CHAPTER 2

Electrochemical Single Base Mismatch Detection Based on Charge Transport Through DNA at DNA-Modified Electrode Surfaces

Improved sensitivity and discrimination with electrocatalysis

Some of these results have been previously published in Kelley, S.O., Boon, E.M., Barton, J.K., Jackson, N.M., Hill, M.G. (1999) *Nucleic Acids Research 27*, 4830.

INTRODUCTION

The most prevalent mechanisms leading to mutations in DNA are direct misincorporation of bases during replication and sustained chemical damage. Under normal circumstances, the cell corrects these problems using DNA polymerase proofreading mechanisms as well as the complex repair machinery of the cell. In certain tissues that contain mismatch repair deficiencies, DNA mispairs may accumulate (1). Even in healthy cells, however, mismatches and lesions can sometimes go unchecked, resulting in permanent alterations in the gene sequence for subsequent generations. Identification of genetic variations (single nucleotide polymorphisms, SNPs) among individuals and populations has implications in understanding human disease and treatment, as well as the interaction of the environment and multiple genes during evolution (2). Once these SNPs are identified and understood, rapid and reliable detection of them will be critical for the study, diagnosis, and treatment of genetically linked disease.

SNPs are detected as mismatches in heteroduplexes formed from a known copy of a gene and the test gene. Consequently, there is great interest in the development of DNA mismatch biosensors (3-4). Biochemical assays (5-10), traditional separation methods (11), gravimetric analyses (12-15), and spectroscopic probes (16-23) have all been employed in the construction of DNA biosensors. Sophisticated analytical schemes involving high resolution microscopy to assay the hybridization of DNA target sequences with arrays

of immobilized single stranded oligonucleotides have been developed for highly parallel genomic sequencing and the detection of mutations (16-17).

More recently, similar electrochemical schemes have also been explored (24-35). Mikkelsen and coworkers attach single stranded DNA probe sequences to glassy carbon electrodes and then hybridize test DNA samples to this DNA-modified electrode. If hybridization occurs, the electrochemistry of a positively charged, redox active, reporter molecule in solution ($Co(phen)_3^{2+}$) shows an enhanced response owing to its increased attraction to the more negatively charged duplex-modified surface (24-25). Ferrocene-linked threading intercalators that bind only to thermodynamically open places in DNA (such as mismatches) have also been utilized to electrochemically detect DNA mismatches (27). Heller et al. immobilizes single stranded probe DNA on a redox polymer and attaches soybean peroxidase (SBP) to target DNA. If hybridization occurs, SBP comes into contact with the polymer and hydrogen peroxide is produced, which is electrochemically detected (34). Clinical Microsensors (Motorola) is a biochip company employing bioelectronics, that is forming electronic circuits using biological molecules. Single stranded target probes are attached to an insulated electrode surface. Test DNA must be complementary to the immobilized target as well as a single stranded ferrocene-tethered signaling probe in solution (a "sandwich" hybridization assay). If hybridization of all three oligonucleotides occurs, the signaling probe brings a ferrocene molecule to an electronic pad imbedded within the otherwise insulated electrode surface, which completes the circuit (29).

Electrochemical methods have been used to directly oxidize DNA as

well. Wang's group concentrates DNA on an electrode surface and purine bases are directly oxidized via an adsorptive square wave voltammetry method, providing femptomole sensitivity for the presence of DNA (30-31). Thorp and coworkers attach single stranded probe DNA to metal oxide electrodes and monitor oxidation of guanine using a redox catalyst such as $Ru(bpy)_3^{3+}$ (28). The electrochemical rate of guanine oxidation varies from single stranded to duplex DNA and thus hybridization can be monitored.

Applied to base mismatch detection, hybridization assays are inherently limited in sensitivity; detection of a point mutation in the test sequence (e.g., a small segment of genomic DNA) requires a distinguishable difference in pairing energies between the probe sequence and a completely complementary versus mutated target strand. With only a single mutation in an extended oligonucleotide, these differences can be very small. Moreover, duplex stabilities for oligonucleotides of a fixed length can vary considerably as a function of base content; GC-rich sequences are significantly more stable than AT-rich analogues. Within a library of immobilized oligonucleotides, hybridization energies for adjacent probe sequences may vary more than the differential binding energies between a particular probe and its complementary versus mutated test sequence. As a result, detection of point mutations may require extensive manipulation of hybridization conditions as well as sophisticated deconvolution algorithms. The ability to detect mutations within intact duplexes would greatly simplify this analysis. Samples could then be prepared under strongly hybridizing conditions, allowing both native and mutated test strands to bind to the probe sequences,

regardless of the overall base composition of individual addresses in the array.

In this thesis we take advantage of a distinctly different characteristic associated with mismatches, the perturbation of the electronic structure of DNA at the mismatched site, to develop a practical and sensitive assay for mismatch discrimination. While single base mismatches cause only subtle changes in overall duplex stability and structure (36-37), they induce significant perturbations in the electronic structure of the base stack. Photoinduced charge transport through donor/acceptor-labeled duplexes has been observed for a variety of systems (38-42), and these DNA-mediated reactions are exceptionally sensitive to perturbations in the base stack: intervening bulges inhibit long range photochemical guanine oxidation (43) and single base mismatches markedly decrease photoinduced charge transport yield (39). Thus, monitoring charge transport through DNA may offer an alternative approach to point mutation detection.

