. We show in the current work that electrodes modified with DNA oligonucleotides show sensitive responses towards micromolar range concentrations of PON. Additionally, we found that the mismatch oligonucleotides immobilized on the electrode surfaces are more sensitive biosensors for detection of peroxynitrite. To this end, we have used chronocoulometry, cyclic voltammetry, and square wave voltammetry to monitor the effect of the mismatch on the sensitivity of the modified electrodes towards peroxynitrite through defined electrocatalytic processes mediated by the grafted oligonucleotides.
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CHAPTER I

General Background

1.1. Introduction

DNA determines genetic characteristics in living beings. Many chemical and physical effects can cause damage in DNA molecules. Physical influences include high temperature[1], ionizing radiations[2], and ultraviolet radiations[3]. While chemical factors include interaction with reactive oxygen species (ROS) and other chemicals generated during biological metabolism under normal function or induced by pathological states[4]. Peroxynitrite (ONOO−) is one of these chemical agents that induce chemical damage in DNA.

ONOO− is a strong oxidizing and nitrating agent that correlated with many pathological conditions. It forms in vivo through a fast reaction between nitric oxide and superoxide in various pathological conditions. For example, ONOO− leads to the generation of reactive oxygen species (ROS) in human leukemia cells[5]. Additionally, in acute and chronic inflammation, ONOO− causes oxidation and nitration in proteins, DNA bases modification, and DNA strand breaks. DNA damage frequently leads to chromosome aberrations and mitotic faults.
Accumulation of mutations and/or other kinds of DNA damage represent a carcinogenic or teratogenic risks[6]. Various DNA damage detection techniques have been the research interest of many researchers such as chromatographic or electrophoretic separation, and autoradiograms[4].

Electrochemical methods gained more interests as they are simple, fast, highly sensitive, and inexpensive. Development of analytical techniques capable of rapid and sensitive detection of various types of DNA damage has been the focus of many laboratories. However, the use of interfaces modified with DNA for the development of analytical tools to determine reactive analytes such as PON has been overlooked. This flipped route represents a creative way to repurpose what we already know about DNA-modified interfaces and effect of base mismatch or DNA damage for the determination of peroxynitrite and, by extension, other reactive analytes.

The goal of our work is to detect and quantify peroxynitrite using DNA films as sensing platforms.

1.2. Peroxynitrite

1.2.1. Formation & reactivity of peroxynitrite

Nitric oxide and superoxide combine rapidly to form a toxic reaction product, peroxynitrite anion (ONOO\(^{-}\)) [7-9]. The proportion of both reactants decides the oxidant reactivity of peroxynitrite. Excess nitric oxide or superoxide decreases the oxidation processes induced by peroxynitrite[10, 11]. The oxidant reactivity of peroxynitrite is affected by an intermediate that has similar biological activity to the hydroxyl radical.
However, this product is not a hydroxyl radical, but peroxynitrous acid (ONOOH) or its activated isomer (ONOOH*) [12].

Various pathological conditions produce peroxynitrite, such as reperfusion injury, chronic inflammation, atherosclerosis, rheumatoid arthritis, neurodegenerative diseases, and some cancer diseases [7, 9, 12-16]. For example, ONOO− is generated as one of the reactive oxygen species (ROS) in human leukemia cells[5]. As a highly reactive species, it causes rapid oxidation of sulfhydryl groups and thioethers, as well as nitration and hydroxylation of aromatic compounds, such as tyrosine and tryptophan[8, 9]. Peroxynitrite also distorts the DNA via several mechanisms. DNA bases modification is one of these mechanisms initiated upon exposure to peroxynitrite. Guanine bases are found to be the most sensitive to peroxynitrite through the formation of 8-nitroguanine and 8-oxoguanine. Additionally, peroxynitrite induces DNA single-strand breakage. Many secondary processes follow the process of DNA damage, such as activation of the nuclear enzyme poly (ADP-ribose) synthetase (PARS) and apoptosis[17]. Peroxynitrite, therefore, may result in DNA or tissue damage, contributing to multistage carcinogenesis processes.

1.2.2. DNA damage caused by peroxynitrite

As we stated earlier, peroxynitrite promotes various DNA modifications. Yermilov et al. studied various reactions of peroxynitrite with nucleobases and nucleosides in vitro[18]. They found that the reaction with peroxynitrite affected more the purine bases (guanine and adenine) than the other bases. The analysis of the reaction
products by high-performance liquid chromatography (HPLC) and Thin Layer Chromatography (TLC) revealed that the major product is 8-nitroguanine. The formation of 8-nitroguanine from guanine increased proportionally in a dose-response manner with peroxynitrite concentration[19]. Only peroxynitrite, but not nitrous acid, and NO-releasing compounds formed 8-nitroguanine. Therefore, 8-nitroguanine is considered a specific marker of DNA damage induced by peroxynitrite. Also, peroxynitrite can instigate DNA strand breaks in vitro. DNA cleavage caused by peroxynitrite was seen at almost every nucleotide with a little dominance at guanine residues[20]. Peroxynitrite induced single strand breaks at acidic pH rather than at neutral or alkaline pH, suggesting that hydroxyl radical-like intermediate(s) (ONOOH*) or peroxynitrous acid (ONOOH) are responsible for the damage[17, 20, 21]. Antioxidants (urate, ascorbate, N-acetylcysteine) inhibit this modification[19]. The development of analytical techniques capable of rapid and sensitive detection of various types of DNA damage and DNA damaging agents is therefore the focus of many laboratories. Scheme 1.1 shows the mechanism of DNA damage by peroxynitrite
1.2.3. Methods to detect DNA damage induced by peroxynitrite

There are a number of reports that studied oxidative DNA damage induced by ONOO$. Various methods have been developed to investigate oxidative DNA damage, such as gel electrophoresis [22-24] and high-performance liquid chromatography with an electrochemical detection system (HPLC-EC)[25, 26]. However, these methods may require sophisticated or expensive equipment and some are time-consuming. In addition, ONOO$ present major challenges in its analysis as it decays fast via many routes with, sometimes, an additional complexity of cross-talk between these decomposition paths.
Therefore, the studies on ONOO\textsuperscript{−} are fewer than hydroxyl radical (OH) and superoxide radicals (O\textsubscript{2}\textbullet{−}). The challenges in the detection and quantification of peroxynitrite require developing other methods. In this regard, electrochemical methods have gained considerable importance as they are simple, fast, and sensitive [27-30]. Electrochemical biosensors have been effectively utilized for various applications including the detection of DNA damage, investigation of the interactions of DNA with different genotoxic substances (such as cancer-causing agents, mutagens, reactive species, or pharmaceuticals and drugs), and also for the detection of specific mutations in DNA sequences.

