Published in final edited form as:
Bioconjug Chem. 2008 November 19; 19(11): 2110–2112. doi:10.1021/bc800339y.

Redmond Red as a Redox Probe for the DNA-mediated Detection of Abasic Sites

Marisa C. Buzzeo and **Jacqueline K. Barton***
Division of Chemistry and Chemical Engineering California Institute of Technology, Pasadena, California 91125

Abstract

Redmond Red, a fluoropore containing a redox active phenoxazine core, has been explored as a new electrochemical probe for the detection of abasic sites in double stranded DNA. The electrochemical behavior of Redmond Red-modified DNA at gold surfaces exhibits stable, quasi-reversible voltammetry with a midpoint potential centered around -50 mV versus NHE. Importantly, with Redmond Red positioned opposite an abasic site within the DNA duplex, the electrochemical response is significantly enhanced compared to Redmond Red positioned across from a base. Redmond Red, reporting only if well stacked in the duplex, represents a sensitive probe to detect abasic sites electrochemically in a DNA-mediated reaction.

Since its discovery, the ability of DNA to conduct charge has been exploited in a variety of arenas, including time resolved spectroscopy (1-3), conductivity measurements (4-7), and the development of biosensors (8). Our group, in particular, has explored the versatility of this intrinsic property of DNA by performing extensive electrochemical studies on surfaces modified with DNA duplexes (9-15). Typically in such systems, thiol-modified oligonucleotides are tethered to a gold electrode at the 5′ terminus by a Au-S bond and further modified at the opposite end with a redox probe, thus allowing the duplexes to act as an extension of the conducting medium. The reduction of the attached probe, as observed voltammetrically, serves as an indicator of the transport of charge via the DNA duplex. Importantly, it has been shown that DNA-mediated charge transport is remarkably sensitive to the integrity of the base stack; upon introduction of a single base mismatch located between the electrode and the redox probe, measured currents are dramatically diminished (9-10). This behavior not only provides conclusive evidence that the observed electrochemical reactions are indeed DNA-mediated but also allows naturally occurring perturbations, which may only subtly interrupt π-stack overlap, to be detected (11-15).

Among these possibly destabilizing defects are abasic sites, which appear naturally and frequently among DNA sequences as the result of hydrolytic cleavage of the glycosidic bond (16-23).Specific enzymes are responsible for correcting these damaged sites prior to transcription and replication. When this repair pathway is delinquent, however, the persistence of these defects can impart deleterious effects on genetic encoding, leading to the development of cancer, among other diseases (24-26). Because they represent a potential threat, the reliable and specific detection of abasic sites is critical, although still very much under exploration. Development of an electrochemical system to detect these unwanted sites specifically could

E-mail: jkbarton@caltech.edu
*Author to whom correspondence should be addressed

Supporting Information Available: Synthetic details, electrochemical experimental conditions, and characterization data (HPLC, MS, UV-vis, T_m) of modified DNA. This material is available free of charge via the Internet at http://pubs.acs.org/BC.

contribute to the understanding of their occurrence and repair. Here, we employ Redmond Red (RR), obtained as a commercially available phosphoramidite and used commonly as a fluorophore, as a new redox probe for the detection of abasic sites. We find that the width of the covalently tethered phenoxazine derivative is particularly well accommodated when positioned opposite an abasic site within a DNA duplex, thus facilitating efficient DNA-mediated reduction as a "turn on" probe for their presence (Figure 1).

Alkane thiol-modified DNA strands were synthesized as described previously (14,15) with Redmond Red internal to the sequence and hybridized to a complement containing an abasic site in the opposite position. All syntheses were performed using standard phosphoramidite chemistry. Redmond Red-modified strands were cleaved from the solid support resin using 0.05 M K_2CO_3 in MeOH at ambient temperature for 12 – 17 h. Complementary strands were cleaved using concentrated NH_4OH at 60 °C for 8 – 12 h. Samples were purified twice by reversed-phase HPLC and characterized by MALDI -TOF mass spectrometry and UV-visible spectroscopy (see Supporting Information). For those oligonucleotides containing an abasic site, the commercially available tetrahydrofuranyl analogue was used (27); the naturally occurring, hemiacetal abasic residue is unstable and reactive on the time scale of DNA synthesis.

