ELECTRICAL DETECTION OF DNA BINDING PROTEINS

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ABSTRACT

The base pair stack of double helical DNA has proven to be an effective medium for charge transport. The π-stacked DNA base pairs can mediate charge transport (CT) chemistry over distances as long as 20 nm, and the reaction is exquisitely sensitive to DNA sequence-dependent conformation and dynamics. This sensitivity to perturbations in DNA structure and base pair stacking makes DNA-mediated charge transport chemistry an ideal methodology for the electrical detection of base mismatches, lesions, and protein binding. Efforts toward expanding the scope of electrochemistry at DNA-modified surfaces for biosensing applications are presented here.

The first conclusive study of the single-molecule conductivity of DNA is demonstrated. A pristine single DNA duplex in its native conformation can be utilized to covalently bridge an oxidatively etched, single-walled carbon nanotube gap. The effective resistance is on the order of ~ 1 MΩ, which is similar to the c-axis resistance of kish graphite. Furthermore, the inclusion of either the CA or GT mismatches within the DNA duplex is seen to increase the resistance of DNA by over two orders of magnitude. This study underscores the fact that conductivity is an intrinsic property of each individual DNA molecule and not, in fact, an ensemble property of the DNA monolayer.

The characterization of DNA monolayers at high oriented pyrolytic graphite is described. Graphite electrodes are modified with duplex DNA containing a pendant pyrene; the yield of electrochemical reduction/oxidation to DNA-bound probes on graphite provides an indicator of base stacking within the DNA. The inclusion of even a single thermodynamically stable yet poorly stacked GT mismatch dramatically attenuates

CT through the monolayer. Graphite also affords a potential window which is not limited by the chemistry/stability of thiols on gold. This broad window facilitates the exploration of DNA monolayers containing either disulfides or a TEMPO moiety. In addition, graphite offers the opportunity of interrogating the electrochemistry of DNA-binding proteins containing iron sulfur clusters both in the presence and absence of DNA. Indeed, upon DNA binding, the redox potential of the Endo III, a base excision repair enzyme, shifts by –200 mV, and the redox potential of SoxR, a transcription factor, shifts by +500 mV. Such explorations underscore the importance of investigating DNA-binding enzymes in their biologically relevant conformations.

The electrochemistry of several phenoxazine- and anthraquinone-based probes of DNA structure is presented. Particular emphasis is given to a Nile Blue (NB) derivative which is connected to the base pair stack via a partially saturated linker and has a midpoint potential of ~ 0 mV versus NHE. NB can be attached to the DNA in a simple fashion and offers the dual advantages of excellent stability and electrocatalytic activity in the presence of ferricyanide. These features facilitate the extensive exploration of DNA-mediated charge transfer to NB at DNA-modified microelectrodes, with a scanning electrochemical microscope, and in a multiplexed format. The application of NB in the detection of transcription factor binding at DNA monolayers is also presented. Nanomolar concentrations of the ubiquitous TATA binding protein transcription factor can be rapidly and sensitively detected from complicated mixtures, including whole-cell lysates.

The efficient transport of charge through self-assembled DNA monolayers on both gold and graphite therefore offers an extremely sensitive tool for investigations of

DNA integrity. As such, novel methodologies for assaying protein/DNA interactions at DNA modified surfaces are now possible.