1.3. **Significance of DNA films interfaces**

DNA treatment with peroxynitrite prompts considerably more damage than an equal dosage of nitric oxide. Therefore, peroxynitrite is of greater reactivity than nitric oxide intrinsically[31]. In fact, many kinds of cellular chemical damage originally attributed to nitric oxide turned out to be the result of peroxynitrite as a product of nitric oxide and superoxide under oxidative stress conditions. Because DNA is very sensitive to ONOO\textsuperscript{−} we hypothesized that DNA films on interfaces can be used for its specific detection and quantification. Also, the base sequence on these films can be systematically varied to determine the level of chemical damage as a function of base content and base sequence. In the long term, this system can be used to explain the differential predisposition of specific parts of the genome to peroxynitrite damage.

In this work, our aim is to use the DNA films as sensing platforms to detect and quantify ONOO\textsuperscript{−}. To this end, we applied different DNA immobilization techniques and used...
various electrochemical techniques to develop this new approach of peroxynitrite sensing.

1.4. Immobilization methods

1.4.1. Grafting

Grafting is a technique for immobilization of molecules on the surface of electrodes through the creation of a covalent bond. The bond formation results from electrochemical generation of an organic radical near the electrode surface by reduction of a suitable precursor of the molecule targeted for attachment. Precursors include aryl diazonium salt and diaryliodonium salts, among other possibilities. The formed radical attaches on the electrode surface, resulting in the formation of a covalently bonded film [32-35].

1.4.1.1. Pyrolytic graphite (PG)

PG is not naturally found [36]. It is fabricated by heating hydrocarbon gas to a very high temperature around 3000°C, where the graphitization process occurs. Vacuum furnace is used for the graphitization process, and the graphite is allowed to crystallize (pyrolysis). An ultra-pure conductive carbon material forms as layers of carbon deposits on top of others layers. As such, PG electrodes have two types of surfaces: the basal plane and the edge plane. The edge plane sites have more reactivity toward chemical reactions than the basal graphite site because of the relative abundance of oxygen functionalities on the edge plane [37-39]. The low density of electronic states is the cause of low reactivity of the graphite basal plane due to the lack of functional groups and adsorption sites [40-42]. The edge plane graphite allows faster electron transfer at its surface plane [40, 42]. Basal planes have a low local density of states near the Fermi energy, whereas the edges have much higher local densities of states due to bonds and/or
terminating impurity groups[43]. Therefore, there is a higher probability that electrons will be available for redox reaction at the edge rather than on the basal planes. However, Unwin and co-workers[44] suggested that this may not be correct. More work is required to understand electron transfer processes at graphite surfaces.

PG is pre-treated either electrochemically or mechanically to acquire a reproducible electrode surface. Electrochemical pre-treatment is usually performed by cyclic voltammetry scanning over a wide potential range. Mechanical polishing is performed by polishing the PG electrodes on a micro-fabric cloth with alumina slurry with decreasing grain size (~ 1 micron and smaller) as well as diamond paste, followed by sonication in an appropriate solvent to remove attached particles. This mechanical pre-treatment was used in this work to prepare PG surfaces before modification.

1.4.1.2. Diazonium formation

The formation of aryldiazonium is called diazotization. This process was founded by Peter Griess in 1858 [45]. Diazotization process is carried out by the addition of nitrous acid, formed in situ, to aromatic amines at about 0°C. The nitrous acid is formed through the reaction between sodium nitrite and a strong mineral acid, usually hydrochloric acid. The aryldiazonium salts formed are organic compounds with a general formula R-N\(^2+\)X\(^-\), where R is an aryl group, and X is an inorganic or organic anions such as Cl\(^-\) or BF\(_4\)\(^-\). The diazotization process can also be carried out in acetonitrile in the presence of tertbutyl nitrite. Scheme 1.2 shows the diazotization method of aromatic amines.
In this work, trans-4-cinnamic acid diazonium tetrafluoroborate is synthesized and purified according to published procedures [46]. The trans-4-cinnamic acid reacts with nitrous acid, formed \textit{in situ} from hydrochloric acid and sodium nitrite in the presence of sodium tetrafluoroborate at 0°C.

![Scheme 1.2. Diazotization scheme of aromatic amines](image)

1.4.1.3. \textbf{Types & applications of grafting methods}

Generation of an organic radical in solution near a surface can lead to an attack of the radical on the surface, resulting in the formation of a covalently bonded film at the surface [32-35]. The most common method used to generate radicals is the reduction of aryl diazonium salts. Grafting from diazonium salt solution is typically carried out using electrochemical means [47-51] although non electrochemical routes also exist [52-55]. In the electrochemical method, the aryl diazonium cation is reduced by the application of an external potential, leading to an aryl radical that is grafted onto the electrode surface through a covalent bond (Scheme 1.3). Electro-grafting of a wide range of aryl diazonium salts has been shown to proceed at carbon [32, 50, 56], metal [33, 57] and semiconductor surfaces [49].

![Scheme 1.3. Proposed mechanism for modification of surfaces via reduction of aryl diazonium salts](image)
There are many applications of modified surfaces with specific functionality including sensors (chemical and biological) [58-62] and molecular electronics[63, 64]. Typically, a sensor consists of an electrode (signal transducer), a recognition element, and a chemical layer that enables the immobilization of the recognition species on the electrode surface[60]. The recognition species must have high selectivity for the target analyte and is effectively immobilized onto the electrode surface to ensure long-term performance of the sensor.

Many approaches have been used to covalently immobilize biological molecules on surfaces for biosensor applications based on aryldiazonium chemistry. In the first approach, the surface is modified with amine or carboxylic acid functionalities via diazonium ions; the biomolecules are then covalently immobilized on the surface via the formation of amide bonds between the surface’s amine/carboxylic acid groups and the biomolecules’ carboxylic acid/amine groups (Scheme 1.4)[65]. Coupling agents, such as carbodiimide and N-hydroxysuccinimide, are usually added to the reaction to facilitate the formation of the amide bond. We used this process to our work as it is efficient, requires mild reaction conditions which preserves the integrity of the biomolecules targeted for immobilization.
Scheme 1.4. Schematic representation of immobilization of biological molecules on a surface via surface-bound carboxylic functionalities.