Charge transduction through double stranded DNA has been observed at DNA-modified gold electrodes (Figure 2.1), which provides a practical platform for the development of the sensitivity of DNA-mediated charge transport into a DNA mismatch sensor. The properties of these DNA surfaces and the charge transfer reactions of redox active DNA intercalators bound to these monolayers have been explored previously (44-47). In the first electrochemical studies of mismatches in these DNA films, the intercalator binding site (> 35 Å from the electrode surface) was fixed by site selectively crosslinking daunomycin (DM) to guanine residues in the duplex (45). In DNA surfaces that were completely Watson-Crick base paired, a well

resolved DM peak was observed in the cyclic voltammagram (CV). However, the presence of a single mispaired base (within the fully hybridized duplex) between the electrode and the site of intercalation switched off the electrochemical response entirely. We have now established that in well packed films of DNA duplexes, the DNA-mediated reaction can proceed using noncovalently bound intercalators such as DM or methylene blue (MB^+) (44,47). Furthermore, these DNA films can be cycled by denaturation of the film leaving surface bound single stranded probes, followed by *in situ* hybridization with a single stranded test sequence (47).

These previous observations demonstrate that mismatches can be detected at a modified electrode using charge transport to noncovalent reporters as the signaling device. The absolute electrochemical signals are limited by the surface concentration of the intercalator (~ 50 pmol/cm^2), and the noncovalent probes could make direct contact with the electrode surface or bind deep within the monolayer (below a mismatch site), thus compromising analytical mismatch detection. A system that augments the signal from probes bound only to the top of the DNA films, as well as increases the absolute observed signals and improves mismatch discrimination would be a great advantage in the development of a reliable clinical device.

Thus, to enhance the sensitivity of our mismatch detection strategy, we have investigated MB^+ electrocatalysis as is described in this chapter. Here we couple the direct electrode to intercalator electron transfer to an electrocatalytic cycle involving a nonintercalating substrate in solution. The resulting assay exhibits greatly enhanced differentiation between

complementary versus mismatched duplexes, and allows the ready detection of point mutations in DNA oligonucleotides. This technology could lead to a selective, inexpensive, and reliable device amenable to widespread application of DNA charge transport in mismatch detection.

MATERIALS AND METHODS

Materials

All DNA synthesis reagents were obtained from Glen Research. Daunomycin was obtained from Fluka; methylene blue, potassium ferricyanide, and ruthenium pentamine chloride were purchased from Aldrich and used as received.

Preparation of DNA-modified surfaces

Thiol-modified oligonucleotides were prepared as described in the Appendix; thiol-terminated linkers were attached to single stranded oligonucleotides, which after stringent purification were hybridized to unmodified complements. The resultant duplexes were deposited on polycrystalline gold electrodes for 24 hours. Before electrochemical measurements, the electrodes were rinsed thoroughly with 5 mM phosphate, 50 mM NaCl buffer (pH 7). As electrodes containing a high surface coverage of DNA were most useful for our experiments, surfaces were routinely assayed for coverage by monitoring the attenuation of the oxidation of

ferrocyanide. Comparable results were obtained with commercial polycrystalline electrodes (BAS) or Au(III) films vapor deposited on mica substrates (Molecular Imaging).

Electrochemical measurements

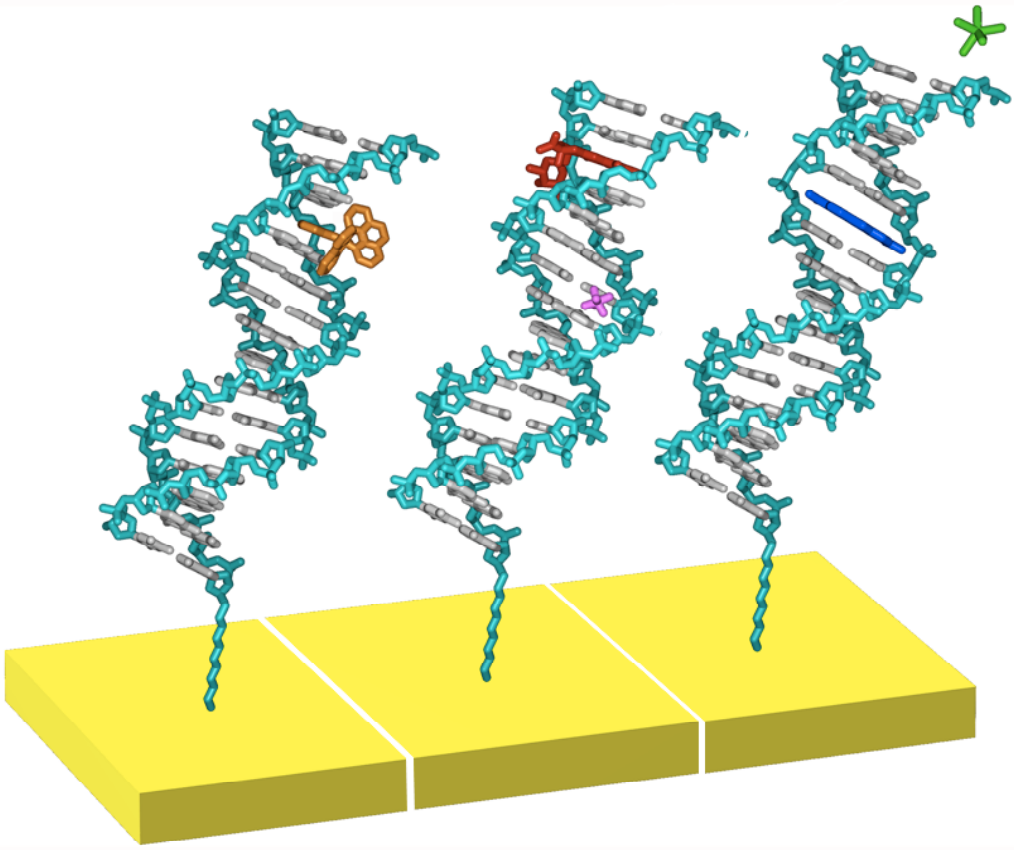
Cyclic voltammetry and chronocoulometry were carried out on 0.02 cm^2 gold electrodes using a Bioanalytical Systems (BAS) Model CV-50W electrochemical analyzer. A normal three electrode configuration consisting of a modified gold disk working electrode, a saturated calomel reference electrode (SCE, Fisher Scientific), and a platinum wire auxiliary electrode was used. A modified Luggin capillary separated the working compartment of the electrochemical cell from the reference compartment. Potentials are reported versus SCE. Volumes of 2.5 mL were typically employed. Unless specifically noted, all measurements were recorded at 23 ± 2˚C in 5 mM sodium phosphate buffer containing 50 mM NaCl, pH 7 that had been thoroughly degassed with Ar.

