CHAPTER 8

An Electrochemical Probe of Protein-DNA Interactions on DNA-Modified Surfaces

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Mike Hill assisted in the interpretation of electrochemical data and Donato Ceres assisted in obtaining AFM images.

INTRODUCTION

Protein-DNA interactions play important roles in processes such as transcription, replication, recombination, and repair. As a result, the development of general and efficient assays of protein-DNA interactions is an important goal in structural biology and proteomics. As we analyze and catalogue the proteome, what features common to proteins that regulate and process DNA can we exploit? One structural parameter for cataloguing protein/DNA binding is the extent to which different proteins bend or distort the DNA helix. Structurally well characterized and frequently observed DNA binding motifs of proteins include base flipping, kinking, and groove binding. However, currently, distinguishing between these binding interactions structurally requires high resolution x-ray crystallography or NMR methods.

DNA-mediated charge transport (CT) is exquisitely sensitive to π-stacking and has yielded sensitive assays in mutational analysis based upon the detection of base stacking perturbations such as mismatches (1-5). In electrochemistry experiments using DNA films, redox active intercalators, such as daunomycin, covalently crosslinked to the modified surface or noncovalently bound, can be efficiently reduced. The presence of a mispaired base between the electrode and the site of intercalation, however, switches off the electrochemical response (6). By coupling the reduction of the intercalator to an electrocatalytic cycle, remarkably, all single base mismatches, including thermodynamically stable GT and GA mismatches, as well as many DNA

base damage products can be easily discriminated (Chapters 2 and 3, refs. 5,7). Spectroscopic and biochemical studies of oxidative damage at a distance also have revealed a sensitivity to disruptions in the intervening π-stack, as are present with mismatches or base flipping enzymes (8-11).

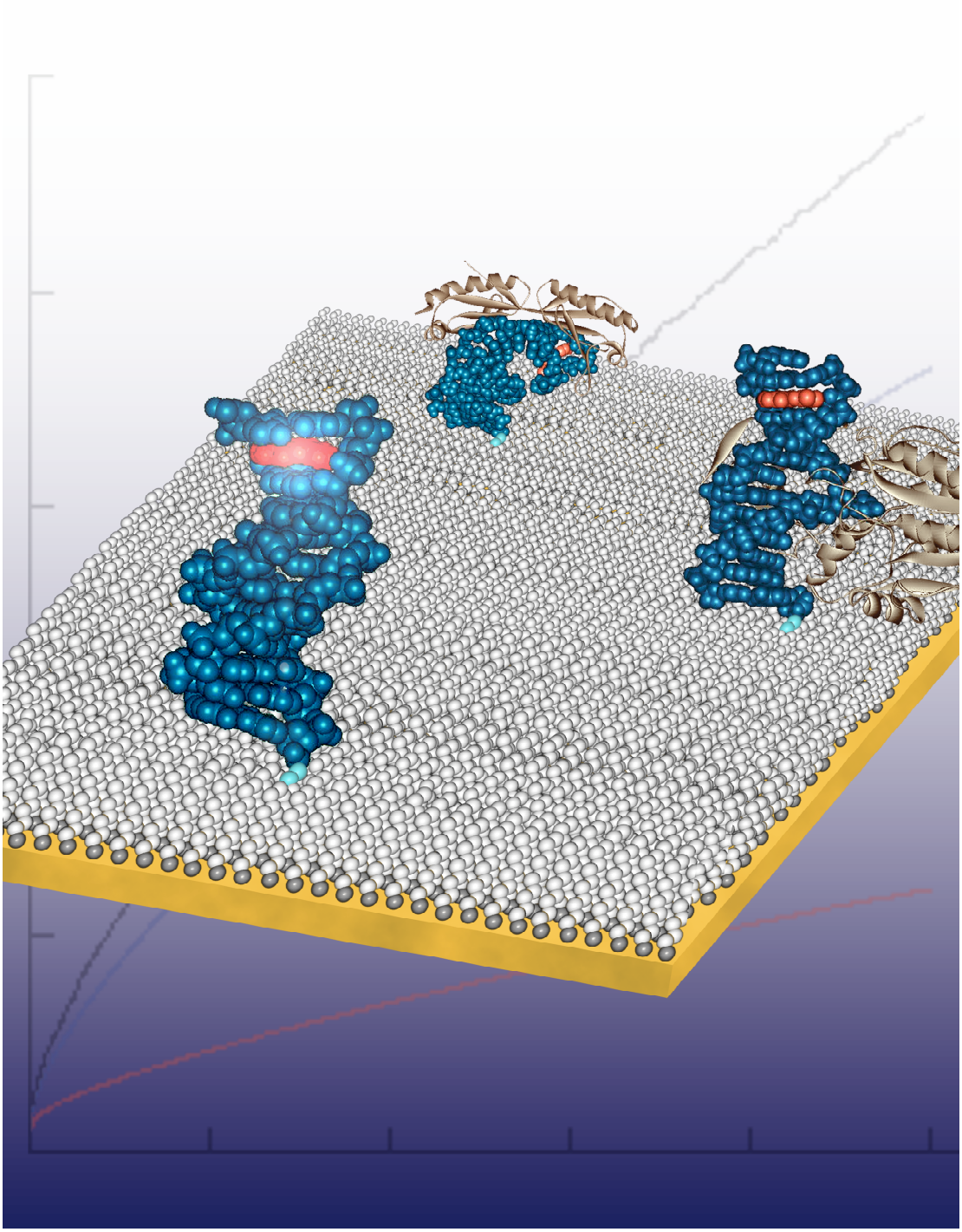
Here we exploit the sensitivity of DNA CT to base stacking to develop a novel probe for protein binding (Figure 8.1). In general, electrochemical detection schemes for DNA have proven to be particularly sensitive (12-15). Now we apply the electrochemical detection of DNA base stacking perturbations to the analysis of protein-DNA interactions and reactions. Such sensitive detection of protein dependent perturbations to DNA on surfaces offers a new approach not just to cataloguing proteins but also to the selection of proteins based upon their sequence specific reactions with DNA and to the real time monitoring of these reactions and their inhibitors.