In another approach, the biomolecules are first modified with p-carboxymethyl aniline, which is then diazotized into aryl diazonium ions before the immobilization on the surface (Scheme 1.5) [65-67]. The limitation of this approach is that the chemical modification of biomolecules, unless carefully targeted to a specific site, may damage the biological activity of the molecules. It is also relatively time-consuming because the chemical modification of the biological molecule will entail some form of separation before use.

Scheme 1.5. Schematic representation of immobilization of biological molecules on a surface via diazonium functionalities.
In general, covalent immobilization of biological molecules through surface-modified diazonium functionalities has been investigated by Viel and co-workers[68]. This method appears to provide cleaner products and is less time-consuming than the alternative methods. In our study, we used the electrodes modified with a 4-amino-cinnamic diazonium salt to generate carboxylic acid groups on the electrode surfaces.

1.4.2. Layer by layer method (LBL)

The layer-by-layer (LBL) assembly is a technique of depositing multilayers of oppositely charged materials leading to films (micron thick or less) held by electrostatic attraction between layers. Although it has gained a rapid progress recently, the research can be traced back to the pioneering work of Ilver in 1966, who reported for the first time the fabrication of multilayers by alternating the deposition of positively and negatively charged colloid particles, as shown in scheme 1.6. As Ilver pointed out, a similar technique could be applied not only to colloid particles, but to polyvalent ions, surfactants, water-soluble polymers, and even proteins[69].

The LBL technique, as schematically shown above, has shown many advantages over other methods for multilayer fabrication[69]. No complicated instruments are needed; thus, it is a simple method for fabricating multilayers. For example, Caruso et al. have demonstrated the LBL deposition on a global template. After dissolving the template, microcapsules were obtained.

As stated earlier, the principal mechanism of the LBL technique is an electrostatic interaction among the multilayers on the electrode surface. Electrostatic interaction requires the use of water-soluble and multi-charged species, such as
polyelectrolytes, proteins and enzymes, colloid particles, and oligo-charged organic compounds [70-72]. Therefore, it does not require an exact positional matching of the charged groups, which facilitates incorporation of more than one building block in the multilayer. The LBL electrostatic assembly is performed in an aqueous solution; thus, it is convenient to fabricate LBL multilayers in an automated way by using a LBL deposition machine. It should be noted that there is no clear interface structure for the polyelectrolyte multilayer. In other words, the adjacent layers may be interdigitated to some extent in polyelectrolyte multilayers.

![Scheme 1.6. Schematic cross-section of a multilayer film](image)

1.5. **Electrochemical methods used**

1.5.1. **Cyclic voltammetry**

Cyclic voltammetry (CV) is a potentiodynamic electrochemical technique in which the working electrode potential is scanned linearly versus time. At a certain set potential, the potential is scanned back in the opposite direction. The cyclic voltammogram is a relationship between the applied potential and the current at the working electrode. Cyclic voltammetry is usually applied to study the electrochemical properties of an analyte in solution[73, 74]. The current results as electrons are transferred to or from the analyte when the electrode reaches a specific voltage that
matches the standard redox potential of the species in the cell. A reduction peak results when electrons are transferred to the analyte in solution or at the interface. For a reversible system, when the voltage is increased back, electrons are transferred from to the reduced analyte back to the electrode and the process results in an oxidation peak. The cyclic voltammogram is the relationship between current as a function of applied potential at a given potential scan rate (Figure 1.1) [75].

![Cyclic Voltammogram](image)

**Figure 1.1.** A typical cyclic voltammogram.

### 1.5.2. Chronocoulometry

Chronocoulometry is an electrochemical technique used for determination of the amount of matter transformed during an electrolysis reaction. Chronocoulometry measures the amount of electricity flowing through working electrode as a function of time at a certain potential [76]. In chronocoulometry, the applied potential on the electrode is jumped from a starting point where no faradaic process occurs to a final point where the redox reaction takes place (Figure 1.2). The transient charge passes through the electrode and is measured as a function of time [75]. Chronocoulometry is very useful for calculating the surface area of sensors and obtaining the amount of adsorbed redox active
molecules on sensor surfaces, as well as in determining mechanisms and kinetics of electron transfer reactions that occur at an electrode surface. Chronocoulometry coupled with other techniques can also be used to determine diffusion coefficients of different analytes.

Figure 1.2. Typical chronocoulogram

1.5.3. Square wave voltammetry

Square wave voltammetry (SWV) is also a technique where the potential is scanned over time. In contrast to CV, in SWV the potential scanning is accomplished by a series of stair steps. The current difference between the cathodic and anodic pulses in each step is measured before the next step is taken. During the cathodic pulse, the electro-active species gets reduced at the electrode, while the anodic pulse oxidizes the reduced species back to the original form. The resultant difference current is measured as a function of voltage potential and is displayed as a square wave voltammogram (Figure 1.3). Since the currents obtained during cathodic and
anodic processes have opposite signs, the difference in current at each step in potential will be higher compared to the currents obtained in CV at a given potential. Thus, SWV is a very sensitive technique because it can detect low currents.

Figure 1.3. A typical square wave voltammogram

1.5.4. Differential pulse voltammetry

Differential Pulse Voltammetry (DPV) is a derivative of linear sweep voltammetry in which regular pulses are superimposed on the linear sweep potential steps. The current is monitored twice: the first sample is taken just before the rise in potential when the pulse starts, while the second is taken at the end of the current pulse just before it decreases back to the baseline. The difference between these two currents is $\Delta I_{\text{pulse}}$. The differential pulse voltammogram results by plotting the current difference versus the potential (Figure 1.4). DPV has high sensitivity as it minimizes the effect of charging current and detects only the faradic current. Therefore, the electrode reaction can be analyzed more precisely, and small amounts of chemicals and biomolecules can be
studied. The height of current peak $\Delta I_{\text{pulse}}$ is proportional to the concentration of the analytes.

Figure 1.4. A typical differential pulse voltammogram
CHAPTER II
Experimental

2.1. Introduction

This chapter outlines the general experimental methods and materials throughout the work of this thesis.