Freshly etched gold macroelectrodes were incubated with 50 μM modified duplex DNA overnight at 5 °C in the presence of 100 mM $MgCl_2$ to encourage formation of densely packed films. Electrochemical measurements were performed at ambient temperature in phosphate buffer (5 mM sodium phosphate, 50 mM NaCl, pH 7) using a CH Instruments 760B potentiostat. A saturated calomel electrode served as a reference electrode, and a platinum wire as a counter. Square wave voltammograms were recorded at a frequency of 15 Hz and an amplitude of 0.025 V. Under such conditions, Redmond Red-modified DNA (^{RR}DNA) displays stable, quasi-reversible voltammetry with a midpoint potential centered around -50 mV versus NHE. The measured peak currents scale linearly with scan rate, indicating that RR behaves as a surface-bound species, as anticipated, and is not under diffusional control.

To test if the observed voltammetric response is in fact dependent on the presence of an abasic site, the same thiolated ^{RR}DNA was then hybridized to complementary sequences containing a guanine (G) or thymine (T) opposite the probe, and the resulting voltammetry recorded (see Figure 2 for sequences). The observed reductive signal is severely affected. Figure 3 shows a comparison of typical square wave voltammograms obtained for both assemblies, and Figure 4 presents a comparison of the average peak areas measured for the different sequences. More than a two-fold increase is consistently observed when RR is situated opposite an abasic site versus an intact DNA strand. It therefore becomes apparent that inclusion of an opposing base precludes well stacked insertion of RR into the duplex, thus preventing efficient reduction through the π-stack. Conversely, when an abasic residue is located on the complementary strand, the phenoxazine derivative, itself having a similar dimension to a base pair, is now able to insert into the duplex and become available for DNA-mediated reduction. The enhancement of signal seen for the well stacked Redmond Red is consistent with earlier results that have shown the redox signal to reflect how well the probe is coupled into the base stack (14,28). This discrimination of abasic sites indicates that the DNA-mediated reduction of RR can provide a convenient means by which to detect the abasic defect.

We also investigated whether or not RR would respond voltammetrically to a base deletion. A complementary strand was synthesized in which the position opposite the RR was occupied not by an abasic site, but instead by the base that corresponds to the next position on the RR strand; in short, no complementary base was assigned to RR (Figure 2). Under these conditions, we considered that the RR may be pinched out of the sequence, allowing the bases above the probe to fully hybridize and thus inhibiting reduction of the phenoxazine moiety. For these

experiments, the length of the oligonucleotide was extended by six bases in order to prevent fraying above the probe site while keeping the position of Redmond Red relative to the electrode surface constant. As observed by both cyclic and square wave voltammetry, duplexes containing the deleted site do indeed consistently yield diminished currents, and the magnitude of the effect (300 % increase for an abasic site) is in line with those results observed when a G or T is situated opposite RR (Figure 4). This indicates that the probe is, in fact, excluded from the base stack in order to allow for the remaining bases to hybridize and is therefore not available for reduction in a DNA-mediated fashion.

Early work first demonstrated the sensitivity of electrochemical techniques to the presence of abasic sites resulting from depurination in chromosomal DNA: differential poloragraphic responses were observed for non-denatured DNA versus the apurinic form (29-31). More recently, studies have reported the detection of these damaged sites specifically in synthetic oligonucleotides using chronopotentiometric and square wave voltammetric techniques (32-35). These systems, however, possess intrinsic limitations that prevent them from serving as practical diagnostics. In the first example, the target DNA must be consumed via acid-induced oligonucleotide digestion (32), while the other detection schemes are specific to thymine-related mutations (33-35). Here, the redox probe is covalently attached to the DNA, the target oligonucleotide remains intact, and the electrochemical measurement is fast, simple, and selective. Moreover, electrocatalytic coupling may provide enhanced signal discrimination, thus affording the increased sensitivity needed on the genomic scale (11,12).

The studies presented here once again highlight how sensitive DNA-mediated charge transport chemistry can be in reporting on DNA structure. Earlier we used DNA-mediated electrochemistry to characterize perturbations in the *intervening* DNA structures using a well stacked probe, but here how well the probe is itself stacked serves as a specific reporter of local conformation. The selective enhancement found with RR opposing an abasic residue confirms that to detect efficient reduction of the probe at a distance from the electrode, the reduction must be DNA-mediated. The dependence of probe detection on the spatial constraints of the duplex underscores the general sensitivity of DNA-mediated charge transport chemistry to local DNA conformation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are grateful to the National Institutes of Health (Grant GM 61077) for their financial support.