TABLE OF CONTENTS

Chapter 1: Introduction to DNA-Mediated Electrochemistry **1**

1.1 Introduction **2**

1.2 Characterization of DNA Monolayers **5**

1.2.1 Thiolated-DNA Monolayers on Gold

1.2.2 Pyrenated-DNA Monolayers on Graphite

1.3 Electrochemistry of Small Molecules Non-covalently Bound to DNA **10**

1.3.1 DNA Mediated Electrochemistry on Gold

1.3.2 DNA Mediated Electrochemistry on Graphite

1.4 DNA-Mediated Electrochemistry of Small Molecules Covalently Bound to DNA **17**

1.4.1 Electrochemistry of Daunomycin

1.4.2 Electrochemistry of Anthraquinone

1.4.3 Electrochemistry of Nile Blue and Redmond Red

1.5 Electrochemical Monitoring of Protein/DNA Interactions **22**

1.5.1 Detection of Base Flipping Enzymes

1.5.2 Detection of Transcription Factors that Distort DNA

1.5.3 Electrical Monitoring of Photolyase in Real Time

1.5.4 Electrochemistry of Repair Enzymes Containing Iron Sulfur Clusters

1.5.5 Electrochemistry of an Iron Sulfur Transcription Factor Bound to DNA

1.6 Conclusion **33**

References **35**

Chapter 2: Conductivity of a Single DNA Duplex Bridging a Carbon Nanotube Gap 41

Abstract 42

2.1 Introduction 43

2.2 Experimental Section 45

2.2.1 Fabrication of Cut Carbon Nanotube Devices

2.2.2 Synthesis of Amine-Modified DNA

2.2.3 Reconnection of Cut Carbon Nanotube Devices with DNA

2.2.4 Dehybridization/Rehybridization of DNA Bridging the SWNT Gap

2.2.5 Enzyme Restriction of DNA Bridging the SWNT Gap

2.3 Results and Discussion 49

References 66

Chapter 3: Electrochemistry Using Self-Assembled DNA Monolayers on Highly Oriented Pyrolytic Graphite 69

Abstract 70

3.1 Introduction 71

3.2 Experimental Section 73

3.2.1 Materials

3.2.2 Synthesis of DNA and Redox-Active Probes

3.2.3 Preparation of DNA-Modified Electrodes

3.2.4 Electrochemical Experiments

3.2.5 Atomic Force Microscopy Experiments

3.2.6 Radioactive Labeling and Quantification

3.3 Results and Discussion 76

3.3.1 Surface Characterization

3.3.2 Electrochemistry at a DNA-Modified HOPG Surface

3.3.3 Electrochemistry on HOPG Is DNA-Mediated

3.3.4 DNA Electrochemistry with Various Intercalators

3.4 Implications 91

References 92

Chapter 4: DNA-Mediated Electrochemistry of Disulfides on Graphite **96**

Abstract **97**

4.1 Introduction **98**

4.2 Experimental Section **99**

4.2.1 Materials

4.2.2 General Oligonucleotide Synthesis

4.2.3 Synthesis of 5'-Pyrene-Modified Oligonucleotides

4.2.4 Synthesis of 5'-Thiol-Modified Oligonucleotides

4.2.5 Synthesis of 3'-Thiol-Modified and 5'-Pyrene-Modified Oligonucleotides

4.2.6 Preparation of Duplex DNA

4.2.7 Melting Temperatures

4.2.8 Preparation of DNA-Modified Electrodes

4.2.9 Electrochemical Experiments

4.2.10 X-Ray Photoelectron Spectroscopy Experiments

4.3 Results and Discussion **102**

4.3.1 Surface Characterization by X-Ray Photoelectron Spectroscopy

4.3.2 Thermal Stability of Thiol Modified Duplexes

4.3.3 Electrochemistry of Disulfides Incorporated in the Phosphate Backbone

4.4 Implications **112**

References **113**

Chapter 5: Coupling into the Base Pair Stack is Necessary for DNA-Mediated Electrochemistry **117**

Abstract **118**

5.1 Introduction **119**

5.2 Experimental Section **122**

5.2.1 Materials

5.2.2 Oligonucleotide Synthesis

5.2.3 Synthesis of Pyrene-Modified Oligonucleotides

5.2.4 Synthesis of Thiol-Modified Oligonucleotides

5.2.5 Synthesis of TEMPO-Modified Oligonucleotides

5.2.6 Synthesis of Anthraquinone-Modified Oligonucleotides

5.2.7 Formation of DNA Monolayers

5.2.8 Electrochemical Measurements

5.2.9 Surface Characterization

5.3 Results **128**

5.3.1 Electrochemical Probes and DNA Sequences

5.3.2 Electrochemistry of the Acetylene-Linked Probes

5.3.3 Electrochemistry of the Poorly Coupled Probes

5.3.4 Surface Characterization

5.4 Discussion **139**

5.4.1 Electrochemical Reduction of the Acetylene-Linked Probes Is Mediated by the DNA Duplex

5.4.2 Electrochemical Reduction of Alkane-Linked Probes Do Not Depend on DNA-Mediated Electrochemistry

5.4.3 Electronic Coupling into the Base Pair Stack Governs the Size of the Electrochemical Signal

5.4.4 DNA Charge Transfer with Acetylene and Alkane-Linked Probes

5.5 Implications **145**

References **147**

Chapter 6: Direct Electrochemistry of Endonuclease III in the Presence and Absence of DNA **152**