MATERIALS AND METHODS

Materials

All DNA synthesis reagents, including all unnatural nucleic acid phosphoramidites, were obtained from Glen Research (Sterling, VA). Daunomycin was purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO) and used as received.

Figure 8.1. Schematic illustration of proteins bound to a DNA-modified surface for electrochemical analysis of binding and reaction.



Preparation of DNA-modified surfaces

Thiol-modified oligonucleotides were prepared using phosphoramidite synthesis as described in the Appendix. Thiol-terminated linkers were attached to single stranded oligonucleotides, HPLC purified, and hybridized to DM-modified complements. Self-assembly was carried out (100 μM duplex, 5 mM phosphate, 50 mM NaCl, pH 7, ambient temperature) without added Mg^{2+} (normally 100 mM). Daunomycin was covalently crosslinked to the top of the DNA film. The covalent adduct is formed by reaction with the exocyclic amine in guanine residues in the presence of formaldehyde (33). All guanines in the duplex were therefore replaced by inosines (I; guanine without the exocyclic amine) except those at the end of the duplex where daunomycin adduct formation is intended. Inosine pairs through two hydrogen bonds to cytosine, and therefore replacement of guanine with cytosine somewhat reduces the melting point of the duplexes; all of the sequences used here, however, are stable at ambient temperatures. After assembly of the duplexes, the remaining exposed surface was filled with mercaptohexanol (100 μM) to prevent daunomycin or protein from directly accessing the electrode. After backfilling, the electrode is rinsed in 5 mM sodium phosphate buffer containing 50 mM NaCl, pH 7, and used for protein binding and electrochemical experiments.

Electrochemical measurements

Cyclic voltammetry, square wave voltammetry and chronocoulometry were carried out on 0.02 cm^2 gold electrodes (Bioanalytical Systems (BAS) Inc., West Lafayette, IN) using a BAS Model CV-50W electrochemical

analyzer. Buffer and electrolyte conditions were 5 mM sodium phosphate buffer containing 50 mM NaCl, pH 7, ambient temperature. A normal three electrode configuration consisting of a modified gold disk working electrode, a silver wire reference electrode, and a platinum wire auxiliary electrode were used. Ag wire was used rather than a saturated calomel electrode due to volume constraints in the protein experiments. We have also used Ag/AgCl and SCE reference electrodes in some of the experiments under these conditions and although there is a potential shift, as expected, the shift was the same for all samples examined. Ag wire is a stable reference in these experiments.

Protein binding and electrochemical conditions were 5 mM phosphate (pH 7), 50 mM NaCl, 50 μM EDTA, and 5% glycerol at ambient temperature. In the case of TBP, buffer solutions also included 4 mM spermidine, 4 mM $MgCl_2$, and 75 mM potassium glutamate. Wildtype and mutant M.*Hha*I was generously donated by New England Biolabs (Beverly, MA), TBP was isolated from *E.coli* using standard procedures, and UDG and R.*Pvu*II were purchased from New England Biolabs. For electrochemical analysis of R.*Pvu*II kinetics, the experimental conditions were 5 mM phosphate (pH 7), 50 mM NaCl, 50 μM EDTA, and 5% glycerol at ambient temperature. Gel experiments were performed in solution under the same conditions with the same DNA sequence (10 μM; with a ^{32}P label rather than the alkanethiol tether) incubated with and without 1 μM R.PvuII then eluted on a 20% dPAGE gel.

RESULTS AND DISCUSSION

DNA-modified films for protein binding and reaction

Our standard procedure for fabricating DNA-modified films was optimized for assaying base stacking perturbations associated with protein binding (Figure 8.2). DNA-modified duplexes are prepared by derivatizing short single stranded oligonucleotides at the 5′ end with a thiol-terminated alkyl chain and hybridizing them to their unmodified complements. In assays for mutational analysis, the thiol-modified duplexes are then packed tightly by self-assembly onto the gold surface, using Mg^{2+} (100 mM) to neutralize the repulsion between closely packed helices; a noncovalent intercalator, serving as the redox probe, is bound near the top of the film (16). Instead, here, to provide accessibility to the proteins, the self-assembly on the surface is carried out without Mg^{2+}, and the electrochemical probe, daunomycin (DM), is covalently crosslinked to a guanine residue near the duplex terminus (DM-DNA). To eliminate direct contact between the DM or the protein and Au surface, we then backfill the Au surface with mercaptohexanol (17). In all cases, the DM binding site is at least three base pairs removed from the protein binding site, based upon the DNA cocrystal structures for all of the proteins studied here. Thus protein binding should not alter the environment of the DM binding site.

These surfaces have been characterized by scanning probe microscopy with chemically modified Si_3N_4 tips (Figure 8.3) (18). Surfaces with bound 15mer oligonucleotide duplexes formed in the presence of Mg^{2+} produce

Figure 8.2. Schematic representation of the fabrication of DNA-modified gold electrodes for electrochemical analysis of protein binding and reaction. The DNA duplexes must be well spaced on the electrode surface in order to allow room for protein binding, so self-assembly is carried out without added Mg^{2+} (normally 100 mM); the electrostatic repulsion between neighboring duplexes is not shielded and the duplexes pack loosely. Daunomycin (shown in red) is covalently crosslinked to the top of the DNA film. After assembly of the duplexes, the remaining exposed surface is filled with mercaptohexanol to prevent daunomycin or protein from directly accessing the electrode. Once the gold electrode modification is complete, the electrode is incubated with approximately 1 μM of a test protein for 20 minutes and then interrogated by chronocoulometry at –575 mV vs. Ag. At these negative potentials, either with or without protein, the DNA helices are found to be in an almost upright position owing to repulsions between the negative electrode and the DNA (19). By chronocoulometry, if the amount of charge accumulated after five seconds is significantly less than the control without protein, we conclude that particular protein disrupts the base stack of DNA upon binding.