REFERENCES

(1). Wan C, Fiebig T, Kelley SO, Treadway CR, Barton JK, Zewail AH. Femtosecond Dynamics of DNA-Mediated Electron Transfer. Proc. Natl. Acad. Sci. U.S.A 1999;102:6014–6019. [PubMed: 10339533]

(2). Takada T, Kawai K, Cai X, Sigimoto A, Fujitsuka M, Majima T. Charge Separation in DNA via Consecutive Adenine Hopping. J. Am. Chem. Soc 2004;126:1125–1129. [PubMed: 14746481]

(3). Lewis FD, Zhu H, Daublain P, Cohen B, Wasielewski MR. Hole Mobility in DNA A Tracts. Angew. Chem., Int. Ed 2006;45:7982–7985.

(4). Hihath J, Xu B, Zhang P, Tao N. Study of Single-Nucleotide Polymorphisms by Means of Electrical Conductance Measurements. Proc. Natl. Acad. Sci. U.S.A 2005;102:16979–16983. [PubMed: 16284253]

(5). van Zalinge H, Schiffrin DJ, Bates AD, Starikov EB, Wenzel W, Nichols RJ. Variable-temperature Measurements of the Single-Molecule Conductance of Double Stranded DNA. Angew Chem. Ed. Int 2006;45:5499–5502.

(6). Wierzbinski E, Amdt J, Hammond W, Slowinksi K. In situ Electrochemical Distance Tunneling Spectroscopy of ds-DNA molecules. Langmuir 2006;22:2426–2429. [PubMed: 16519433]

(7). Guo X, Gorodetsky AA, Hone J, Barton JK, Nuckolls C. Conductivity of a Single DNA Duplex Bridging a Carbon Nanotube Gap. Nat. Nanotechnol 2008;3:163–167. [PubMed: 18654489]

(8). Drummond TG, Hill MG, Barton JK. Electrochemical DNA Sensors. Nat. Biotechnol 2003;21:1192–1199.1199 [PubMed: 14520405], and references therein.

(9). Kelley SO, Jackson NM, Hill MG, Barton JK. Long Range Electron Transfer through DNA Films. Angew. Chem., Int Ed 1999;38:941–945.

(10). Kelley SO, Boon EM, Jackson NM, Hill MG, Barton JK. Single-Base Mismatch Detection Based on Charge Transduction Through DNA. Nucleic Acids Res 1999;27:4830–4837. [PubMed: 10572185]

(11). Boon EM, Ceres DM, Drummond TG, Hill MG, Barton JK. Mutation Detection by Electrocatalysis at DNA-Modified Electrodes. Nature Biotechnol 2000;18:1096–1100. [PubMed: 11017050]

(12). Boal AK, Barton JK. Electrochemical Detection of Lesions in DNA. Bioconjugate Chem 2005;16:312–321.

(13). Gorodetsky AA, Dietrich LEP, Lee PE, Demple B, Newman DK, Barton JK. DNA binding shifts the redox potential of the transcription factor SoxR. Proc. Natl. Acad. Sci. U.S.A 2008;105:3684–3689. [PubMed: 18316718]

(14). Gorodetsky AA, Green O, Yavin E, Barton JK. Coupling into the Base Pair Stack is Necessary for DNA-mediated Electrochemistry. Bioconjugate Chem 2007;18:1434–1441.

(15). Gorodetsky AA, Ebrahim A, Barton JK. Electrical Detection of TATA Binding Protein at DNA-Modified Microelectrodes. J. Am. Chem. Soc 2008;130:2924–2925. [PubMed: 18271589]

(16). Vesnaver G, Chang C-N, Eisenberg M, Grollman AP, Breslauer KJ. Influence of Abasic and Anucleosidic Sites on the Stability, Conformation, and Melting Behavior of a DNA Duplex: Correlations of Thermodynamic and Structural Data. Proc. Natl. Acad. Sci. U.S.A 1989;86:3614–3618. [PubMed: 2726738]

(17). Lindahl T. Instability and Decay of the Primary Structure of DNA. Nature 1993;362:709–715. [PubMed: 8469282]

(18). Krokan HE, Standal R, Slupphaug G. DNA Glycosylases in the Base Excision Repair of DNA. Biochem. J 1997;325:1–16. [PubMed: 9224623]