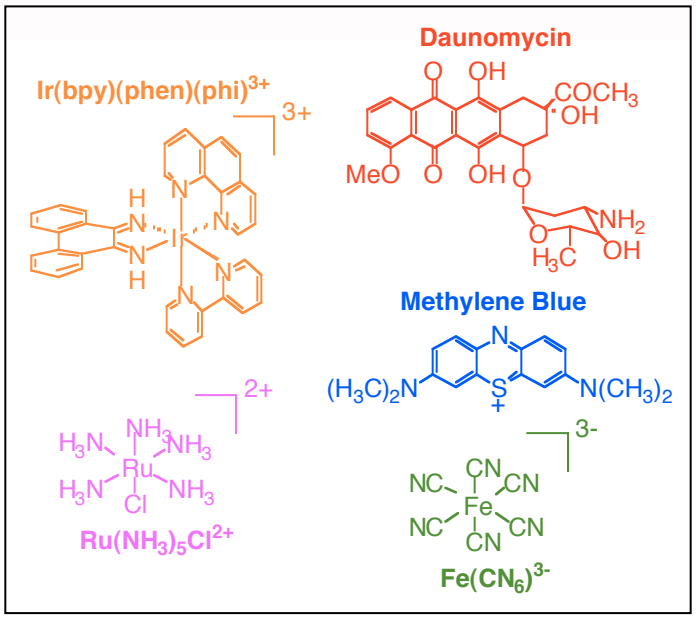
RESULTS AND DISCUSSION

DNA-modified surfaces for single base mismatch detection

Gold electrodes are modified with the duplex 5'-AGTACAGTCATCGCG and all oligonucleotides are labeled at the 5′-end with a linker of the formula $SH(CH_2)_2CONH(CH_2)_6NHCO$ (Figure 2.1).

Figure 2.1. Schematic illustration of DNA duplexes immobilized on a gold surface for use in electrochemical assays. A variety of species are depicted in the monolayer including the intercalators Ir(bpy)(phen)(phi)$^{3+}$ (orange), daunomycin (red), and methylene blue (blue); the groove binder $Ru(NH_3)_5Cl^{2+}$ (purple), and $Fe(CN)_6^{3-}$ (green), which does not associate with the immobilized helices due to its negative charge.





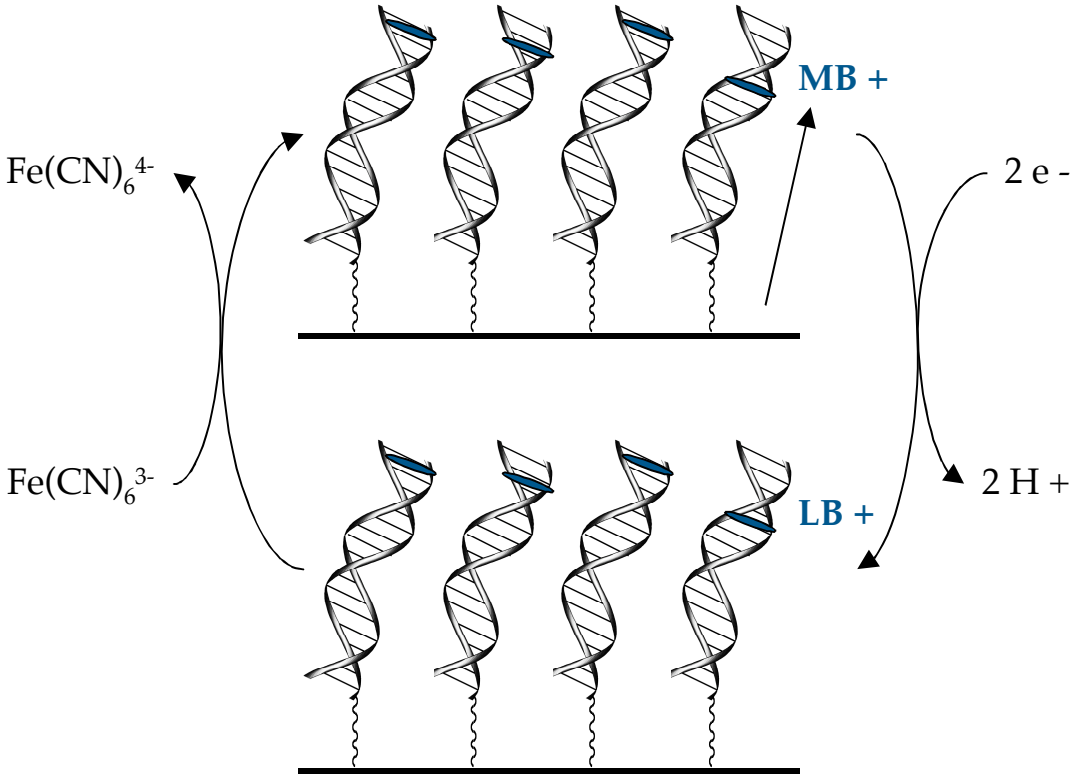
Atomic force microscopy studies have shown that the duplexes form densely packed monolayers with individual helices in an upright orientation with respect to the gold surface (47). Redox active cations (e.g., $Ru(NH_3)_6^{3+}$) and DNA intercalators bind strongly to the modified surfaces and yield well behaved electrochemical signals. Anions (e.g., $Fe(CN)_6^{3-}$) and nonbinding neutral species (e.g., dimethylaminoferrocene) do not associate with the electrodes and are electrochemically silent (44).