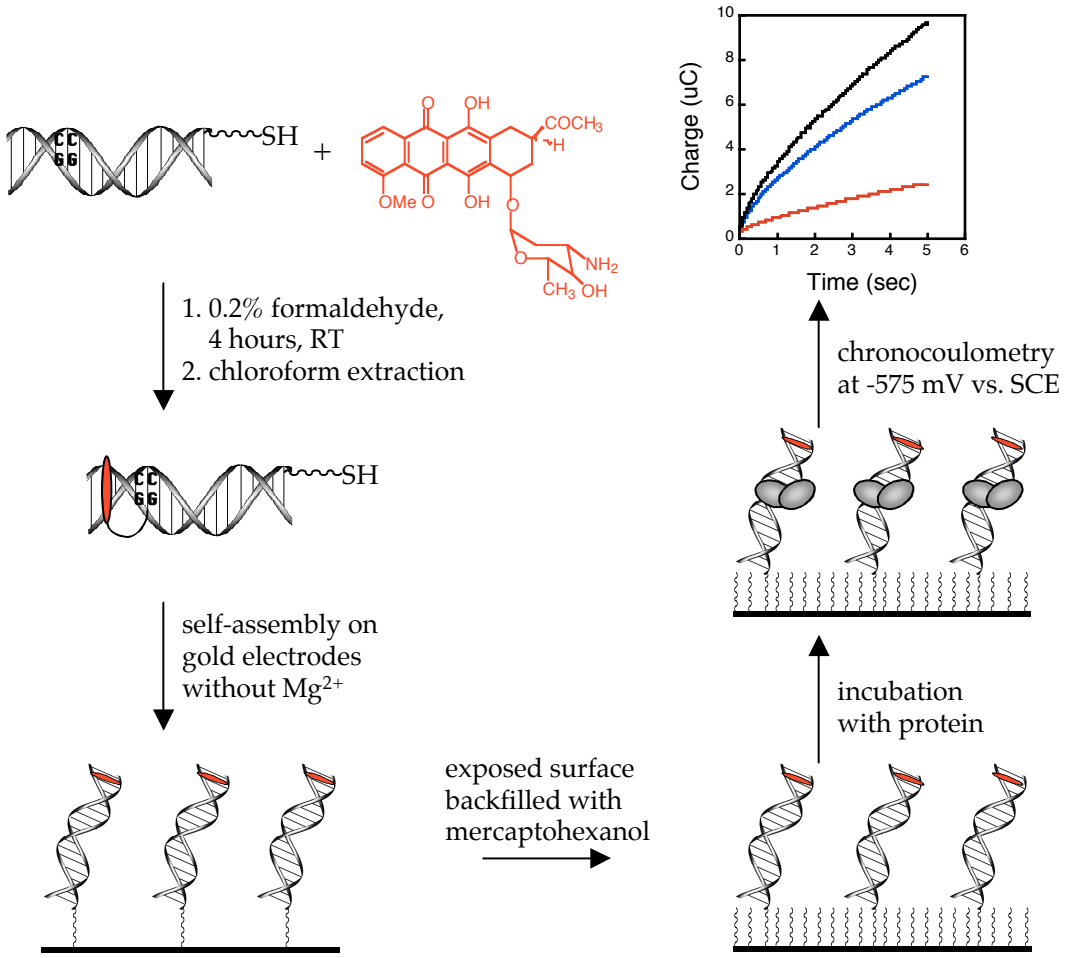
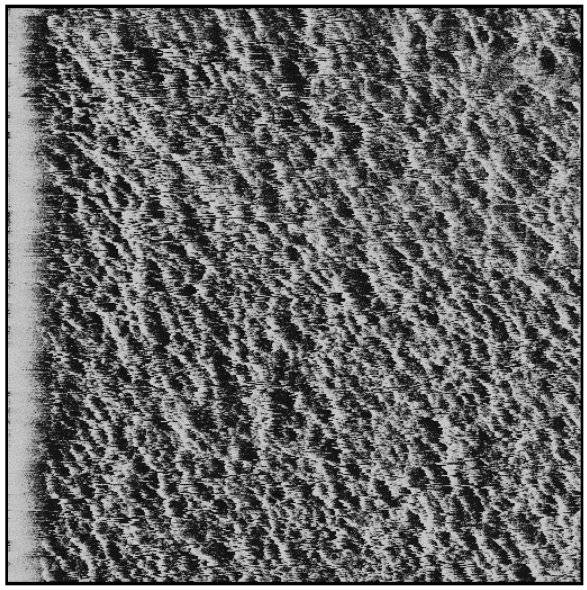
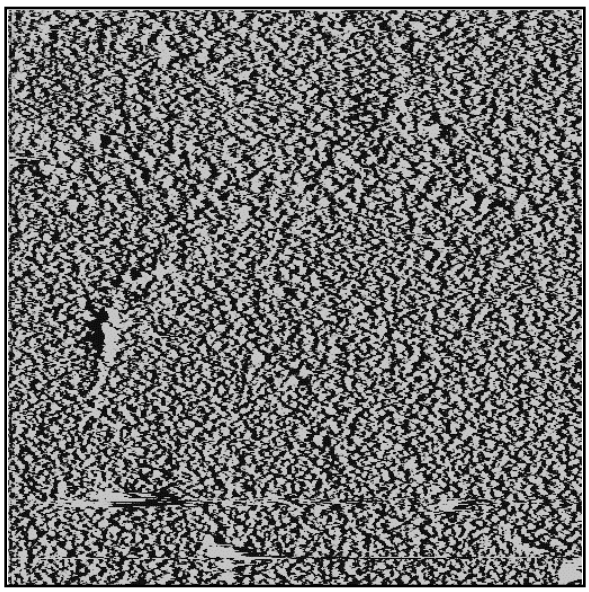


Figure 8.3. AFM images collected of a loosely packed DNA-modified surface (a) before and (b) after incubation with 1 μM M.*Hha*I. Images are taken in buffer solution (height contrast is 2.0 nm, scale is 300 nm x 300 nm). Features in (b) are about 300 Å.