(19). Gelfand CA, Plum GE, Grollman AP, Johnson F, Breslauer KJ. Thermodynamic Consequences of an Abasic Lesion in Duplex DNA are Strongly Dependent on Base Sequence. Biochemistry 1998;37:7321–7327. [PubMed: 9585546]

(20). Lhomme J, Constant J-F, Demeunynck M. Abasic DNA Structure, Reactivity, and Recognition. Biopolymers 1999;52:65–83. [PubMed: 10898853]

(21). Kuzminov A. Single-strand Interruptions in Replicating Chromosomes Cause Double-Strand Breaks. Proc. Natl. Acad. Sci. U.S.A 2001;98:8241–8246. [PubMed: 11459959]

(22). Schärer OD, Jiricny J. Recent Progress in the Biology, Chemistry, and Structural Biology of DNA Glycosylases. BioEssays 2001;23:270. [PubMed: 11223884]

(23). Yu S-L, Lee S-K, Johnson RE, Prakash L, Prakash S. The Stalling of Transcription at Abasic Sites is Highly Mutagenic. Mol. Cell Biol 2003;23:382–388. [PubMed: 12482989]

(24). Loeb LA. Apurinic Sites as Mutagenic Intermediates. Cell 1985;40:483–484. [PubMed: 2982494]

(25). Guillet M, Boiteux S. Origin of Endogenous DNA Abasic Sites in *Saccharomyces cerevisiae.* Mol. Cell. Biol 2003;23:8386–8394. [PubMed: 14585995]

(26). Boiteux S, Guillet M. Abasic sites in DNA: Repair and Biological Consequences in *Saccharomyces cerevisiae*. DNA Repair 2004;3:1–12. [PubMed: 14697754]

(27). Lin Z, Hung KN, Grollman AP, de los Santos C. Solution Structure of Duplex DNA Containing an Extrahelical Abasic Site Analog determined by NMR Spectroscopy and Molecular Dynamics. Nucleic Acids Res 1998;26:2385–2391. [PubMed: 9580690]

(28). Boon EM, Jackson NM, Wightman MD, Kelley SO, Hill MG, Barton JK. Intercalative Stacking: A Critical Feature of DNA Charge-Transport Electrochemistry. J. Phys. Chem. B 2003;107:11805–11812.

(29). Paleček E. Oscillographic Polarography of Highly Polymerized Deoxyribonucleic Acid. Nature 1960;188:656–657. [PubMed: 13732209]

(30). Olińkski R, Walter Z, Wiaderkiewicz R, Lukášová E, Paleček E. Changes in DNA Properties Due to Treatment with the Pesticides Malathion and DDVP. Radiat. Environ. Biophys 1980;18:65–72. [PubMed: 7443982]

(31). Jelen F, Fojta M, Paleček E. Voltammetry of Native Double-Stranded, Denatured, and Degraded DNAs. J. Electroanal. Chem 1997;427:49–56.

(32). Dolinnaya NG, Jan MR, Kawde A-N, Oretskaya TS, Tashlitsky VN, Wang J. Electrochemical Detection of Abasic Site-Containing DNA. Electroanalysis 2006;18:399–404.

(33). Morita K, Sankaran NB, Huang W, Seino T, Sato Y. Electrochemical SNPs Detection Using an Abasic Site-Containing DNA on a Gold Electrode. Chem. Commun 2006:2376–2378.

(34). Morita K, Nishizawa S, Teramae N. Use of an Abasic Site-Containing DNA for Electrochemical SNP Detection. Nucleic Acids Symp. Ser 2006;50:91–92.

(35). Huang W, Morita K, Sankaran NB, Nishizawa S, Teramae N. Electrochemical Detection at Low Temperature for a Specific Nucleobase of Target Nucleic Acids by an Abasic Site-Containing DNA Binding Ligand. Electrochem. Comm 2006;8:395–398.