Electrocatalysis of methylene blue

Although mismatched DNA can be distinguished by direct voltammetry of noncovalent intercalators (47), there are inherent problems with the sensitivity of such assays. In order to increase the viability of electrochemical mismatch detection via charge transport through DNA, we have coupled the direct electron transfer event to an electrocatalytic process involving a species freely diffusing in solution (Figure 2.2). This effectively amplifies the signal corresponding to intercalator bound only to the top of the monolayer, and improves the discrimination between fully basepaired and mismatched duplex films.

Methylene blue (MB^+) was chosen as the intercalated catalyst. MB^+ binds readily to the DNA-modified surfaces with an association constant of $3.8(5) \times 10^6\ M^{-1}$ (44). Potassium ferricyanide was chosen as the solution-borne substrate. Possessing a large negative charge, $Fe(CN)_6^{3-}$ is electroinactive at the DNA-modified surface even at overpotentials as high as ~ 1 volt (Figure 2.3), yet its chemical reduction by reduced MB^+ is thermodynamically favored by ~ 0.6 eV. Given the low reorganization energy expected for this process

Figure 2.2. Schematic representation of electrocatalytic reduction of $Fe(CN)_6^{3-}$ by MB^+ at a DNA-modified electrode. Electrons flow from the electrode surface to intercalated MB^+. Once reduced, LB^+ can easily reduce $Fe(CN)_6^{3-}$ and regenerate MB^+ that can continue on in the catalytic cycle, thus repeated interrogation of the DNA monolayer is achieved. MB^+ binding is primarily constrained to the top of the densely packed DNA monolayer, requiring charge transport through the DNA film and electrostatic repulsion keeps $Fe(CN)_6^{3-}$ away from the interior of the anionic DNA film.



(48), the cross reaction between the electrochemically generated catalyst and substrate should be very rapid. Depending on the various steps in the overall reaction, the signals may now be limited by the concentration of substrate in solution.

Addition of micromolar MB^+ to a 2.0 mM ferricyanide solution causes a pronounced electrochemical signal at the DNA-modified surface (Figure 2.3). Notably, this signal comes at the reduction potential of MB^+ and is completely irreversible. Electrons flow from the Au electrode to intercalated MB^+ and then are accepted by $Fe(CN)_6^{3-}$ in solution (thus no electrochemical oxidation peak is observed). Chemically oxidized MB^+ is again available for electrochemical reduction and the catalytic cycle continues as long as the potential of the gold electrode is sufficiently negative to reduce MB^+. Because the presence of mismatches effectively decreases the amount of reduced intercalator bound to the film, they should also decrease signals obtained from catalytic reactions.

Electrocatalytic detection of a CA mismatch

Incorporation of a mispaired base step into the duplex significantly attenuates the electrocatalytic response obtained with methylene blue (Figure 2.4). Fewer MB^+ molecules are reduced at the mismatched DNA electrode, so the steady state concentration of active catalyst is lower and a diminished overall catalytic rate results. A range of catalyst and substrate concentrations was investigated to maximize the difference in electrocatalytic response at the fully basepaired (TA) and mismatched (CA) duplexes. Under optimized conditions, the presence of a mismatch causes a 6-fold decrease in the

Figure 2.3. Cyclic voltammetry (= 100 mV / s, A = 0.02 cm^2) at a gold electrode modified with DNA (sequence: SH-5'-AGTACAGTCATCGCG-3') of 2.0 mM $Fe(CN)_6^{3-}$ (black), 2.0 μM MB^+ (blue), 2.0 mM $Fe(CN)_6^{3-}$ plus 2.0 mM MB^+ (red).