(a)



(b)



smooth featureless films with a depth of about 45 Å (19). However, images of DNA surfaces formed without Mg^{2+} have a very different morphology. The duplexes are less ordered on the surface and are significantly more loosely packed. For films prepared under low Mg^{2+} concentrations, in the absence of an applied potential, the film height is lower, about 20 Å, indicating the DNA has room to lie on the gold surface and, without the addition of mercaptohexanol, the gold surface is visible in some areas. When these DNA films are incubated with protein, however, the surface distinctly changes: Au is no longer visible; the film height increases; small repeating features are evident; and the morphology becomes more complex. It is noteworthy that, even in the absence of protein, at the negative potentials where the electrochemical experiments are carried out, we have observed an increase in film height to almost 60 Å (19); in the presence of the applied potential, then, electrostatic repulsion between the negatively charged DNA and the electrode surface appears to cause the duplexes to "stand up." Therefore the schematic representation in Figure 8.2, although a simplification, contains the critical features for the experiment: (i) DNA helices are distributed across the surface; (ii) protein binding does not interfere sterically with bound DM; and (iii) at negative potentials, the DNA helices with or without protein bound are repelled from the negatively charged surface so that DM is not in direct contact with the gold.

Square wave and cyclic voltammograms obtained for DM-DNA-Au electrodes show a reversible reduction of DM at –580 mV versus Ag, and possess features characteristic of surface-bound species (6,20). DNA adsorbed on Au(111) without Mg^{2+} in the self-assembly solution was

furthermore quantitated in a ^{32}P radioactive labeling experiment (16). The assay yielded an average surface coverage of 12 pmol/cm^2 after 24 hours of modification, corresponding to a fractional coverage of 0.19. As expected, these values reflect a much lower surface coverage than previous studies with monolayers formed in the presence of high Mg^{2+} concentrations (fractional coverage of 0.75) (16). The stoichiometry of crosslinked DM to DNA is 1:1 as confirmed by UV-vis spectroscopy. All data indicate that the crosslinked DM is electrochemically active.

Electrical analysis of DNA-protein interactions at DNA-modified electrodes

Chronocoulometry results for DM-DNA-modified surfaces in the absence and presence of different DNA binding proteins are shown in Figures 8.4-8.9. For the well matched DNA duplex sequences tested, in the absence of bound protein, chronocoulometry at –575 mV (versus Ag) yields a substantial signal upon integration over 5 seconds. In the presence of some proteins, however, signal attenuation is observed, and this diminution in signal depends upon whether the protein structurally perturbs the DNA.

The slopes of the chronocoulometry plots are indicative of electrocatalysis. Experiments in our laboratory using DM bound to DNA-modified surfaces in the presence and absence of oxygen further indicate that the DM reduced by DNA-mediated CT is electrocatalytically reducing oxygen in solution. Plots of charge versus $t^{1/2}$ for all the chronocoulometry data shown in Figures 8.4-8.9 support this mechanism (21). Given that the protein and DM are well separated on the DNA duplex, protein binding is not expected to inhibit the interaction of oxygen with DM. This electrocatalytic

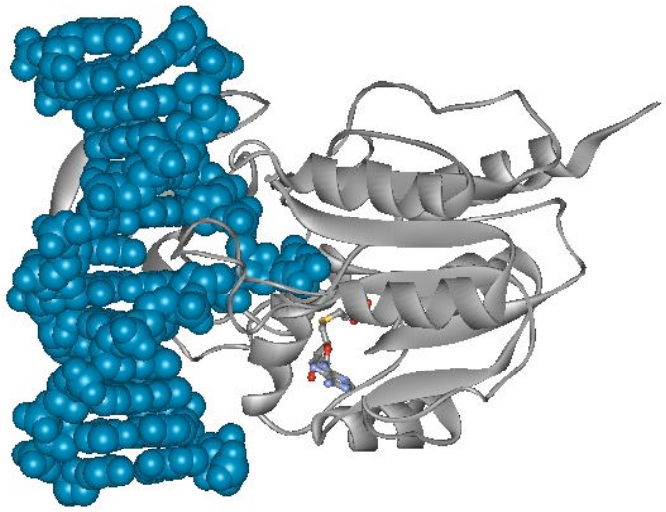
reaction effectively amplifies the DM signal and improves the discrimination between signals obtained for structurally intact versus structurally perturbed DNA films. Similar signal enhancement and increased sensitivity by electrocatalytic amplification of DNA-mediated electrochemistry has been used to detect DNA base stacking perturbations such as mismatches and lesions (Chapters 2,3, refs. 5,7).

These protein dependent changes in DNA-mediated reduction of DM are illustrated clearly for DNA films containing the methyltransferase *Hha*I (M.*Hha*I) target sequence in the presence and absence of M.*Hha*I. This enzyme catalyzes the methylation of cytosine in the sequence 5'-GCGC-3', and a M.*Hha*I-DNA cocrystal structure reveals that M.*Hha*I flips the cytosine out from the duplex and inserts glutamine 237 into the resulting space in the base stack (22-25). In order to examine this phenomenon electrochemically, an electrode was prepared containing the duplex sequence SH-5'-AIAIAT**ICIC**AIAT*CC(DM)*T-3' (protein binding site in boldface, DM binding site in italics). The methyl group source cofactor, S-adenosylmethionine, was omitted to test protein binding but not reaction on the electrode surface. As is evident in Figure 8.4, with M.*Hha*I bound to the DNA-modified electrode, the amount of charge passing through the film is greatly diminished. However, when BSA, a protein that does not bind DNA, is tested, no inhibition of current flow results. The diminution in current flow with M.*Hha*I can be understood based upon the interruption in the base pair stack as a result of base flipping and glutamine insertion by M.*Hha*I. We also tested a mutant M.*Hha*I, Q237W that inserts instead an aromatic amino acid side chain, tryptophan, into the base pair stack upon base flipping, maintaining the

integrity of the π-stack. With binding of this mutant protein, and restoration of the π-stack, current flow is restored. It is noteworthy also that a small drop in charge is observed in the binding of the mutant enzyme but not with BSA (*vide infra*). These results support previous observations made in solution, where CT was measured through studies of long range oxidative damage on DNA assemblies containing guanine doublets, as sites of oxidation, and a spatially separated rhodium photooxidant (8-11). Oxidative damage at the site distal to protein binding was substantially diminished in the presence of native M.*Hha*I but was restored in the presence of the Q237W mutant. It is remarkable that parallel results are obtained between these experiments irrespective of whether the reaction being monitored involves oxidation chemistry, to damage guanine bases, or reduction chemistry, here to reduce DM.