2.2. Synthesis and General Solutions

2.2.1. Phosphate Buffer Solution

Phosphate buffer saline (PBS) of 0.05 M, was prepared by dissolving 3.28 g of sodium di-hydrogen phosphate (NaH$_2$PO$_4$), 4.12 g of disodium hydrogen phosphate (Na$_2$HPO$_4$) and 5.85 g NaCl in deionized (DI) water (final volume, 1000 mL) to give pH 7.0.

2.2.2. MES Buffer Solution

2-(n-morpholino)ethane sulfonic (MES) buffer of 0.05 M, was prepared by dissolving 4.88 g of MES acid in 450 ml of DIH$_2$O, add 3.45 g NaCl. The pH 6.5 is then adjusted with monovalent strong acid or base as needed. Finally, the volume is adjusted to 500 ml with DI H$_2$O.
2.2.3. Acetate buffer solution

Acetate buffer of 0.1 M was prepared by adding 18 ml of 0.1 M acetic acid with 400 ml of 0.1 M sodium acetate. The pH is then adjusted to 5.5. Water is added to make a final volume to 500 ml.

2.2.4. Preparation of electroactive probes solutions

2.2.4.1. Potassium ferricyanide: 2 mM Potassium ferricyanide $K_3[Fe(CN)_6]$ was prepared in PBS to be used in cyclic voltammetry.

2.2.4.2. 50 µM tris(2,2'-bipyridyl) dichloro ruthenium(II) hexahydrate (Ru(bpy)3) prepared in pH 5.5 0.1M acetate buffer that will be the redox probe in square wave voltammetry.

2.2.4.3. 2 mM ferricyanide and 2µM methylene blue solution prepared in pH 7.0 PBS as a probe in cyclic voltammetry and chroncoulometry.

2.2.5. Preparation of Trans-4-cinnamic acid diazonium tetrafluoroborate

We synthesized this diazonium compound according to published procedures[46]. Briefly, 0.82g of trans-4-cinnamic acid is dissolved in 10 ml of 6M HCl. The solution is cooled in an ice bath. 5mM NaNO₂ is added dropwise to the mixture with stirring over 30 minutes. Another solution of 0.72g (6.5 mM) of sodium tetrafluoroborate in 2 mL of water was then added. The mixture was stirred vigorously for another 10 minutes. The solid diazonium tetrafluoroborate was filtered quickly and washed immediately once with 5 mL of cold 5% sodium tetrafluoroborate solution and then with 5 mL of cold ethanol, and finally several times with ether. The pure product was dried in a desiccator.
2.2.6. Preparation of double-stranded oligonucleotides

15’mer- single-stranded oligonucleotides, C6 5’ amino modified were purchased from BioSynthesis. (1. 5’-amino-C6-AGT ACA GTC ATC GCG-3’, 2. 5’-CGC GAT GAC TGT ACT-3’, 3. 5’-CGC GAT GAA TGT ACT -3’) were suspended in deionized water to prepare stock solutions of 2 μg/μl concentrations. Equal volumes of each ssDNA (1+2: complementary oligos and 1+3: mismatched oligos) were hybridized in pH 7.0 phosphate buffer (5 mM phosphate, 0.1 mM NaCl) by heating for 5-8 min at 95°C in a heat block and cooling slowly to room temperature over 3-4 hrs. We used absorption spectroscopy to estimate the final DNA concentration using a known DNA extinction coefficient at 260 nm.

2.3. Electrochemical Methods

2.3.1. Instrument and Software

Electrochemical measurements were performed using CHI-440 electrochemical workstation potentiostat interfaced to PC system.

2.3.2. Electrodes

Pyrolytic graphite (PG) electrodes were cleaned before any measurement. The PG was hand-polished with 0.3 and 0.05 micron alumina slurries on Buehler microcloth, followed by sonication in H₂O for 15 min before grafting procedures to ensure the elimination of the alumina particles from the surface. This procedure was repeated for each set of experiments.
2.3.3. Cell Setup

Standard glass multi-armed electrochemical cells were used. The cells were thoroughly rinsed with de-ionized water before use. The three electrodes consisted of the PG as the working electrode, a platinum wire as an auxiliary electrode, and Ag/AgCl (3.0 M KCl) was used as the reference electrode.

2.4. Surface modification procedures

Two strategies have been applied for modification procedures; grafting strategy and layer-by-layer (LBL):

2.4.1. Grafting Strategy

2.4.1.1. Chemicals and biomolecules

Double stranded oligonucleotides (1+2: complementary oligos and 1+3: mismatched oligonucleotides) were prepared as in 2.2.6. All other chemicals; trans-4-aminocinnamic acid, sodium nitrite, 1-Ethyl-3-(3- dimethyl aminopropyl)carbodiimide Hydrochloride (EDAC), 2-[N-morpholino] ethane sulfonic acid (MES), N-hydroxy-succinimide (NHS), methylene blue, Potassium hexacyanoferrate (III), and tris(2,2′-bipyridyl) dichloro ruthenium(II) hexahydrate were reagent grade and purchased from Sigma-Aldrich. Deionized water was obtained from a Barnstead ultra-pure water purification system (specific resistance >18.2 MΩ•cm).

2.4.1.2. Procedures

The electrode modification process is made in 2 steps:

Grafting with carboxylic acid group

Pyrolytic graphite electrodes were polished with 0.3 and 0.05 μm alumina slurries respectively on a Buehler microcloth and ultra-sonicated in deionized water for
15 min before grafting. Two electrochemical cycles performed in 10 mM of the synthesized diazonium compound in acetonitrile containing 0.1 M Bu₄NBF₄ as the supporting electrolyte. Cyclic Voltammetry was carried out on the PG in a potential window of +0.8 V to -0.8 V (scan rate 0.2 V/s). Two consecutive scans were used in these conditions (Scheme-2.1, Figure-2.1). Ferricyanide and ruthenium redox probes have been used to test the formation of the film on the surface of electrodes.