Abstract **153**

6.1 Introduction **154**

6.2 Experimental Section **156**

6.2.1 Materials

6.2.2 General DNA Synthesis and Preparation

6.2.3 Expression and Purification of Endonuclease III

6.2.4 Preparation of Bare and DNA-Modified HOPG Electrodes

6.2.5 Preparation of Didodecyldimethylammonium-Modified Pyrolytic Graphite Electrodes

6.2.6 Electrochemical Measurements on HOPG

6.2.7 Electrochemical Measurements on Pyrolytic Graphite

6.3 Results **161**

6.3.1 Electrochemistry of Free and DN- Bound Endo III on HOPG

6.3.2 Electrochemistry of Free and DN- Bound Endo III on Pyrolytic Graphite

6.4 Implications and Discussion **171**

References **174**

Chapter 7: DNA Binding Shifts the Redox Potential of the Transcription Factor SoxR 179

Abstract 180

7.1 Introduction 181

7.2 Experimental Procedures 185

7.2.1 Materials

7.2.2 Oligonucleotide Synthesis

7.2.3 Expression and Purification of SoxR

7.2.4 Formation of DNA Monolayers and Electrochemical Measurements

7.3 Results 188

7.3.1 Experimental Strategy Used to Probe SoxR Electrochemically

7.3.2 SoxR Binding Is Reported Through the Redmond Red Electrochemical Signal

7.3.3 Electrochemistry of P. aeruginosa SoxR

7.3.4 Comparison of the Voltammetry of E. coli and P. aeruginosa SoxR

7.3.5 Electrochemistry of the P. Aeruginosa S95L SoxR Mutant

7.3.6 Comparison of the Voltammetry of SoxR to that of Free Iron

7.4 Discussion and Implications 199

References 205

Chapter 8: Scanning Electrochemical Microscopy of DNA Monolayers Modified with Nile Blue **210**

Abstract **211**

8.1 Introduction **212**

8.2 Experimental **214**

8.2.1 Materials

8.2.2 Synthesis of Thiol and Nile Blue-Modified DNA

8.2.3 Preparation of Backfilled DNA/11-mercaptoundecylphosphoric Acid Monolayers

8.2.4 Electrochemistry/Scanning Electrochemical Microscopy Experiments

8.2.5 TATA Binding Protein Experiments

8.3 Results **220**

8.3.1 Electrochemistry of Backfilled DNA Monolayers Modified with Nile Blue

8.3.2 SECM of DNA Monolayers Modified with Nile Blue in Feedback Mode

8.3.3 SECM Imaging of DNA Monolayer Morphology

8.3.4 Detection of TATA Binding Protein by SECM

8.4 Discussion **238**

References **241**

Chapter 9: Electrical Detection of TATA-Binding Protein at DNA-Modified Microelectrodes 246

Abstract 247

9.1 Introduction 248

9.2 Experimental 250

9.2.1 Materials

9.2.2 Synthesis of Thiol and Nile Blue-Modified DNA

9.2.3 Protein Preparation and Storage

9.2.4 Preparation of Backfilled DNA Monolayers

9.2.5 Electrochemistry and Protein Detection Experiments

9.3 Results 254

9.4 Implications 265

References 266

Chapter 10: Electrochemical Detection of TATA-Binding Protein from a Cell Lysate 270

Abstract 271

10.1 Introduction 272

10.2 Experimental 275

10.2.1 Materials

10.2.2 Synthesis of Thiol and Nile Blue-Modified DNA

10.2.3 Preparation of Epithelial Cell Lysates

10.2.4 Gel Electrophoresis and Immunoblot Analysis

10.2.5 Manufacture of Gold Electrodes

10.2.6 Preparation of Backfilled DNA Monolayers

10.2.7 Electrochemical Experiments

10.3 Results 281

10.3.1 Experimental Strategy for the Detection of TATA Binding Protein

10.3.2 Nile Blue as a Redox Probe for the Detection of Protein Binding

10.3.3 10.3.3 Self Assembly of Nile Blue-DNA Monolayers

10.3.4 Detection of Human TATA Binding Protein from Cell Lysate Solutions on a Single Substrate

10.3.5 Detection of Murine TATA Binding Protein from Whole Cell Lysates

10.4 Implications and Conclusion 292

References 294

LIST OF TABLES AND FIGURES

Chapter 1: Introduction to DNA-Mediated Electrochemistry **1**

Figure 1.1: Schematic illustration of DNA duplexes in a close-packed monolayer **4**

Figure 1.2: Schematic illustration of the modulation of the height of a DNA monolayer 9

Figure 1.3: Chemical structures of redox-active probes **12**

Figure 1.4: Schematic illustration of the reduction of methylene blue **13**

Figure 1.5: Schematic illustration of the methylene blue/ferricyanide electrocatalytic cycle at a DNA monolayer **15**

Figure 1.6: A schematic illustration of DNA duplexes containing covalently cross-linked daunomycin **18**