Analogous results are also observed on DNA films containing an abasic site within the protein binding site (5′-GAbGC-3′). M.*Hha*I binds more tightly to the abasic substrate (26), but because of helix disruption, the presence of the abasic site *without* protein leads to a diminution in integrated charge compared to the well matched substrate. As can be seen in Figure 8.5, binding of the mutant protein with insertion of the Trp residue, however, completes the π-stack and again restores current flow. Thus, the efficient DM reduction observed with Q237W cannot be explained by a loss of protein binding affinity. Square wave voltammetry studies also yield consistent results. Without protein or with Q237W bound to the film, a peak is observed at –580 mV, the reduction potential of DM, but there is no peak with the wildtype enzyme.

Figure 8.4. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound wildtype and mutant Q237W M.*Hha*I (1 μM) protein using a well matched DNA binding site. The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT), blue (demonstrating efficient CT), or green for BSA (a protein that does not bind DNA). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding. The cocrystal structure of M.*Hha*I with DNA is also pictured (22).



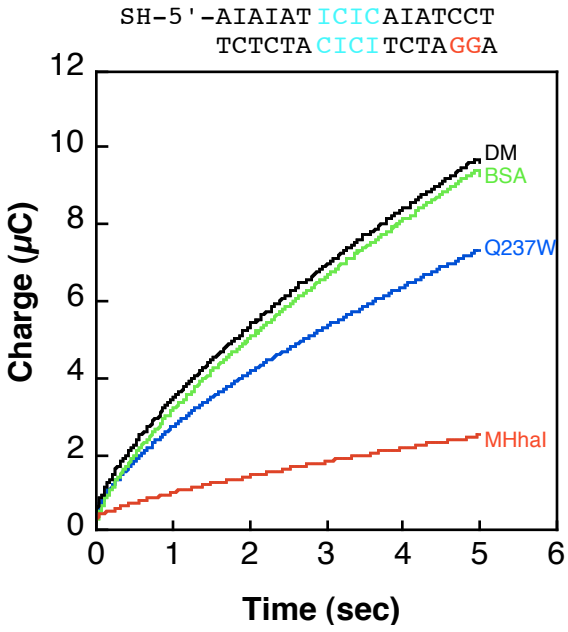
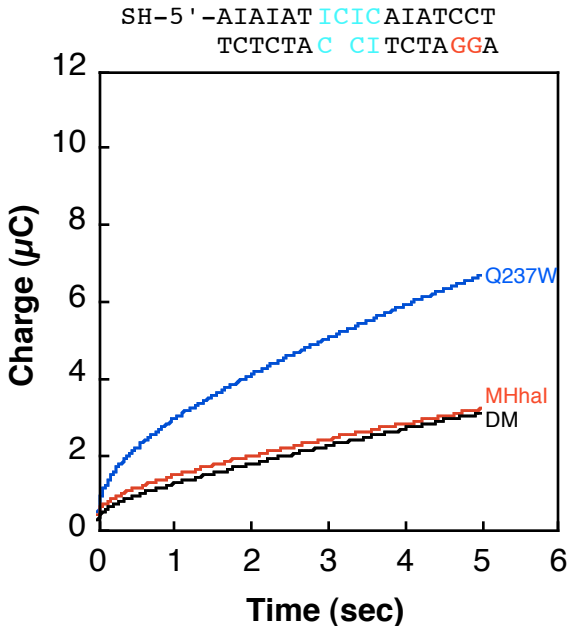


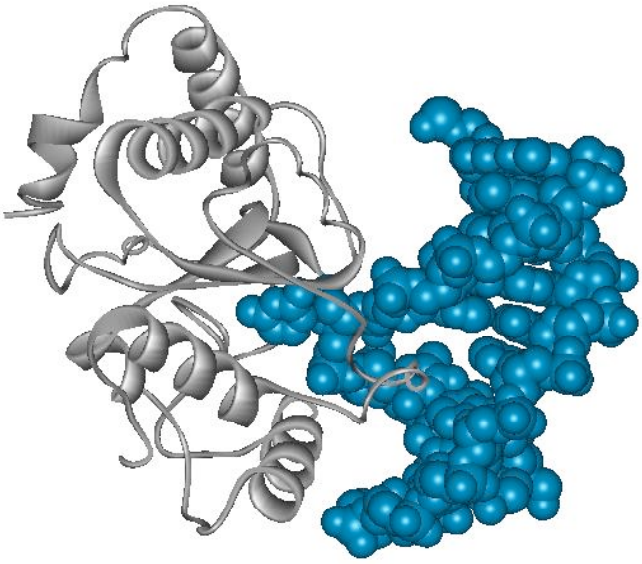
Figure 8.5. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound wildtype and mutant Q237W M.*Hha*I (1 μM) protein using an abasic DNA binding site. The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT) or blue (demonstrating efficient CT). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding.



These experiments effectively demonstrate that we are able sensitively to assay the base stack of DNA within protein-DNA complexes. In these experiments a continuing concern is whether the path of charge transport to effect the electrochemical reduction of DM is through the DNA base stack. These data clearly support that pathway. If instead the DNA were lying down so that the DM could touch the electrode surface directly, there would not be a differential effect with native M.*Hha*I versus the Q237W mutant, where the only change in the protein is in its interior, inserted in the DNA. The comparison of the results with both M.*Hha*I enzymes bound to the well matched DNA substrate and to the abasic DNA substrate is particularly compelling (Figures 8.4 and 8.5). Again, if the electron flow to reduce DM were not through the base stack of DNA, we would not observe a diminution of current without protein for the abasic substrate that is then restored upon binding by Q237W, which inserts an aromatic, heterocyclic tryptophan to complete π-stacking. Thus, this system effectively measures the integrity of the base stack of DNA. Perturbations, such as are associated with protein binding or the presence of an abasic site, that disrupt the structure of the base stack are easily detected.

Several other structurally well characterized protein-DNA complexes were also probed. Uracil DNA glycosylase (UDG) (27), is a base flipping enzyme important in base excision repair. The Au electrode surface was modified with the sequence SH-5′-AICTIA**A**TCAIT*CC(DM)*T-3′ with a 2′-fluoro-uracil, to prevent enzyme turnover (28), incorporated onto the complementary strand opposite the boldface A. After backfilling with mercaptohexanol and incubation with 1 μM UDG, we interrogated the

Figure 8.6. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound UDG, another baseflipping enzyme (1 μM). The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT) or blue (demonstrating efficient CT). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding. The cocrystal structure of UDG with DNA is also pictured (27).