**DNA immobilization on the electrode surface**

The modified electrode grafted with carboxylic acid groups was washed thoroughly with acetone followed by deionized water before proceeding with activation of the carboxylic groups for DNA immobilization (Scheme 2.1). A 50 μl drop of EDC/NHS solution (10:1 mg of EDC to NHS in 1.0 ml solution of 50 mM MES buffer, pH 6.5) carefully cast on the surface of the electrode. The electrode was incubated in this mixture in a closed environment for 20 min. After incubation, the modified PG electrode was washed immediately with deionized water and 50 μl of 50 μM amino terminated oligonucleotides in 5 mM phosphate buffer (pH 7.0) containing 50 mM NaCl, and 0.1 M MgCl₂ was cast on the electrode surface in a closed chamber for 2 hours. After immobilization of the oligonucleotides, the electrode was thoroughly washed with 5 mM pH 7.0 phosphate buffer (50 mM NaCl) followed by deionized water.
Scheme 2.1. Immobilization of DNA on electrode surfaces.

Figure 2.1. Cyclic voltammogram for the first and second scans of 15 mM trans-4-cinnamic acid diazonium tetrafluoroborate in acetonitrile.
2.4.1.3. Effect of DNA films on redox probe voltammetry

The presence of immobilized DNA is characterized electrochemically using different redox probes.

2.4.1.4. Ferricyanide redox probe

Ferricyanide reversible redox couple is normally observed around 200 mV/Ag-AgCl. Derivatization of the electrode surface with carboxylic acid groups drastically reduces the electrochemical signal due to electrostatic repulsion between the negatively charged probe and the electrode surface. DNA immobilization further enhances the negative charge density on the electrode surface, which results in a further reduction of the electrochemical signal. Figure 2.2 shows the cyclic voltammograms in 2mM potassium ferricyanide for bare, electrode for the electrode grafted with carboxylic groups, and for a DNA-modified electrode.
Figure 2.2. Cyclic voltammograms for bare, carboxyl grafted, and DNA-modified electrode surfaces in 2 mM Fe(CN)$_6^{4-}$ in pH 7.0 phosphate buffer.
2.4.1.5. Tris(2,2'-bipyridyl) dichloro ruthenium(II) complex \([\text{Ru(BPY)}_3]^{2+}\)

DNA immobilization on the electrode surface is characterized using tris(2,2’-bipyridyl) dichloro ruthenium(II) complex as a redox probe. This transition metal complex has a positive charge, and shows enhanced electrochemical oxidation current on immobilized DNA[56]. The ruthenium complex is first oxidized via charge transfer from the electrode surface. The oxidized ruthenium probe is reduced back to Ru\(^{2+}\) form by guanine sites in attached DNA layer[56, 77] (Scheme2.2). This two-step catalytic mechanism of Ru(bpy)\(_3\)\(^{2+}\) mediated DNA oxidation leads to a significant increase in catalytic current as shown in the voltammogram for the DNA-modified electrode. Figure 2.3 displays the square wave voltammograms for the bare, for the carboxylic acid modified surface, and for the DNA-modified electrodes in 50 \(\mu\)M Ru(bpy)\(_3\) in pH 5.5 acetate buffer. DNA immobilization shifts the redox peak of the ruthenium complex towards positive values while increasing the peak current compared to bare electrode in the same solution as a result of the oxidative catalytic process described above.
Figure 2.3. Square wave voltammograms for bare, carboxyl modified, and DNA-modified electrodes in 50 µM Ru(bpy)$_3^{2+}$ complex in pH 5.5 acetate buffer.
Scheme 2.2 Ruthenium-mediated guanine base oxidation
2.4.2. Layer-by-Layer immobilization

2.4.2.1. Chemicals and biomolecules

Poly-(diallyl dimethyl ammonium) (MW 200,000-350,000) was purchased from Aldrich. Double-stranded Calf thymus DNA (ds-CT DNA) was purchased from Sigma-Aldrich. Water used in all experiments was purified in a Barnstead Nanopure purification system and had a resistivity higher than 18.2 MΩ•cm.

2.4.2.2. Procedures

The other method for DNA immobilization on the surface of the electrodes is through Layer-by-layer (LBL) assembly[71, 78]. It is a simple method that depends on adsorbing a charged macromolecule on oppositely charged under layer that has been adsorbed on the electrode surface. In our case, poly-(diallyl dimethyl ammonium) (MW 200,000-350,000) (PDDA) was used as a positively charged component. The PG is first treated electrochemically in acidic solution to generate negative charges on the surface. It is then soaked in 1mg/ml the positively charged PDDA for 20 min. The electrode surface is then washed with deionized water and dried under a stream of N₂ gas. The second layer consisted of the negatively charged calf thymus DNA. 50µL of 1mg/ml DNA solution was cast on the electrode surface and allowed to incubate for 30 min. Scheme 2.3 shows the procedure of DNA immobilization using LBL assembly procedure.
Scheme 2.3. Layer-by-layer modification of an electrode surface with PDDA and calf thymus DNA.

2.4.2.1. Effect of DNA films on redox probe voltammetry

The effect of DNA adsorption by LBL method on the electrode has been studied using 50 μM Ru(bpy)$_3$ as a reporting redox probe in pH 5.5 acetate buffer. Ru(bpy)$_3$ oxidation typically gives an oxidation peak at 1.05 V vs. Ag/AgCl in square wave voltammetry. Presence of the first layer of PDDA with a positive charge on the electrode surface causes a decrease in peak current due to repulsion between the positive charged electrode surface and the positively charged ruthenium complex probe. Adsorption of calf thymus DNA on the PDDA-modified surface increases the peak oxidation peak current upon of the redox probe. Scheme 2.2 illustrates the catalytic mechanism of interaction between the guanine bases of the DNA calf thymus on the surface of the electrodes and the ruthenium redox probe[79, 80]. This interaction is the cause of increase of the peak current upon DNA adsorption as shown in Figure 2.4.
Figure 2.4. Square wave voltammetry for bare, PDDA modified, and DNA modified-layer by layer electrodes in 50 μM Ru(bpy)$_3^{2+}$ complex in pH 5.5 acetate buffer.
CHAPTER III

Grafted DNA oligonucleotides as peroxynitrite sensing elements

3.1. Introduction

DNA/oligonucleotides immobilization on the surface of electrodes have a variety of applications in a number of areas. For instance, immobilization of hybridized oligonucleotides on the surface of electrodes has been used to detect the single base mismatch in DNA sequences. Also, genomic DNA adsorbed on electrode surfaces with enzymes has been used to study the predisposition of DNA to chemical damage by reactive species generated by the adsorbed enzymes in situ. In this work, we developed a new application that consists of using DNA films as sensing platforms for the reactive metabolite peroxynitrite. This chapter addresses this new analytical route that capitalizes on established methods of DNA immobilization on one hand, and known reactivity of DNA and peroxynitrite on the other, to provide a proof of principle of using this system to develop DNA-based sensor for peroxynitrite’s selective detection and sensitive quantification.
3.2. Detection of mismatch oligonucleotides