Figure 1.7: Illustration of the fabrication of DNA monolayers on gold and the subsequent electrochemical analysis of protein binding **24**

Figure 1.8: Schematic illustration of the electrochemical monitoring of the repair of thymine dimers in a DNA monolayer by photolyase **30**

Chapter 2: Conductivity of a Single DNA Duplex Bridging a Carbon Nanotube Gap **41**

Figure 2.1: A method to cut and functionalize individual SWNTs with DNA strands **46**

Figure 2.2: Device characteristics for individual SWNTs connected with DNA. **50**

Table 2.1: Summary of DNA resistance values **52**

Figure 2.3: Device characteristics for individual SWNTs reconnected with well matched DNA **53**

Figure 2.4: Device characteristics for individual SWNTs reconnected CA mismatched DNA **54**

Figure 2.5: Mismatches have a large effect on DNA conductance **56**

Figure 2.6: Effect of CA mismatches on DNA conductance **57**

Figure 2.7: Effect of GT mismatches on DNA conductance **58**

Figure 2.8: The electrical characteristics of a device rejoined with single-stranded DNA **60**

Figure 2.9: Control experiments using partially cut devices **61**

Figure 2.10: Enzymes can be used to cleave the DNA between the ends of the SWNTs **63**

Figure 2.11: Restriction by enzymes is sequence specific **64**

Chapter 3: Electrochemistry Using Self-Assembled DNA Monolayers on Highly Oriented Pyrolytic Graphite **69**

Figure 3.1: Schematic illustration of DNA duplexes modified with a pyrene moiety assembled on an HOPG surface **78**

Figure 3.2: AFM characterization of DNA-modified HOPG **79**

Figure 3.3: Cyclic voltammetry of methylene blue **81**

Figure 3.4: Cyclic voltammetry of methylene blue at DNA monolayers with and without a mismatch **84**

Figure 3.5: Cyclic voltammetry of methylene blue and ferricyanide at DNA monolayers with and without a mismatch **86**

Table 3.1: Summary of mismatch detection with various linkers on DNA-modified HOPG electrodes **87**

Figure 3.6: Cyclic voltammetry of metallointercalators on bare and DNA-modified HOPG **89**

Figure 3.7: Cyclic voltammetry of metallointercalators in the presence and absence of mismatches **90**

Chapter 4: DNA-Mediated Electrochemistry of Disulfides on Graphite 96

Figure 4.1: Schematic illustration of charge transfer to thiols incorporated into the DNA backbone **103**

Figure 4.2: XPS spectra of HOPG modified with pyrenated DNA **105**

Figure 4.3: Melting temperatures of DNA duplexes containing thiols **106**

Figure 4.4: Square wave voltammetry at various DNA monolayers modified with disulfides **108**

Figure 4.5: Cyclic voltammetry of an electrode modified with duplex DNA featuring a disulfide at various scan rates **109**

Figure 4.6: Cyclic voltammetry of well-matched DNA featuring a disulfide under various buffer conditions **111**

Chapter 5: Coupling into the Base Pair Stack is Necessary for DNA-Mediated Electrochemistry 117

Scheme 5.1: Conditions for synthesis of 2-Iodoanthraquinone and its coupling to ethynyluracil-modified DNA **126**

Figure 5.1: Sequences and probes used during the course of these studies **131**

Figure 5.2: Square wave voltammetry of AQ and TEMPO-modified DNA **132**

Figure 5.3: Cyclic voltammetry of AQ-modified DNA at various scan rates **133**

Figure 5.4: Square wave voltammetry of well-matched DNA modified with an acetylene-TEMPO label on HOPG **135**

Table 5.1: Electrochemical characteristics of DNA monolayers modified with electroactive moieties **136**

Figure 5.5: Tapping mode AFM characterization of DNA-modified and bare Graphite **138**

Figure 5.6: Schematic representation of two possible modes of charge transfer from an electroactive label incorporated into DNA **143**

Chapter 6: Direct Electrochemistry of Endonuclease III in the Presence and Absence of DNA **152**

Figure 6.1: Schematic representation of electrochemistry for Endo III on HOPG with and without modification with DNA **157**

Figure 6.2: Cyclic and square wave voltammetry of Endo III in the presence and absence of DNA **162**

Figure 6.3: Square wave voltammetry of Endo III on bare HOPG with and without backfilling **163**

Figure 6.4: Cyclic voltammetry on DNA-modified HOPG with and without Endonuclease III **165**

Figure 6.5: Cyclic voltammetry of higher Endo III concentrations in the absence of DNA **166**

Figure 6.6: Cyclic voltammetry of free iron on bare HOPG in the presence and absence of thiols **168**