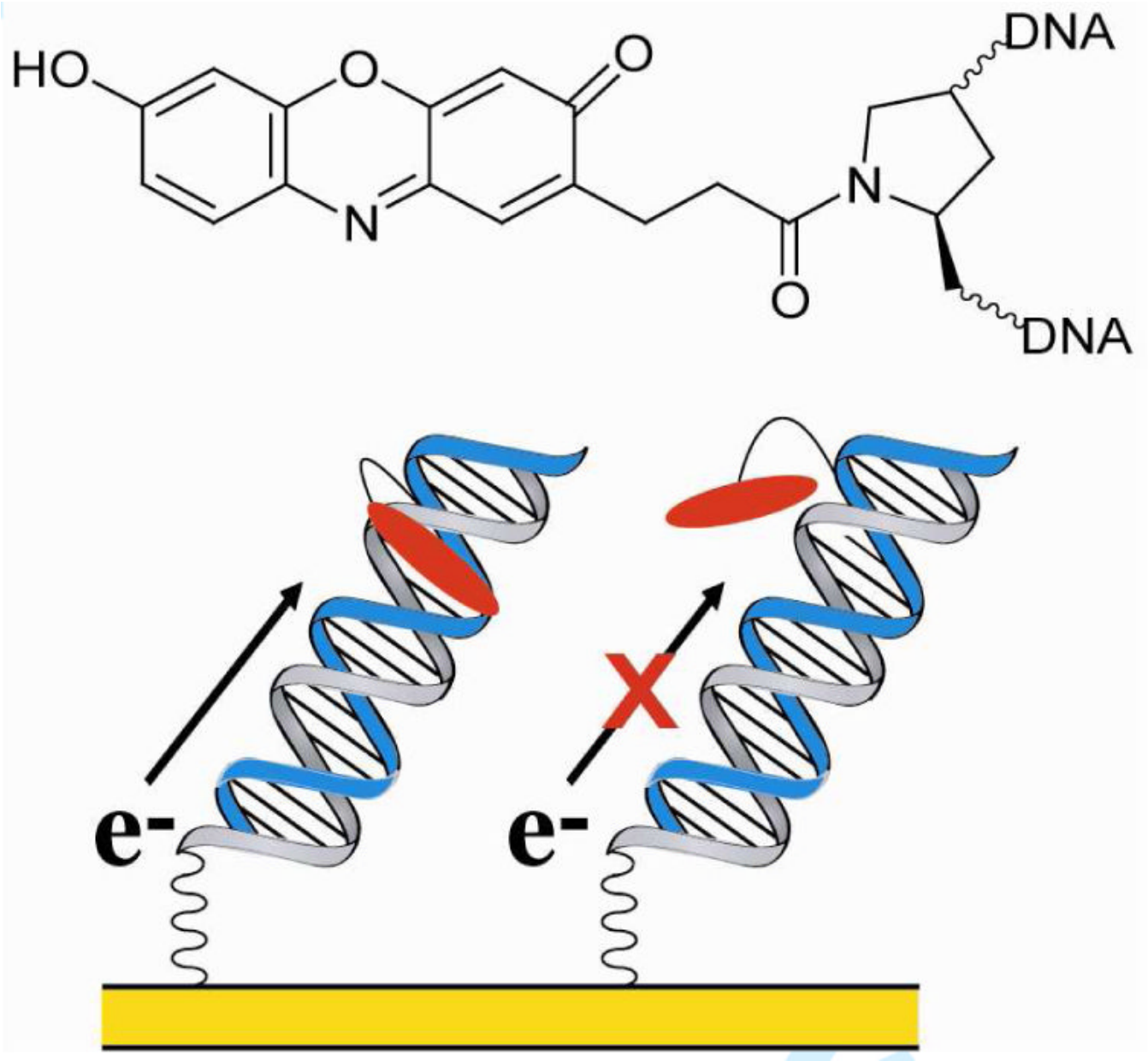

Figure 1.
Structure of Redmond Red (RR), an electroactive phenoxazine derivative covalently tethered to DNA, along with a schematic representation of the basis of the abasic site probe. With Redmond Red placed opposite an abasic residue (left), DNA-mediated reduction can proceed, but if opposite a base (right), RR is excluded from the duplex and DNA-mediated reduction cannot proceed. Note that the three-ring phenoxazine core is comparable in size to a base pair.

