Site-selective electron transfer from purines to electrocatalysts: voltammetric detection of a biologically relevant deletion in hybridized DNA duplexes

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Background: The one-electron oxidation of guanine nucleobases is of interest for understanding the mechanisms of mutagenesis, probing electron-transfer reactions in DNA, and developing sensing schemes for nucleic acids. The electron-transfer rates for oxidation of guanine by exogenous redox catalysts depend on the base paired to the guanine. An important goal in developing the mismatch sensitivity is to identify a means for monitoring the current resulting from electron transfer at a single base in the presence of native oligonucleotides that contain all four bases.

Results: The nucleobase 8-oxo-guanine (8G) is selectively oxidized by the redox catalyst $Os(bpy)_3^{3+/2+}$ (bpy = 2,2′-bipyridine) in the presence of native guanine. Cyclic voltammograms of $Os(bpy)_3^{2+}$ show current enhancements indicative of nucleobase oxidation upon addition of oligonucleotides that contain 8G, but not in the presence of native guanine. As expected, similar experiments with $Ru(bpy)_3^{2+}$ show enhancement with both guanine and 8G. The current enhancements for the 8G/Os(III) reaction increase in the order 8G–C ~ 8G•T < 8G•G < 8G•A < 8G, the same order as that observed for guanine/Ru(III). This site-selective mismatch sensitivity can be applied to detection of a TTT deletion, which is important in cystic fibrosis.

Conclusions: The base 8G can be effectively used in conjunction with a low-potential redox catalyst as a probe for selective electron transfer at a single site. Because of the high selectivity for 8G, rate constants can be obtained that reflect the oxidation of only one base. The mismatch sensitivity can be used to detect biologically relevant abnormalities in DNA.

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Introduction

The electron-transfer reactions of guanine nucleobases and their derivatives are of intense interest because they are probably relevant to natural mutagenesis [1,2] and they are convenient probes for understanding electronic coupling parallel to the long axis of DNA duplexes [3–6]. The redox potential of one-electron guanine oxidation, recently determined using equilibrium titration, is 1.05 V at pH 7 (all potentials versus Ag/AgCl) [7], which is in good agreement with early estimates from our group [8] and others [9,10]. The potential is lowered by as much as 0.4 V when guanine is on the 5′ side of another g uanine in a DNA duplex [3,11,12], which provides a convenient means for engineering low-potential sites into complex DNA structures [13]. The oxidation is also more favorable for 8-oxoguanine (8G) [14–17], which is a product of chemistry that follows one-electron oxidation of guanine itself and is an intermediate in G→T transversions and other natural DNA modifications [18]. The potential for the 8G nucleoside oxidation is approximately 0.85 V in nonaqueous solution [14], and, in fact, an electrochemical detection scheme is normally used to detect small quantities of 8G against a large background of normal guanine [19]. We report here on the use of 8G to follow changes in the DNA secondary structure at a single site using cyclic voltammetry. The observed electron-transfer kinetics provide the basis for an estimate of the potential of 8G oxidation in duplex DNA in aqueous solution.

The majority of guanine electron-transfer reactions studied so far involve photochemically generated oxidants that abstract single electrons [3–6]. We have shown that these reactions can also be studied in the ground state by electrochemistry at potentials (~1.1 V) that can be accessed at metal oxide electrodes in neutral solution [8,20–22]. Electrochemistry is attractive for studying these reactions because it is more sensitive than optical methods. Uncatalyzed electrochemical guanine oxidation is observed only when DNA is strongly adsorbed to carbon electrodes [23]. Metal oxide electrodes, which do not adsorb DNA appreciably [24], show no electrochemical response for direct guanine oxidation; however, electron transfer near the thermodynamic potential can be monitored electrochemically using a redox catalyst that is

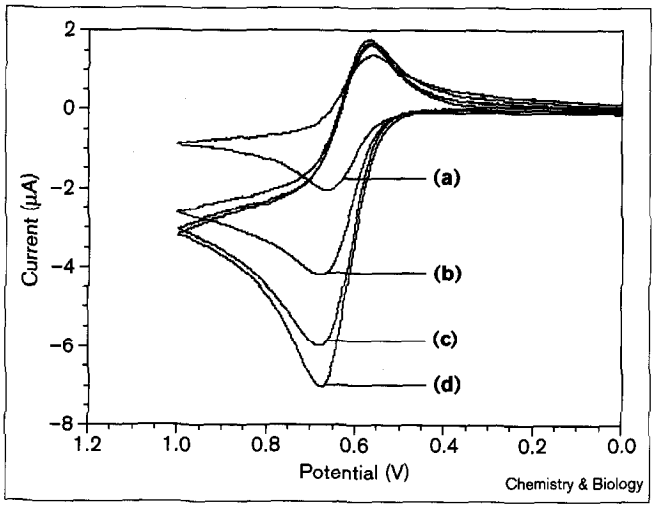
a simple one-electron transfer agent, such as $Ru(bpy)_3^{3+/2+}$ ($E_{1/2}$ = 1.07 V; bpy = 2,2′-bipyridine) [21]. As we have discussed previously, the rate constant for the oxidation of guanine by $Ru(bpy)_3^{3+}$ can be determined by digital simulation of cyclic voltammograms of $Ru(bpy)_3^{2+}$ measured in the presence of DNA; at high salt concentrations (700 mM NaCl), the simulation requires a two-step EC′ mechanism [8,22]:

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^- \quad (1)$$

$$Ru(bpy)_3^{3+} + DNA(G) \rightarrow Ru(bpy)_3^{2+} + DNA(G^+) \quad (2)$$

We have shown previously that the rate constant for equation 2 can be determined independently using pulsed electrochemical techniques and homogeneous stopped-flow spectrophotometry, which give values in good agreement with those from cyclic voltammetry [8]. In oligonucleotides containing a single guanine, the rate constant for equation 2 is different for single-stranded DNA and duplexes by a factor of 150, which arises primarily from changes in the solvent accessibility of the guanine [8]. Furthermore, improper hybridization to produce mismatches at guanine gives rate constants that are between those of duplex and single-stranded guanine; all of the possible mismatches give rate constants that can be distinguished using cyclic voltammetry.

Figure 1



Cyclic voltammograms of $Os(bpy)_3^{2+}$ with double-stranded or single-stranded oligonucleotides (100 μM in 8-oxodeoxyguanosine nucleotide concentration). All hybridizations were carried out in 50 mM sodium phosphate (pH 7.0) with 780 mM NaCl. All scan rates shown were 250 mV/s. **(a)** 50 μM $Os(bpy)_3^{2+}$ alone. **(b)** 50 μM $Os(bpy)_3^{2+}$ with a fully hybridized, matched, double-stranded oligonucleotide (5′-ATGAAG-T**8G**AAGTTTT)•(3′-TACTTCA**C**TTCAAAA). **(c)** 50 μM $Os(bpy)_3^{2+}$ with a fully hybridized, mismatched, double-stranded oligonucleotide (5′-ATGAAGT**8G**AAGTTTT)•(3′-TACTTCA**A**TTCAAAA). **(d)** 50 μM $Os(bpy)_3^{2+}$ single-stranded oligonucleotide (5′-ATGAAGT**8G**AAGTTTT).

We have suggested that the mismatch dependence of the rate constant for equation 2 can serve as a sensing scheme for mismatches and other secondary structure distortions in fully hybridized duplexes [8,25]. Other means for detecting mismatches involve modification of the mismatch site with small molecules or enzymes that specifically recognize the mismatch followed by analysis on sequencing gels [26,27]. To realize an electron-transfer scheme that does not involve molecular recognition, we need a method for following the secondary structure at a specific site in the presence of other guanines that are naturally in the sequence [25,28]. This method is provided by the use of 8G as a secondary structure probe with $Os(bpy)_3^{2+}$, which has a much lower redox potential ($E_{1/2}$ = 0.62 V) than $Ru(bpy)_3^{2+}$, as the redox catalyst. We and others have previously shown that osmium(III) does not oxidize guanine in DNA polymers [24,29,30]; we therefore suspected that $Os(bpy)_3^{3+/2+}$ could selectively catalyze the oxidation of 8G in the presence of guanine:

$$Os(bpy)_3^{2+} \rightarrow Os(bpy)_3^{3+} + e^- \quad (3)$$

$$Os(bpy)_3^{3+} + DNA(8G) \rightarrow Os(bpy)_3^{2+} + DNA(8G^+) \quad (4)$$

We report here that cyclic voltammograms of $Os(bpy)_3^{2+}$ exhibit current enhancement, consistent with equation 4, when 8G is present but not for normal guanine, providing a means for detecting mismatch-selective oxidation at a single base in oligonucleotides containing guanine.

Results

Mismatch-selective electron transfer

Shown in Figure 1 are cyclic voltammograms of $Os(bpy)_3^{2+}$ with and without oligonucleotides containing a single 8G (and three underivatized guanines). The current enhancement is significantly larger for the single-stranded oligomer (Figure 1d) than when hybridized to its complement (Figure 1b), and the duplexes with mismatches at the 8G give intermediate degrees of current enhancement (the voltammogram for the 8G•A mismatch is shown in Figure 1c; other mismatches give current enhancements between that of 8G•A and 8G–C). These secondary structure effects mirror those obtained with $Ru(bpy)_3^{2+}$ and underivatized guanine [8]. The rate constants for equation 4 obtained from simulation of cyclic voltammograms are shown in Table 1. Also shown in Table 1 are earlier data for the analogous reaction of guanine with $Ru(bpy)_3^{3+}$ under the same conditions [8]. The rate of oxidation of 8G in the perfect G–C duplex by Os(III) is approximately an order of magnitude faster than that of guanine by Ru(III).