Figure 6.7: Cyclic voltammetry of Endo III on bare HOPG at slow scan rates **169**

Figure 6.8: Cyclic voltammetry of free and DNA-bound Endo III on didodecyldimethylammonium-modified graphite **170**

Figure 6.9: Illustration of the potentials versus NHE of the couples of Endo III in the presence and absence of DNA **172**

Chapter 7: DNA Binding Shifts the Redox Potential of the Transcription Factor SoxR **179**

Figure 7.1: Schematic illustration of the self-assembly/backfilling of a DNA monolayer followed by incubation with protein **189**

Figure 7.2: Cyclic voltammetry of SoxR at electrodes modified with DNA **192**

Figure 7.3: Binding of SoxR to the DNA-modified film **194**

Figure 7.4: Square wave voltammetry of* P. aeruginosa *and* E. coli *SoxR at DNA-modified graphite electrodes **196**

Figure 7.5: Cyclic voltammetry of a SoxR mutant at an electrode modified with DNA **198**

Figure 7.6: Cyclic voltammetry of free iron in SoxR storage buffer on bare and DNA-modified HOPG **200**

Figure 7.7: Redox potentials of free and DNA-bound SoxR along with those of cellular oxidants/reductants **202**

Chapter 8: Scanning Electrochemical Microscopy of DNA Monolayers Modified with Nile Blue 210

Figure 8.1: Schematic illustration of the coupling of Nile Blue to the NHS-Carboxy-dT-modified DNA on solid support **216**

Figure 8.2: Schematic illustration of SECM imaging of DNA monolayers modified with Nile Blue **221**

Figure 8.3: Cyclic voltammetry of Nile Blue-DNA monolayers **222**

Figure 8.4: Cyclic voltammetry of DNA monolayers modified with Nile Blue in the presence of ferricyanide **223**

Figure 8.5: Successive cyclic voltammograms of ferricyanide at a bare Au electrode in the presence of mercaptoundecylphosphoric acid **224**

Figure 8.6: SECM approach curves obtained for DNA monolayers modified with Nile Blue **226**

Figure 8.7: SECM approach curves at DNA monolayers in the presence and absence of Methylene Blue **229**

Figure 8.8: SECM images obtained for Nile Blue-DNA monolayers **232**

Figure 8.9: Schematic illustration of the SECM-based, sequence-specific detection of TBP binding at DNA monolayers **234**

Figure 8.10: SECM approach curves taken for Nile Blue-DNA monolayers with and without TBP **235**

Figure 8.11: Scanning electrochemical microscopy images of a Nile Blue-DNA monolayer in the presence and absence of various proteins **236**

Chapter 9: Electrical Detection of TATA-Binding Protein at DNA Modified Microelectrodes 246

Figure 9.1: Schematic illustration of the addition of TBP to a DNA monolayer modified with Nile Blue **255**

Figure 9.2: Cyclic and square wave voltammetry at macroelectrodes and microelectrodes before and after addition of TBP **256**

Figure 9.3: Cyclic voltammetry of a Nile Blue-DNA modified microelectrode of a 25 µm diameter for two different buffers **257**

Figure 9.4: The effect of oxygen on the voltammetry at a DNA modified Microelectrode **259**

Figure 9.5: Square wave voltammetry of DNA-modified macroelectrodes and microelectrodes prepared on a single substrate **260**

Figure 9.6: Cyclic voltammetry of a Nile Blue-DNA modified microelectrodes showing reversible binding of TBP **262**

Figure 9.7: Detection of TBP binding at microelectrodes using various concentrations of TBP **263**

Figure 9.8: Detection of TBP at microelectrodes in the presence of various other proteins **264**

Chapter 10: Electrochemical Detection of TATA-Binding Protein from a Cell Lysate **270**

Figure 10.1: Illustration of the self-assembly of DNA monolayers on gold and the subsequent electrochemical analysis of protein binding **280**

Figure 10.2: Cyclic and square wave voltammetry of a Nile Blue-DNA monolayer **282**

Figure 10.3: An illustration of the distribution of integrated surface charge found for NB-DNA monolayers on gold electrodes **284**

Figure 10.4: Square wave voltammetry of Nile Blue-DNA monolayers in the presence and absence of cell lysate/TBP **286**

Figure 10.5: A schematic illustration of the growth and lysis of murine epithelial cells **288**

Figure 10.6: A representation of the signal loss found at Nile Blue-DNA modified surfaces after addition of epithelial cell lysate under various conditions **289**

Figure 10.7: Visualization of TBP levels in cell lysates via western blotting Analysis **291**