The immobilized DNA on the surface of the electrodes has been used to detect the base mismatch using Barton’s intercalator-mediated charge transport technique [81]. The mismatch detection experiment consists of immobilizing two identical 15-base oligonucleotide probe sequences on two identical pyrolytic graphite electrodes, and hybridizing one probe to a complementary target on one electrode and the second probe of the second electrode containing a sequence that results in a mismatched base. We can use both cyclic voltammetry and chronocoulometry to monitor the response of the two systems using a catalytic system using a sequence of two redox probes.

3.2.1. Detection of mismatch using methylene blue

The detection of the single base mismatch is based on the high sensitivity of DNA long-range charge transport to stacking perturbation such as mismatch [82-85]. This sensitivity depends on electronic coupling within the base pair stack rather than on the thermodynamics of base pairing. To exploit this property in functional mismatch detection assays, we have developed a method for electrochemical detection of mismatches based on charge transport through dsDNA monolayers on pyrolytic graphite electrodes [81, 86, 87]. Methylene blue (MB), a redox probe well-known to intercalate in DNA, is used as a redox probe for this procedure. It gives reversible redox couple at -0.2 V vs. Ag/Ag/Cl on a bare electrode. This peak is higher in the presence of complementary DNA due to higher electron transfer through the DNA films a result of π-stacking effect. When a single base mismatch present, the π-way formed by base stacking is interrupted resulting in diminished electron flow and reduction of the peak current.
Figure 3.1. Cyclic Voltammograms in 2 μM methylene blue in pH 7.0 phosphate buffer for the bare electrode and for electrodes modified with complementary and single base mismatched oligonucleotides.
3.2.2. Detection of DNA single base mismatches using ferricyanide/ methylene blue

Methylene blue has been used for detection of mismatch. However another method proved to be sensitive by coupling the charge transfer through methylene blue with a freely diffusing redox probe like negatively charged ferricyanide that interacts with bases only at the top of the negatively charged DNA films. This catalytic charge transfer increase the observed current and thus the sensitivity of the process[88, 89]. Scheme 3.2 illustrates how ferricyanide affects the electrochemical response and increase the sensitivity[90]. It shows the two-step catalytic process that happens in solution. First, methylene blue intercalates the DNA strand and reduced into leuco-methylene blue. In the second step, the reduced methylene blue oxidizes back to methylene blue reducing the ferricyanide in solution resulting in a catalytic response. This can be observed using cyclic voltammetry with complementary DNA and with single base mismatch DNA immobilized on the surface as shown in Figure 3.1. Higher electrocatalytic current is observed in the case of complementary DNA due to efficient charge transfer.
Scheme 3.1. Electrochemical response of the modified electrode in methylene blue/ferricyanide.
3.2.3. Detection of DNA single base mismatch using chronocoulometry

Chronocoulometry technique is another technique that can be used to detect the single mismatch DNA using MB/ferricyanide system. A potential of -0.4 V vs. Ag/AgCl is applied to the modified electrode and the charge flowing through the electrode is monitored. Consistent with cyclic voltammetry, the complementary DNA gives high faradic charge compared to the electrode modified DNA with base mismatch. Figure 3.3 shows the chronocoulometric graphs for immobilized complementary DNA and the electrode modified with the DNA sequence containing a single base mismatch.
3.3. Detection of peroxynitrite using complementary DNA oligonucleotides

DNA determines genetic characteristics in living beings. Many chemical and physical effects can cause damage in DNA molecules. Physical processes include exposure to high temperature[1], ionizing radiations[91], and ultraviolet radiations[3]. Chemical factors include interaction with, reactive oxygen species and other reactive chemicals involved in the biological metabolism [4]. Macrophages and neutrophils also produce reactive oxygen and nitrogen compounds at sites of inflammation and infections[92]. These chemicals attack DNA bases and cause oxidation, nitration of the
bases, or other transformation such as DNA strand break which are difficult to repair and extremely toxic[93]. Such chemical damage impairs base pairing and/or block DNA replication and transcription. Chromosome aberrations and mitotic faults are the common results of DNA damage. Accumulation of mutations and/or other kinds of DNA damage represent a carcinogenic or teratogenic risk[6]. Studies reported that the level of carcinogenesis due to oxidative DNA damage is more than the lesions induced by exposure to exogenous carcinogenic compounds[94].

Peroxynitrite (ONOO$^-$) is one of these reactive chemical agents that inflict irreversible damage on DNA. ONOO$^-$ is a strong oxidizing and nitrating agent that correlated with both cell death, and an increased cancer risk observed in many pathological conditions such as reperfusion injury, chronic inflammation, atherosclerosis, rheumatoid arthritis, neurodegenerative diseases, and an increased cancer incidence in tissues under chronic inflammatory conditions [7, 13-15]. For example, ONOO$^-$ leads to the generation of reactive oxygen species (ROS) in human leukemia cells[5]. Additionally, in acute and chronic inflammation, ONOO$^-$ is a highly reactive species that causes oxidation, nitration for protein, DNA bases modification, and DNA strand breaks. Development of analytical techniques capable of rapid and sensitive detection of various types of DNA damage and DNA damaging agents is therefore in the focus of many laboratories.

In this work, we develop a new method to detect and quantify peroxynitrite using DNA films as a sensing elements immobilized on the electrode surface. We capitalize on the DNA response to the methylene blue/ferricyanide system in cyclic voltammetry and
chronocoulometry to correlate the electrochemical response to exposure to various doses of peroxynitrite.