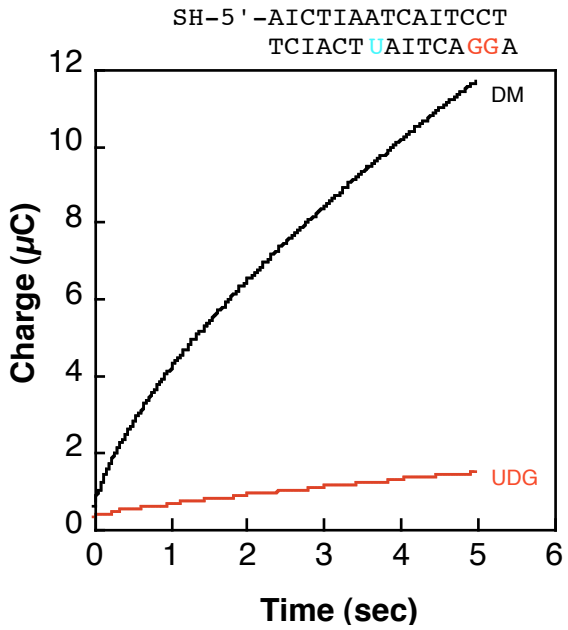
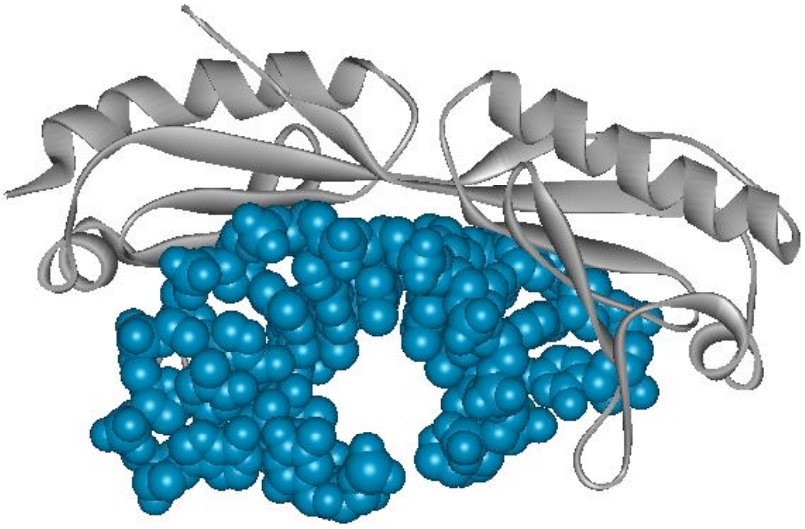
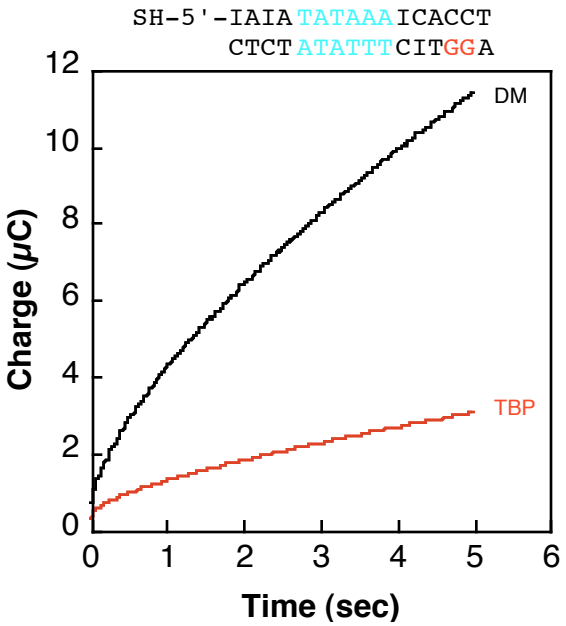


Figure 8.7. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound TBP (1 μM). The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT) or blue (demonstrating efficient CT). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding. The cocrystal structure of TBP with DNA is also pictured (29).





surface at –575 mV by chronocoulometry (Figure 8.6). Again, very little charge was transported through this protein-DNA complex. This result further supports our structural model, where base flipping by uracil DNA glycosylase perturbs base stacking and hence current flow to DM.

The assay, however, is not sensitive only to base flipping. Instead the assay provides a measurement of current flow associated with perturbations to the base stack. The TATA box binding protein (TBP), for example, kinks DNA approximately 90° upon binding to its target site, completely disrupting base stacking but not base pairing (29-30). Binding of TBP to its recognition site was examined on electrodes modified with the sequence SH-5′-IAIA**TATAAA**ICA*CC(DM)*T-3′ and mercaptohexanol. As evident in Figure 8.7, with protein binding (1 μM), the ability to reduce DM through DNA-mediated CT is significantly diminished.

We also investigated DNA binding by the restriction endonuclease *Pvu*II (R.*Pvu*II) at a surface modified SH-5′-TCTT**CAIMTI**AIA*CC(DM)*T-3′, passivated with mercaptohexanol, and incubated with 1 μM enzyme. A methylated cytosine (**M**) was incorporated to prevent cleavage of the DNA film. As predicted from the crystal structure of this enzyme bound to DNA (31), which reveals no significant perturbation of the DNA base stack, efficient reduction of DM is observed by chronocoulometry at –575 mV, both in the absence and presence of R.*Pvu*II (Figure 8.8). However, a small drop in the accumulated charge is observed upon binding R.*Pvu*II to its target.