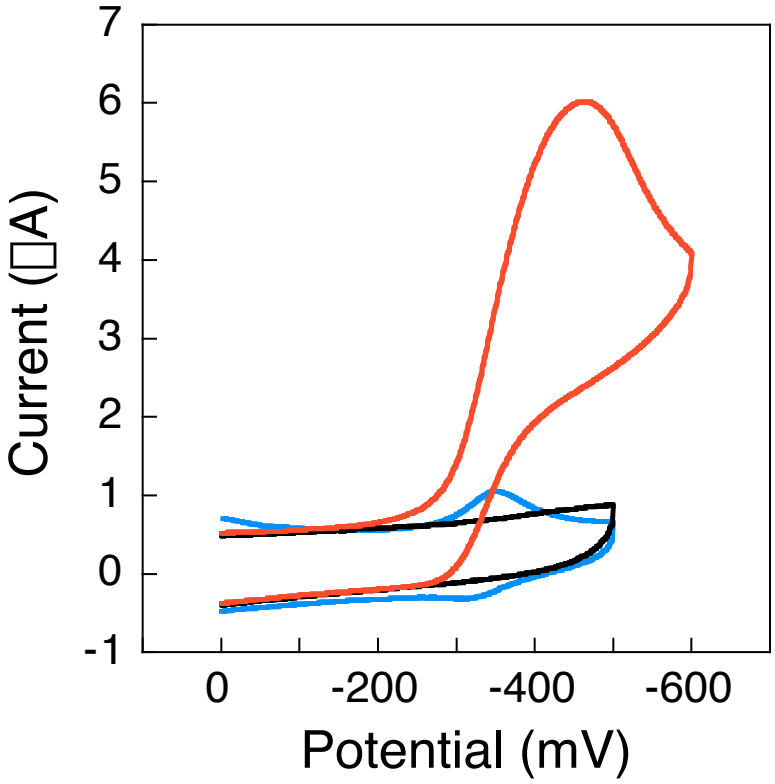
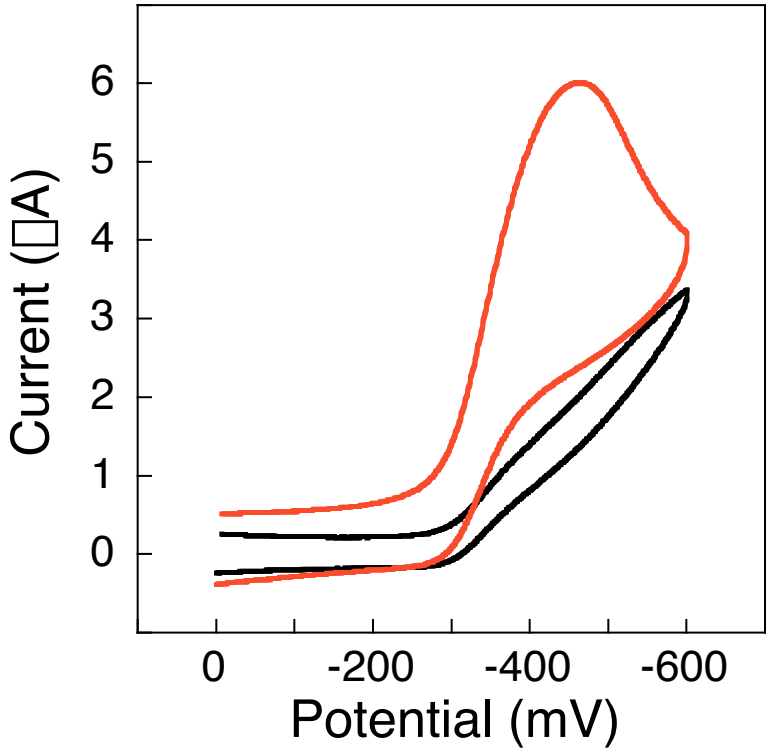


Figure 2.4. Cyclic voltammograms ($\square$ = 100 mV/s, A = 0.02 cm^2) of 2.0 mM $Fe(CN)_6^{3}$ plus 2.0 μM MB^+ at a gold electrode modified with the thiol-terminated sequence SH-5'-AGTACAGTCATCGCG-3' hybridized to a fully basepaired complement (red) and a complement that features an A opposite the underlined C (black).



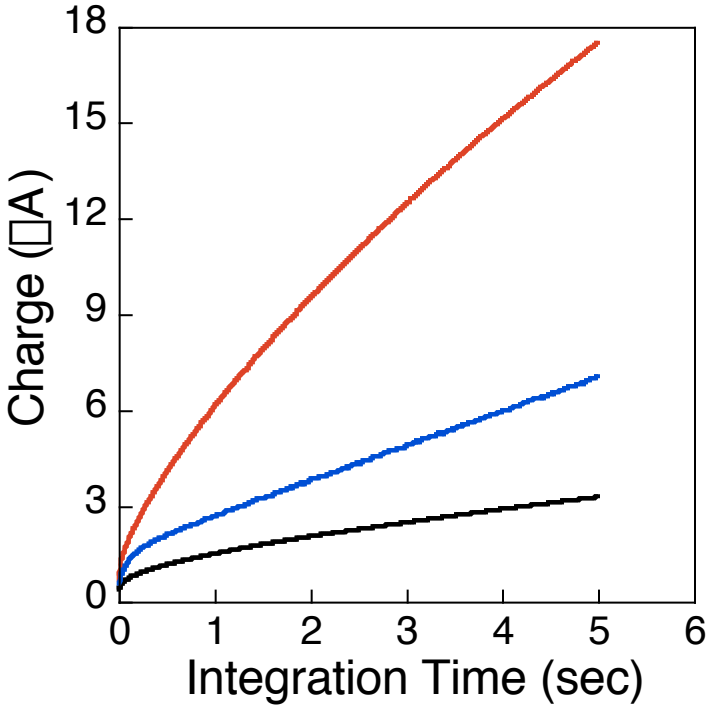
electrocatalytic current, compared to a 2-fold decrease in the peak current obtained by monitoring the direct electrochemistry of methylene blue. Hence, coupling direct electrochemistry of methylene blue to a catalytic event inherently both increases the sensitivity of mismatch detection and provides larger absolute signals. Owing to the catalytic nature of this mismatch detection assay, it should be possible to increase the differentiation between matched and mismatched sequences with longer integration times.

Detection of mismatches using chronocoulometry

Because this charge transport based assay features a catalytic reaction whose rate depends on the degree of complementarily within the individual duplexes, the measured charge resulting from reduced methylene blue at TA versus CA containing films increases disproportionately with longer integration times (Figure 2.5). Thus we can use chronocoulometry to measure the charge at a DNA-modified electrode surface. Here the potential is held at the reduction potential of MB^+ and charge is accumulated over the course of the measurement. MB^+ should be turned over faster in a perfectly matched film than in a mismatched film, resulting in greater mismatch discrimination. In fact, using 0.5 μM MB^+ and 2.0 mM ferricyanide, 5 second potential steps to –350 mV gave faradic charges of 18 and 3 μC, respectively, for matched versus mismatched duplexes. Increased sampling times continue to increase the differentiation of signals obtained with mismatched versus paired complements.