3.3.1. Cyclic voltammetry for peroxynitrite detection

MB/ferricyanide is used as a coupled system of redox probes to detect the difference in electrocatalytic current between the immobilized oligonucleotides before and after exposure to different concentrations of peroxynitrite. First we monitor the current for the immobilized DNA films before exposure to peroxynitrite. As expected, the catalytic current through the undamaged DNA before exposure to PON is higher than the current observed after exposure of the same DNA film to peroxynitrite. Scheme 3.2 shows the different chemical damage effects that peroxynitrite can induce in DNA. ONOO⁻ has three major types of damage on DNA: direct strand breaks due to oxidation of deoxyribose moieties, nitration of guanine, and oxidation of guanine and other nucleobases[95]. The major two products of this damage are; 8-oxodG and 8-nitroG[96]. Figure 3.4 shows the cyclic voltammograms for DNA biosensors before and after exposure to 200 µM ONOO⁻ for 5 minutes using MB/ferricyanide system.
Scheme 3.2. Effect of peroxynitrite on DNA
Figure 3.4. Cyclic voltammograms of 2 mM ferricyanide and 2 μM methylene blue solution in pH 7.0 phosphate buffer on electrodes modified with complementary oligonucleotides before and after exposure to 200 μM PON for 5 minutes.
3.3.2. Cyclic voltammetry for peroxynitrite detection

DNA-mediated charge transport coupled to electrocatalysis is sensitive to perturbation induced by peroxynitrite and, as in cyclic voltammetry, is expected to result in lower flow of faradaic charge. In fact, figure 3.5. shows that the charge observed through attached DNA is substantially lowered after exposure to a solution of 200µM PON for 5 minutes figure 3.5.

![Figure 3.5. Chronocoulometry at – 400 mV in the presence of 2.0 mM ferricyanide and 2µM methylene blue at pH 7.0 on electrodes modified with complementary oligonucleotide before and after exposure to 200µM PON for 5 minutes.](image)

Figure 3.5. Chronocoulometry at – 400 mV in the presence of 2.0 mM ferricyanide and 2µM methylene blue at pH 7.0 on electrodes modified with complementary oligonucleotide before and after exposure to 200µM PON for 5 minutes.
3.4. Mismatch oligonucleotides as ultrasensitive peroxynitrite sensors

The same system used for complementary DNA strands has been applied for the hybridized oligos containing a base mismatch. Cyclic voltammetry and chronocoulometry have been applied using the MB/ferricyanide system of coupled as redox probe. Figures 3.6 and Figure 3.7 show the cyclic voltammetry and chronocoulometry before and after exposure to 200µM of PON for 5 minutes. We found that mismatch DNA immobilized on the electrode surface was significantly more sensitive to PON exposure than on the complementary DNA at any PON concentration. Therefore, modified electrodes with hybridized oligos containing base mismatch can be used as a platform for ultrasensitive peroxynitrite detection.

![Cyclic voltammograms of single base mismatched oligonucleotides](image)

Figure 3.6. Cyclic voltammograms of single base mismatched oligonucleotides in 2 mM Ferricyanide with 2µM methylene blue in pH 7.0 phosphate buffer before and after exposure to 200µM PON for 5 minutes.
Figure 3.7. Chronocoulometry at –400 mV of 2.0 mM ferricyanide in the presence of 2μM methylene blue at pH 7.0 on modified electrodes with mismatched oligonucleotides before and after exposure to 200μM PON for 5 minutes.

3.5. Effect of using different peroxynitrite concentrations

To compare the relative sensitivity of immobilized DNA with complementary sequences versus those containing a base mismatch, we monitored the response of the two types of sensors before and after exposure to increasing concentration of PON. Again, we used both cyclic voltammetry and chronocoulometry using the MB/ferricyanide system. Figure 3.8 shows the chronocoulometric data. As expected, that
data shows that the higher the concentration of PON, the higher the change in the charge observed for both types of DNA as a result of expected increase in DNA chemical lesions due to exposure to higher doses of PON. However, the electrodes with DNA sequences containing a base mismatch show significantly larger change in charge difference at any concentration of peroxynitrite. When we used smaller concentration of PON, we found that the charge difference increased with a linear relationship till 5uM in both complementary and mismatched oligonucleotides as shown in figure 3.9. This shows that the sequences with base mismatch are more sensitive to PON exposure and therefore are amenable to detecting lower concentrations of PON.

Figure 3.8. Effect of different PON concentrations on complementary and base mismatched DNA.
Figure 3.9. Dose-dependent concentration of both complementary and mismatched oligonucleotides.

3.6. Conclusion

Electrodes modified by DNA oligonucleotides show sensitive responses towards micromolar range concentration of PON. Complementary and mismatched oligonucleotides show differential electrochemical responses. The mismatched oligonucleotide is a more sensitive platform to detect low concentrations of PON.
CHAPTER IV
Layer by layer immobilization of genomic DNA for peroxynitrite detection

4.1. Introduction

As we stated in an earlier chapter, different assembly strategies have been developed to modify surfaces and introduce recognition elements that target particular analytes. One of the most widely used method is a layer-by-layer (LBL) method introduced by Iler in 1966[97]. The basic concept behind the LBL was using polyanions and polycations on the surface as alternate layers[98]. The resulting film is held through electrostatic attraction between its oppositely charged layers[99]. The advantages of LBL assembly over many other assembly techniques are many. For instance, it can precisely control the composition of the layers and thickness of films. Also, the LBL is a simple method and can be used on any substrate and for all kinds of components ranging from polyelectrolytes, polymers, proteins, as well as colloidal nanoparticles [100-102].
Recently, other types of the LBL assembly strategies, not necessarily based on electrostatic attraction, have been developed. These included methods based on biological affinity [103-105], covalent binding[106, 107], cross-linking[108, 109], and electrodeposition[110].

Our method of LBL includes the electrostatic attraction between the positively charged polymer on the surface of the electrode, poly(diallyl dimethyl ammonium chloride) (PDDA), and the negatively charged calf thymus DNA cast on the surface of the electrode. Scheme 4.1. Shows an illustration of the proposed layer-by-layer method for biomolecular immobilization.

4.2. Experimental

4.2.1. Chemicals and biomolecules

Calf thymus DNA was purchased from Sigma-Aldrich. Tris(2,2’-bipyridyl)dichloro ruthenium(II) hexahydrate were purchased from Sigma-Aldrich. De-ionized water (resistivity > 18 MΩ.cm) was provided by a Barnstead nanopore water system.

4.2.2. Electrochemical measurements

Square wave voltammetry is carried out using the CHI-440 electrochemical workstation in a three-neck electrochemical cell. Ag/AgCl is used as the reference electrode, platinum wire as a counter electrode, and pyrolytic graphite as a working electrode. All electrochemical measurements are carried out at room temperature in buffers.