Control experiments using an electrode surface modified with *Pvu*II DNA, but incubated with BSA or M.*Hha*I (no binding site on the DNA film; Figure 8.9) indicate that protein present in the electrochemical cell, but not

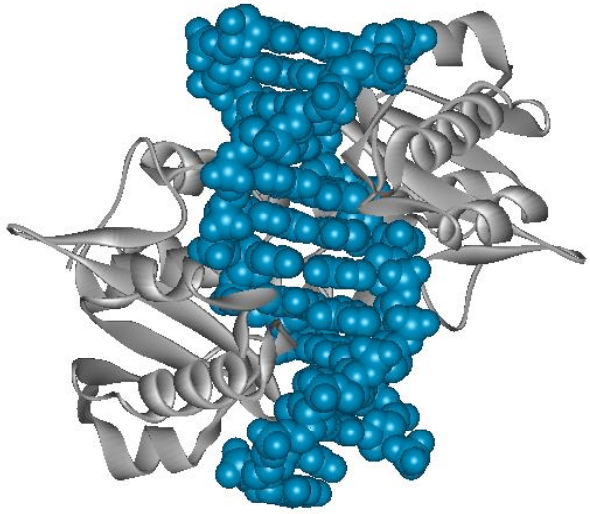
specifically bound to the electrode has no effect on the reduction of DM. It appears that the presence or absence of this small change in chronocoulometry is indicative of protein binding. We see this also in M.*Hha*I binding to an electrode modified with *Pvu*II DNA (Figure 8.9). Here, M.*Hha*I can bind nonspecifically to the DNA surface but does not base flip because there is no specific binding site. BSA, on the other hand, does not bind to DNA and thus no drop in charge is observed.

Indeed, all of these data are consistent with the conclusion that charge transport through DNA-modified surfaces provides a very sensitive method to probe protein-DNA interactions. No similar information as to whether a given protein perturbs the DNA helix at its binding site is available currently without detailed structural characterization using NMR or x-ray crystallography.

Electrical analysis of enzymatic reaction at DNA-modified electrodes

We tested also whether this assay might be amenable to electrical probes of DNA enzyme reaction kinetics (Figure 8.10). The restriction activity of R.*Pvu*II (1 μM) was assayed on the Au surface modified with SH-5'-TCTT**CAICTI**AIA*CC(DM)*T-3', but now with the native nonmethylated restriction site incorporated into the monolayer to monitor endonuclease activity. As the enzyme cuts the DNA, the DM probe is released from the surface, resulting in a diminution in CT in the film. Indeed, the amount of charge accumulated at this surface after five seconds of chronocoulometry decreases with increasing reaction time while the amount of DM reduction in

Figure 8.8. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound R.*Pvu*II using the methylated target (1 μM). The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT) or blue (demonstrating efficient CT). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding. The cocrystal structure of R.*Pvu*II with DNA is also pictured (31).



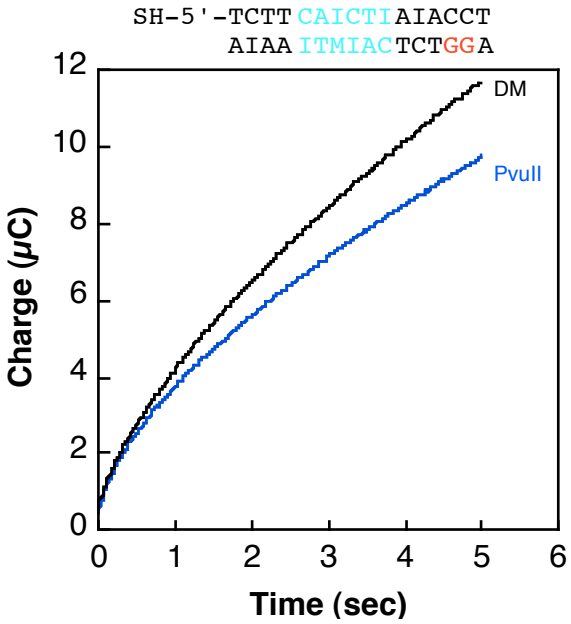


Figure 8.9. Chronocoulometry at –575 mV of DM covalently crosslinked to thiol-terminated DNA films on gold electrodes with and without bound protein (1 μM). The DNA sequence used is shown above the plot with the specific DNA binding site highlighted in cyan and the daunomycin adduct site highlighted in red. Chronocoulometry of the DNA film without protein is in black and the test trace after incubation with protein is either in red (demonstrating inhibition of DNA-mediated CT) or blue (demonstrating efficient CT). This figure illustrates a control experiment where the DNA binding site (for *Pvu*II) and protein (M.*Hha*I wildtype in red, Q237W in blue) do not match or the protein used does not bind DNA at all (BSA in green). CT through DNA-modified surfaces accurately reflects DNA structural perturbation caused by protein binding.