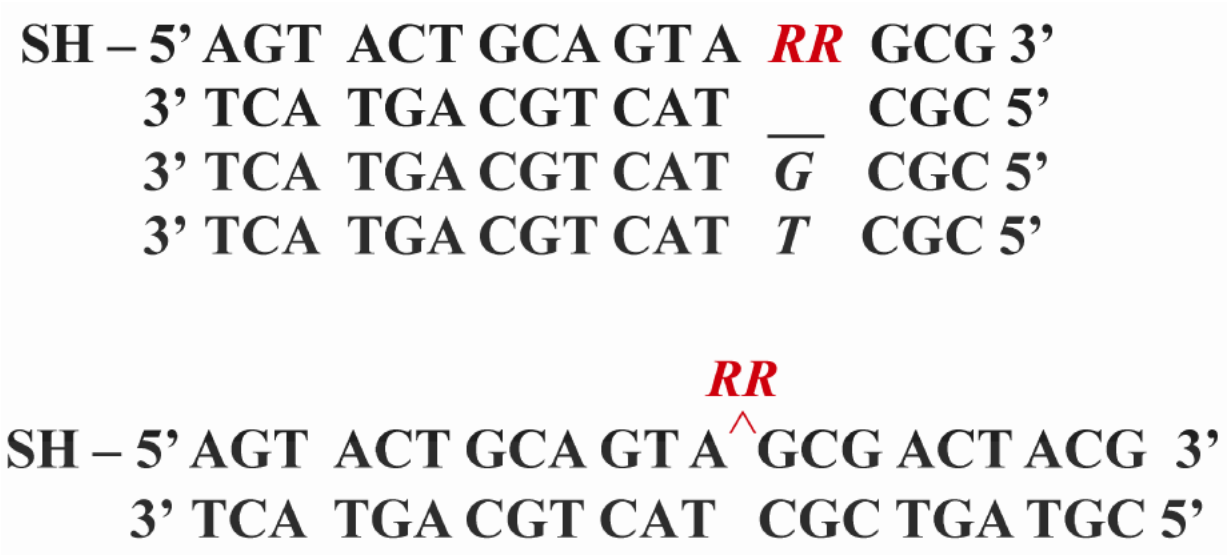

Figure 2.

Sequences employed to determine the sensitivity of the DNA-mediated reduction of Redmond Red to the presence of the opposing base. Shown are the thiol-modified strands, along with the variable complementary strands, where "*RR*" indicates a Redmond Red moiety, and "__" an abasic site.

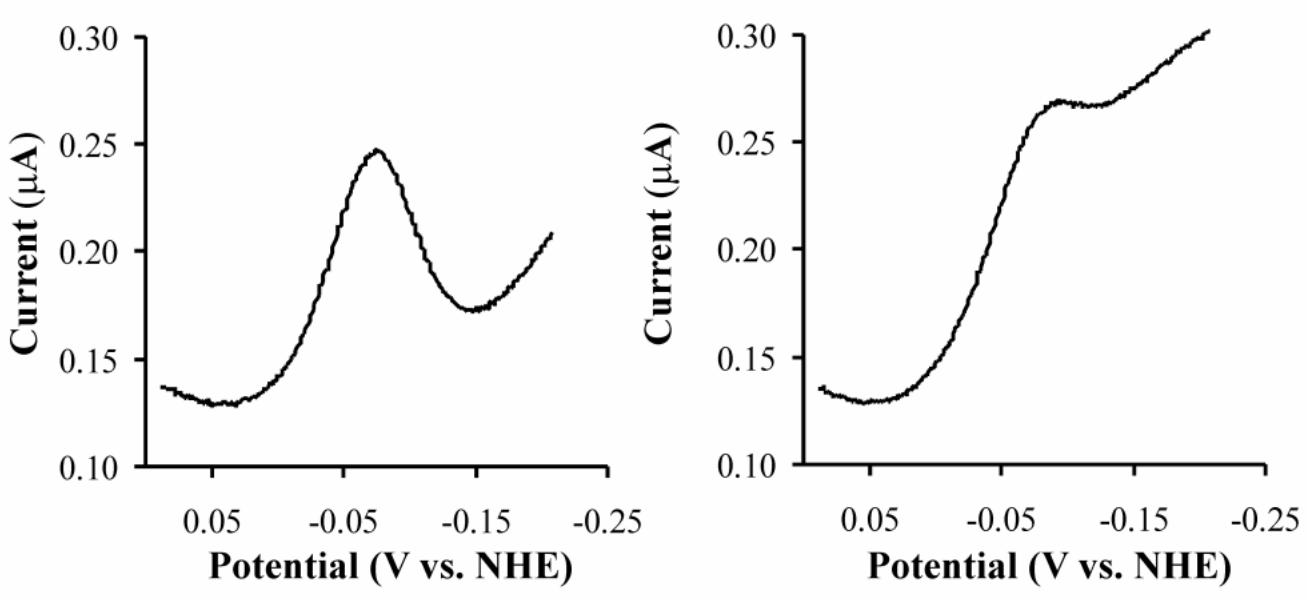

Figure 3.