4.2.3. DNA immobilization procedure for electrochemical characterizations

DNA-modified electrodes are fabricated in two steps. First, the electrode surface is electrochemically cycled in acidic solution to generate negatively charged functionalities on the surface. The electrode is then incubated in a solution containing 10 mg/ml PDDA for 20 minutes. The electrode is thoroughly washed with deionized water and then dried under dry stream of nitrogen gas. Finally, 50 µl of normal calf thymus DNA (0.1 mg/ml in pH 7.4 phosphate buffer) is cast on the positively charged surface and allowed to stand for 30 minutes before thorough washing with deionized water.
4.3. Results & discussion

4.3.1 Characterization of the DNA-modified electrode

The characterization of DNA-modified electrodes is carried electrochemically using square wave voltammetry and Tris(2,2′-bipyridyl)dichloro ruthenium(II) hexahydrate Ru(bpy)$_3^{2+}$ as an electroactive probe. Figure 1 shows the square wave voltammograms for bare electrode, PDDA-modified electrode, and the electrode with a second outer coat of calf thymus DNA immobilized in the presence of in Ru(bpy)$_3^{2+}$ complex as a redox probe in pH 5.5 acetate buffer. Ru(bpy)$_3^{2+}$ gives a distinguished oxidation peak at about 1.05 V vs. Ag/AgCl for the bare electrode. PDDA modification on the surface decreases the oxidation peak due to repulsion between the positively charged PDDA modified surface and the positively charged ruthenium complex. On the other hand, immobilization of the calf thymus DNA on top of the PDDA layer increases the oxidation peaks due to coupled catalytic oxidation of guanine bases using the freely diffusing ruthenium bipyridyl complex[111]. Scheme 4.2. illustrates the interaction between the guanine bases and the ruthenium redox probes.
In this work, we used two different types of layers: thin DNA LBL, and thick LBL. The thickness is proportional to the number of layers used. We found that the sensors with thick DNA layer immobilized on the electrode surface give, as expected, higher current in presence of Ru(bpy)$_3^{2+}$ as a redox probe as shown in Figure 4.1 and Figure 4.2.

![Figure 4.1. Square wave voltammograms for bare, PDDA-modified electrode, and electrodes modified with thin DNA films-using the LBL method in 50 μM Ru(bpy)$_3^{2+}$ complex in pH 5.5 acetate buffer.](image-url)
Figure 4.2. Square wave voltammograms for bare, PDDA-modified electrode and electrodes modified with thick DNA films using the LBL method in 50 μM Ru(bpy)$_3^{2+}$ complex in pH 5.5 acetate buffer.
Scheme 4.2. Guanine bases interaction with ruthenium
4.3.2. Application of the LBL method in peroxynitrite detection

We demonstrated earlier in section 5.3.1.2.1 that a ruthenium bipyridyl electro-active probe could be used to detect immobilized DNA via an increase in oxidative catalytic current. The catalytic current results from guanine base oxidation mediated by the freely diffusing ruthenium redox probe. DNA damage is known to unwind the helices and expose DNA bases, including guanines, making them more accessible to the electro-active ruthenium redox probe, which would increase observed catalytic current. This, in turn can be used as a signal of the extent of DNA damage and, thus, an indication of the concentration of damaging analyte. We take advantage of this difference in catalytic current to apply our new methodology to the determination of peroxynitrite based on exposure of the modified electrodes to peroxynitrite solutions.

Pyrolytic graphite electrodes with normal DNA on are characterized using Ru(bpy)$_3^{2+}$ in pH 5.5 acetate buffer. The same electrode is exposed to PON for a set amount of time, washed, and then tested under the same conditions. The overlaid square wave voltammograms in Figure 4.3 show the difference between the oxidation peaks of damaged DNA versus normal DNA before exposure to 50µM PON for 5 minutes, indicating that our LBL immobilization technique, in conjunction with the use of Ru(bpy)$_3^{2+}$ as an electro-active probe, is also capable of detecting the presence of peroxynitrite. This proof of principle shows that the LBL method and the differential response of adsorbed DNA on the surface to peroxynitrite can also developed into a sensitive platform for the quantification of peroxynitrite in solution. This method has the added advantage of the possibility to modulate the sensitivity of the sensing surface by
changing the number the layers of DNA included as well as using DNA with higher guanine base content.

Figure 4.3. Square wave voltammograms for DNA-modified electrode before and after exposure to 50 µM of PON for 5 minutes in 50 µM Ru(bpy)$_3^{2+}$ complex in pH 5.5 acetate buffer.
4.4. Differential Pulse Voltammetry (DPV) for peroxynitrite detection

Pulse techniques are designed to increase sensitivity by sampling the current and increasing the ratio of faradaic to capacitive current. Differential Pulse Voltammetry (DPV) has high sensitivity as it minimizes the effect of charging current on the faradaic process. Therefore, the electrode reaction can be analyzed more precisely, and small amounts of target analyte can be studied. Here, we apply the DPV on DNA-modified electrode in acetate buffer solution before and after exposure to peroxynitrite. Figure 4.5 shows the typical DPV response of DNA-modified electrode before and after treatment with peroxynitrite. The exposure to peroxynitrite gives rise to a prominent oxidation peak around +0.84 V vs. Ag/AgCl [112, 113]. This peak is very close to reported values for the oxidation of guanine bases in denatured or partially denatured DNA on carbon electrodes. Exposure to peroxynitrite partially denatures DNA on the electrode surface which exposes guanidine bases and facilitates oxidation resulting in the observed peak (Figure 4.5).
Figure 4.4. Differential pulse voltammogram in 0.1 M acetate buffer pH 4.5 obtained with DNA-modified electrodes before and after exposure to 50µM PON for 5 minutes.
This method is now being used to systematically measure the oxidation peak current as a function PON concentration and compare its performance to the other methods discussed earlier.

4.5. Conclusion

The LBL technique can be used as a simple and powerful method to immobilize calf thymus DNA (or other DNA sources) on electrodes surfaces for use as sensing platforms. DNA immobilized by LBL method has different electrochemical behavior before and after exposure to peroxynitrite. Therefore, DNA-modified electrodes can be used as sensors for peroxynitrite detection and quantification.
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