Furthermore, using chronocoulometry, not only can thermodynamically unstable DNA mismatches be detected, but also due to

Figure 2.5. Chronocoulometry of 2.0 mM $Fe(CN)_6^{3}$ plus 0.5 μM MB^+ at a gold electrode modified with the thiol-terminated sequence SH-5'-AGTACAGTC*A*TCGCG-3' hybridized to a fully basepaired complement (red) and a complement that features an A opposite the underlined C (black), and a complement that features a G opposite the italicized *A* (blue).



repeated sampling of a particular sequence (presumably), it is now possible to detect a GA mismatch monitoring the electrocatalytic reduction of methylene blue (Figure 2.5). This is not possible using regular cyclic voltammetry (46), photoinduced electron transfer methods (39), or traditional hybridization schemes, most likely because this thermodynamically stable purine-purine pair is sufficiently well stacked with the DNA helix to support efficient electron transfer (37).

Catalyst and scan rate dependence

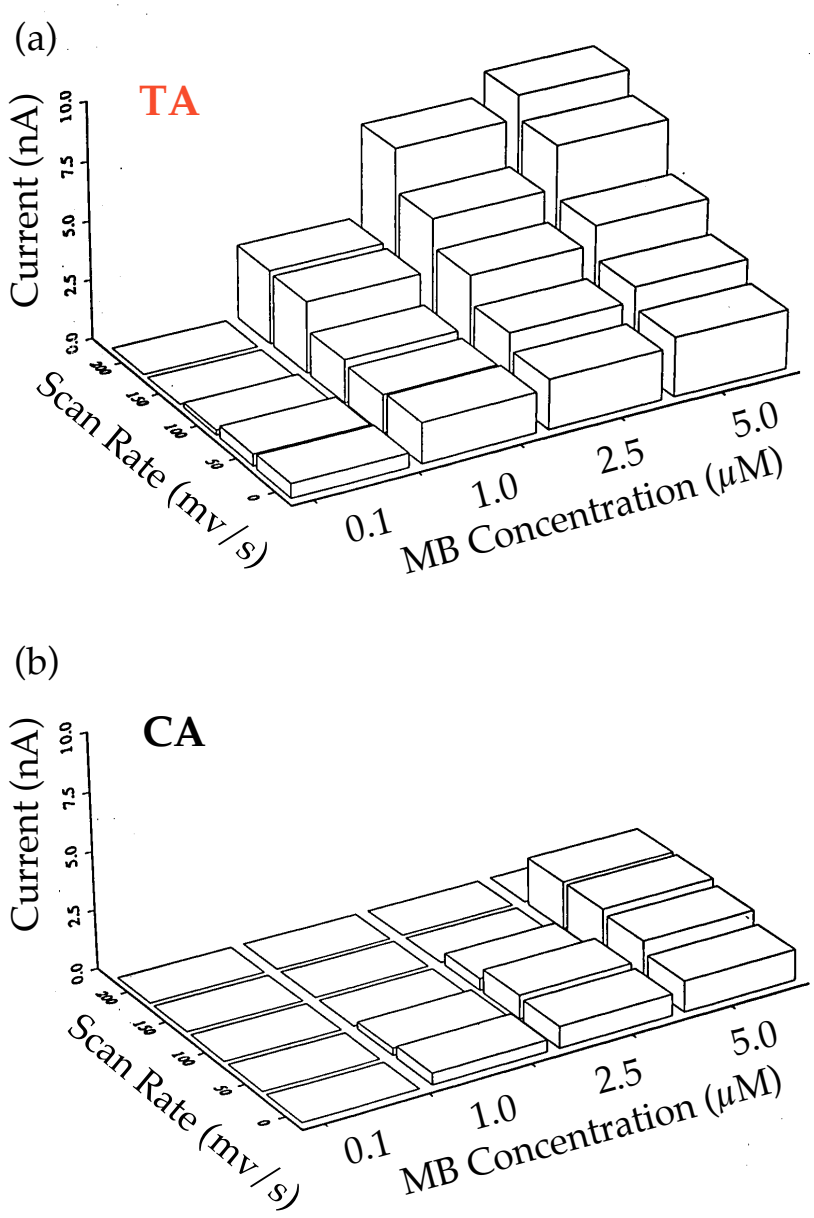
Figure 2.6 illustrates the MB^+ and scan rate dependence of this electrocatalytic reaction. There are clearly at least two trends in these data. At low catalyst concentrations (or in a mismatch film, which effectively lowers the concentration of active catalyst), larger catalytic currents are observed at slower scan rates. This can be explained analogously to the chronocoulometry described above. At slower scan rates, the electrode potential remains sufficient to reduce MB^+ longer and thus more catalytic cycles can be completed. Essentially, the charge measured increases with increased sampling time. The slower scan rate provides longer integration times and thus greater absolute signals that increase disproportionately for fully complementary versus mismatched DNA.

At high catalyst concentrations, however, this effect is overcome, and larger currents are observed at faster scan rates. At a given potential, the ratio of the concentration oxidized ([O]) to reduced ([R]) MB^+ is governed by the Nernst equation ($E = E^o - (0.0592/n) \log ([R]/[O])$ at 25°C, n = number of electrons involved in the reduction). Thus at faster scan rates, larger currents

are required to maintain this ratio of oxidized to reduced MB^+; more charge has to pass through the surface to "keep up" with the quickly changing potential. The differing scan rate dependence at differing MB^+ concentrations suggests that MB^+ may diffuse away from the monolayer to reduce ferricyanide in solution. Thus at high catalyst concentrations, as soon as one MB^+ leaves, another one immediately binds to the vacated duplex and is thus immediately reduced due to Nernstian behavior. In other words, at high MB^+ concentrations, even larger currents need to be passed to maintain the ratio of [R]/[O] bound to the electrode surface, as O is continually being replaced with R. Likewise, at low MB^+ concentrations, the ratio of [R]/[O] may be in a steady state as there is not as much excess MB^+ in solution, and thus reduced MB^+ bound to the monolayer is not constantly available.