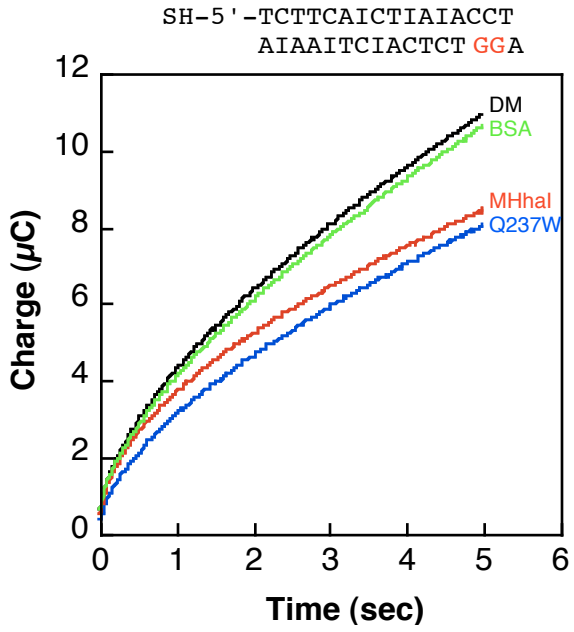
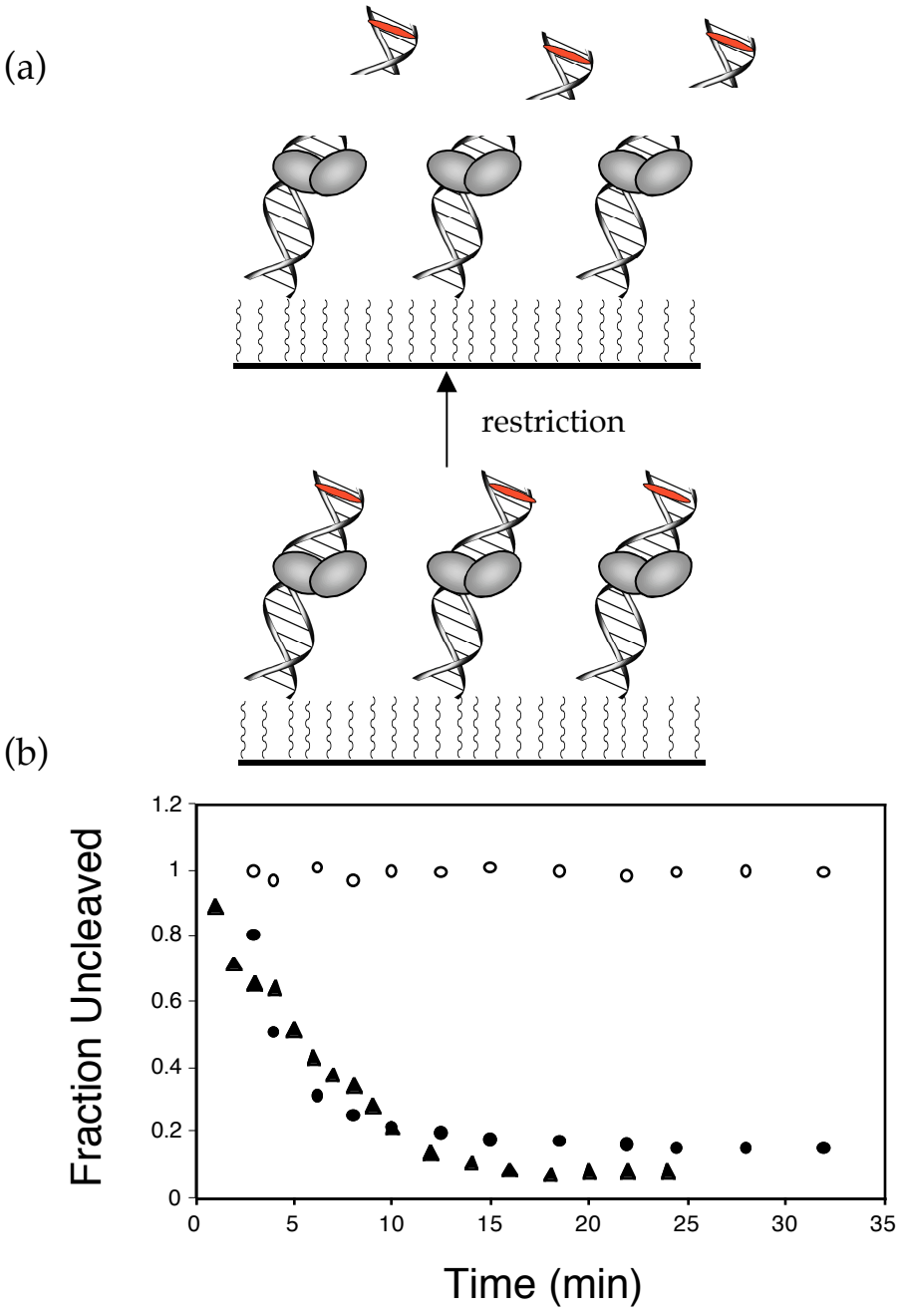


Figure 8.10. Kinetics of DNA restriction monitored electrically. Panel (a) shows a schematic representation of enzyme restriction at a DM-DNA surface and panel (b) is a plot of fraction DNA cleaved by R.*Pvu*II determined by either the amount of charge accumulated at the DNA film (closed circles) or using gel electrophoresis (triangles) as a function of enzyme reaction time. Also shown is chronocoulometry in the absence of protein (open circles). Chronocoulometry took place at –575 mV with and without bound R.*Pvu*II (1 μM) at a gold electrode modified with the unmethylated thiol-terminated sequence SH-5′-TCTT**CAICTI**AIA*CC(DM)*T-3′ and mercaptohexanol.



an identical surface without protein remains constant. The decrease in charge is exponential as expected for the kinetics of R.*Pvu*II (32).

As a comparison, we also assayed the reaction under parallel conditions by gel electrophoretic analysis of the DNA restriction products. The electrochemical data agree with the gel based cleavage assay under the electrochemical conditions (Figure 8.10). These data clearly show that DNA-modified films may be employed also in electrical monitoring of DNA enzymatic reactions. The electrochemical assay offers the advantage of real time monitoring and of sensitivity.

An electrical probe for DNA-protein interactions and reactions

These results establish a new, sensitive assay for sequence specific protein-DNA interactions and reactions. DNA CT chemistry allows the rapid determination of structural perturbations in a DNA site associated with binding of a given protein. CT chemistry also facilitates the real time monitoring of enzymatic reactions on DNA. DNA-modified electrodes are furthermore amenable to an array format and thus provide a practical tool for the selection and assay of proteins based upon their sequence specific interactions with DNA. Moreover the electrochemical assay on DNA monolayers provides a sensitive route to test for inhibitors of such protein-DNA interactions. Hence DNA CT chemistry not only provides a novel strategy for the structural analysis of how individual proteins bind DNA but also a remarkably sensitive tool in real time for DNA based proteomics.

SUMMARY

DNA charge transport chemistry is found to provide a novel, sensitive method for probing protein dependent changes in DNA structure and enzymatic reactions. The development of an electrochemical assay of protein binding to DNA-modified electrodes based upon the detection of associated perturbations in DNA base stacking is described. Gold electrode surfaces modified with loosely packed DNA duplexes covalently crosslinked to a redox active intercalator and containing the binding site of the test protein were constructed, and charge transport through DNA as a function of protein binding was assayed. Substantial attenuation in current is seen in the presence of the base flipping enzymes, *Hha*I methylase and uracil DNA glycosylase, as well as with TATA binding protein. On binding the endonuclease R.*Pvu*II to its methylated target, little base stacking perturbation occurs and little diminution in current flow is observed. Importantly, the kinetics of restriction by R.*Pvu*II of its nonmethylated target is also easily monitored electrochemically. Hence a completely new approach to assaying protein-DNA interactions and reactions on surfaces is now available.

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