Electrochemical reduction at a ^{RR}DNA-modified electrode, observed by square wave voltammetry, when RR is positioned opposite an abasic site (left) or a guanine residue (right); the reductive signal is significantly attenuated in the latter case. Peak areas were determined by integrating above the baseline of the voltammograms, thus accounting for differences in the background responses.

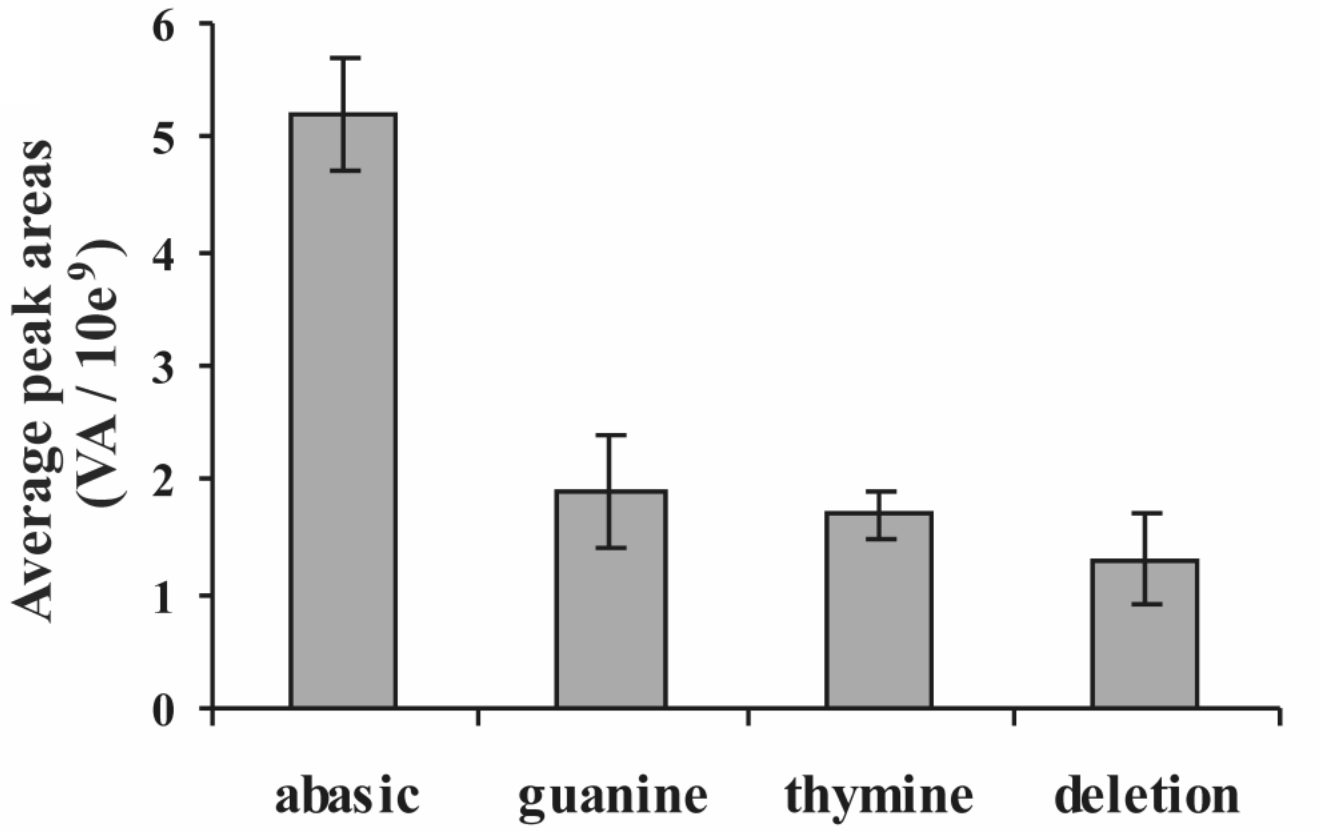

Figure 4.
Bar chart representation of the differences observed in peak areas for the reduction of Redmond Red when positioned opposite an abasic residue, a base, or a deletion site within a DNA duplex. Peak areas, determined from square wave voltammetry, have all been normalized to the area of the gold surface as determined by sulfuric acid etches performed immediately prior to measurement. Error bars shown represent one standard deviation of the measurement.