From previous studies of the electrochemistry of MB^+ bound to DNA-modified electrodes, it is known that these DNA monolayers are not saturated with MB^+ until ~ 2 μM bulk concentration and that the stoichiometry of MB^+ to DNA is 1 to 1 independent of bulk MB^+ concentration (44). This is fully consistent with the concentration at which the scan rate dependence of electrocatalysis changes in this experiment. These data alone are insufficient to support a mechanism of electrocatalysis that involves dynamic shuttling of MB^+ between the monolayer and solution, but data to support this hypothesis are presented below in the variation of catalyst section of this chapter, as well as in Chapter 3 and Chapter 5.

Figure 2.6. Plot of the catalytic current of 2.0 mM $Fe(CN)_6^{3}$ plus various MB^+ concentrations at various scan rates at a gold electrode modified with the thiol-terminated sequence SH-5'-AGTACAGTCATCGCG-3' hybridized to (a) a fully basepaired complement (TA) and (b) a complement that features an A opposite the underlined C (CA).



Variation of catalyst

A range of intercalators and groove binders (Figure 2.1) were examined as catalysts for the reduction of ferricyanide in the detection of DNA mismatches (Table 2.1 and Figure 2.7). The efficiencies of mismatch detection using the various reporter molecules reveal several important characteristics of this assay.

Interestingly, $Ru(NH_3)_5Cl^{2+}$ (a groove binder with approximately the same potential as MB^+) is an effective electrocatalyst for the reduction of ferricyanide at DNA-modified surfaces, but is insensitive to mismatches in the film (Figure 2.7). It appears that intercalation into the DNA base stack is necessary for mismatch detection. Probes such as $Ru(NH_3)_5Cl^{2+}$ (or $Ru(NH_3)_6^{3+}$) that associate with DNA through purely electrostatic interactions (49) do not yield measurable differences in the electrochemical response in the presence of base mismatches, while the electrochemical signals obtained from the intercalators methylene blue (51) and $Ir(bpy)(phen)(phi)^{3+}$ (52) are affected by the presence of a mismatch in the film (Table 2.1). The reduction of $Ru(NH_3)_5Cl^{2+}$ likely proceeds through the facilitated diffusion of the ruthenium complex along the grooves of the immobilized helices, while the intercalated species may participate in charge transport through the stacked bases. Therefore, because single base mismatches do not affect the overall structure of the DNA helix (36-37), but do affect the local stacking of the DNA base, it seems intercalated probes may be better suited for reporting perturbations in the electronics of the base stack. This result is fully consistent with the results obtained with the direct voltammetry of intercalated versus nonintercalated probes (46) as well as mismatch detection

in other DNA-mediated charge transport assays (39). The importance of intercalation in these experiments is further investigated in Chapter 4 of this thesis.

Among the intercalators bound to DNA-modified electrodes, electrocatalysis appears to require a species that can dynamically shuttle electrons to solution-borne ferricyanide, as suggested above by the concentration and scan rate dependence. Daunomycin is a very poor electrocatalyst (Figure 2.7). This is consistent with the observation that it has a stronger affinity for DNA (53) than does methylene blue (54) (Table 2.1) and may have slower exchange dynamics which would not allow the transfer of electrons to the acceptor, ferricyanide. Furthermore, methylene blue is a smaller and more mobile species than daunomycin; this may facilitate its travel between the base stack and solution. Likewise, direct electrochemical studies of MB^+ bound to DNA-modified electrodes (44) indicate that leucomethylene blue (LB^+, reduced MB^+) has less affinity for the DNA monolayer than MB^+ (see Figure 2.3, blue trace), which should also promote the cycling of MB^+ between DNA and the solution. $Ir(bpy)(phen)(phi)^{3+}$ (52) has bulky ancillary ligands that likely prohibit its binding deep within the monolayer. As such it probably binds at the solvent exposed periphery of the monolayer and thus can easily sample the base stack and solution. In conclusion, it appears that electronic coupling with the electrode surface and the solution-borne acceptor simultaneously is important to achieve catalysis.

While these experiments begin to address some of the mechanistic issues of electrocatalysis, rotated disk electrochemistry (RDE) experiments

have been pursued as a means to understand the kinetics and mechanism of this electrocatalytic reaction in greater detail (Chapter 5).

The efficiency of charge transport through DNA films offers a new approach to DNA based sensors. Using this methodology, a broad range of point mutations can be detected within heterogeneous DNA sequences, irrespective of base composition. Monitoring electrochemical signals at addressable electrodes, as opposed to detecting fluorescence by high resolution microscopy or radioactive labeling, may provide a practical detection system for inexpensive devices to search for known mutations on targeted genes. While others have explored electrochemical schemes for the development of DNA biosensors, the reliance of these schemes on hybridization assays does not offer the same advantages as a charge transport based approach. The discovery that DNA-mediated charge transport reactions are exquisitely sensitive to the stacking of the intervening bases has provided insight into the role of the DNA base stack in modulating this reactivity. As a result, we can now exploit this sensitivity to stacking in the development of a practical assay for single base changes in DNA sequence.

Figure 2.7. Cyclic voltammograms (= 100 mV/s, A = 0.02 cm^2) of 2.0 mM $Fe(CN)_6^{3}$ plus 28 μM $Ru(NH_3)_5Cl^{2+}$ (black) or 2.0 μM MB^+ (blue) or 2.0 μM DM (red) at a gold electrode modified with the thiol-terminated sequence SH-5'-AGTACAGTCATCGCG-3' hybridized to a fully basepaired complement (a) and a complement that features an A opposite the underlined C (b).

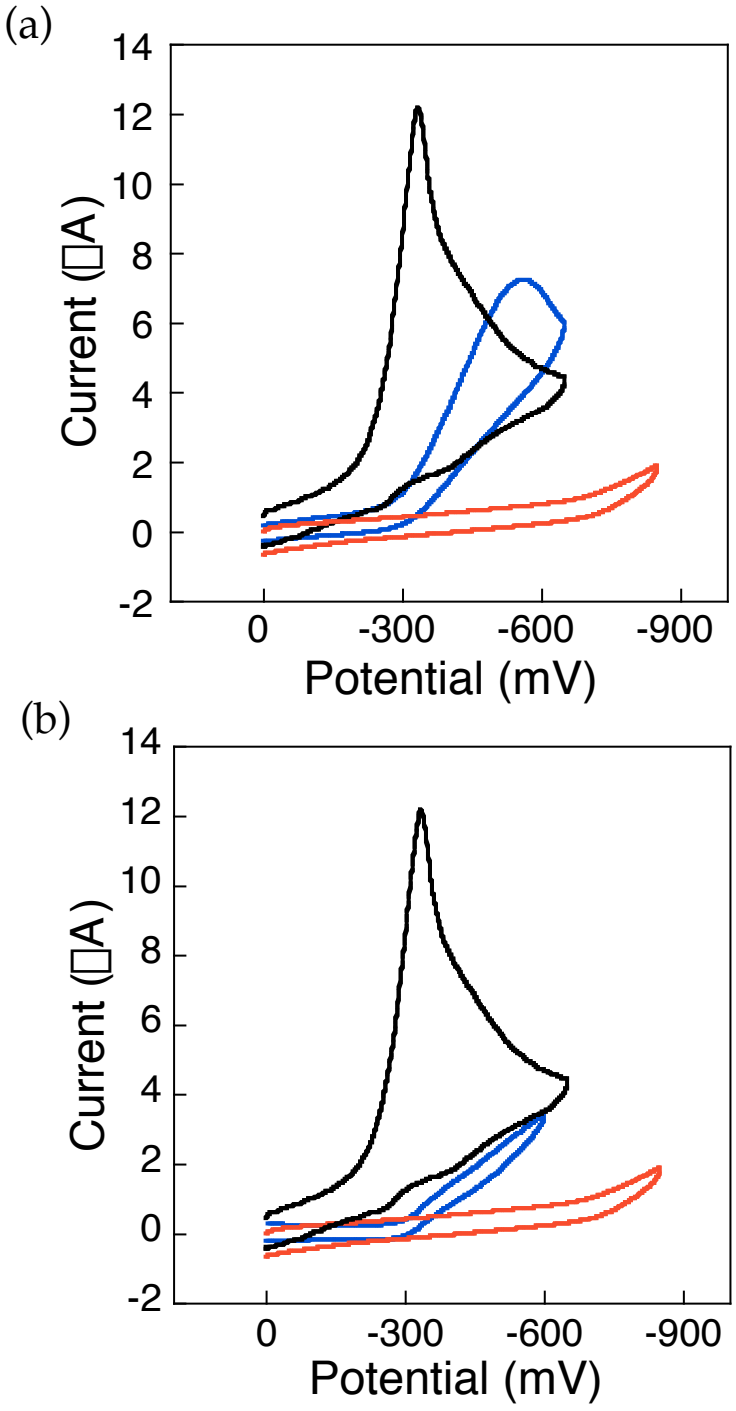
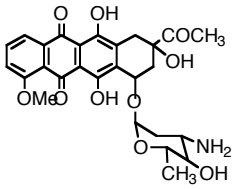
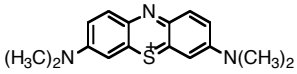
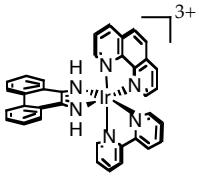
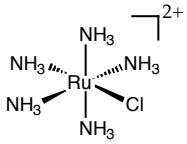


Table 2.1. Electrocatalytic mismatch detection. Dependence on DNA binding probe.

Probe	DNA-binding mode	Q_{CA}/Q_{TA}	Disassociation Constant (x 10^6 M^{-1})
	intercalation	1.0(1)	1.9(4)
	intercalation	0.17(3)	3.8(5)
	intercalation	0.25(6)	2.2(3)
	groove-binding	1.0(1)	

Sequence is SH-5'-AGTACAGTCATCGCG-3' hybridized to a fully basepaired complement and a complement that features an A opposite the underlined C. Values are based on cyclic voltammograms measured for various probes noncovalently bound to duplex-modified electrodes. Values are based on >3 trials each, and the results are comparable for experiments run side by side, or from different sample preparation. Disassociation constants are measured electrochemically by absorption isotherms, see reference 55.

SUMMARY

We have developed a method for electrochemical detection of mismatches based on charge transport through double stranded DNA monolayers on gold electrodes. As lesions to the base stack effectively block the charge transfer pathway to intercalating probe molecules, this assay reliably reports on single base mismatches even under strongly hybridizing conditions. Exploiting the intrinsic ion exchange properties of these films (possessing a 2- charge for each base pair in the duplex), it is possible to significantly enhance the sensitivity of this assay by coupling the through film electron transfer to an electrocatalytic cycle involving a negatively charged ion in solution. The resulting signals (as large as ~100 μA at 1 mm diameter electrodes and integration times of less than one minute) offer a practical means to detect DNA mutations at the single basepair level. These experiments suggest the base stack of DNA as the pathway for the charge transport and illustrate the extreme sensitivity of the π-stack to small perturbations. The self-assembly of thiol-modified duplexes on gold, coupled to efficient charge transport through the resulting films, offers an alternative approach to hybridization based DNA sensors.

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