The lack of an effect of the presence of three native guanines in the structure demonstrates that background oxidation of guanine by $Os(bpy)_3^{3+}$ is slow, as expected [24,29,30]. To further demonstrate that only oxidation

Table 1

Rate constants for oxidation of guanine and 8-oxo-guanine in oligonucleotides by $Ru(bpy)_3^{3+}$ and $Os(bpy)_3^{3+}$.

Oligonucleotide	k Os^{3+}/8G (M^{-1} s^{-1})*	k Ru^{3+}/G (M^{-1} s^{-1})†
G–C pair	2.2×10^4	1.2×10^3
G•T mismatch	3.8×10^4	5.1×10^3
G•G mismatch	6.3×10^4	1.0×10^4
G•A mismatch	1.6×10^5	1.9×10^4
G single-stranded	1.4×10^6	1.8×10^5

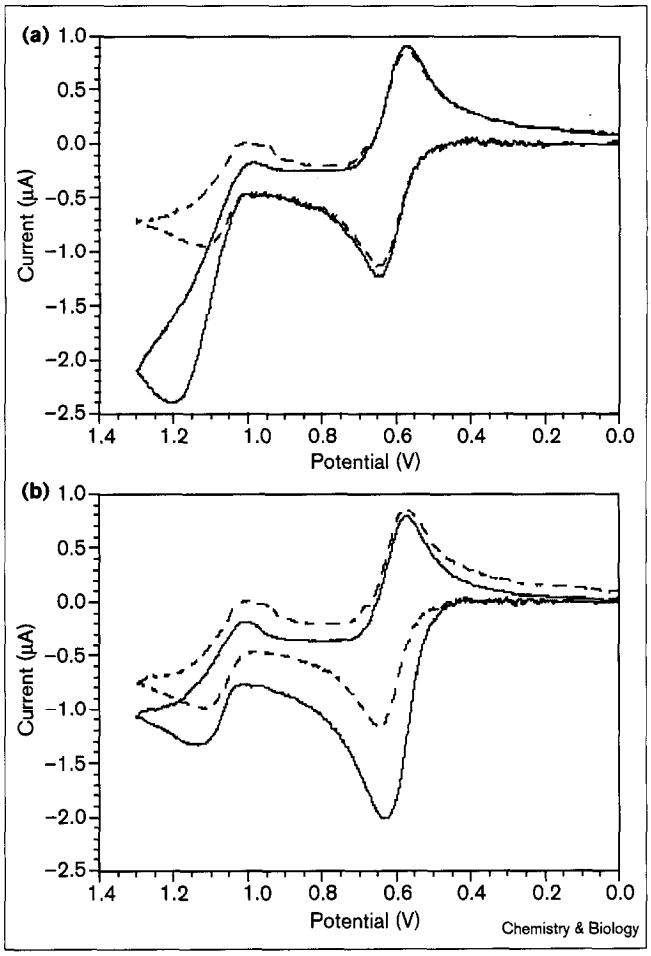
*Rate constants for equation 4 determined from digital simulation of cyclic voltammograms of $Os(bpy)_3^{2+}$ in the presence of the indicated oligonucleotides. Measurements were made on the sequence 5′-d[ATG-AAG-T8GA-AGT-TTT], which was used either as a single strand or hybridized to a complementary strand (5′-d[AAA-ACT-TXA-CTT-CAT] where X is the indicated nucleotide). Values are averages of rate constants obtained from five trials with scan rates of 25 mV/s and 250 mV/s at 780 mM NaCl, 50 mM phosphate buffer, pH 7. Error limits and other conditions as in [8]. †Rate constants for equation 2 from [8].

of 8G occurs with $Os(bpy)_3^{3+}$, cyclic voltammograms were measured on mixtures of $Os(bpy)_3^{2+}$, which we suspect only oxidizes 8G, and $Ru(bpy)_3^{2+}$, which should be able to oxidize both 8G and normal guanine. Figure 2a shows the cyclic voltammograms of $Os(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$ with and without an oligonucleotide that contains guanine but no 8G. This reaction gives current enhancement only for the $Ru(bpy)_3^{3+/2+}$ wave and not for the $Os(bpy)_3^{3+/2+}$ wave, showing that Os(III) is not an effective oxidant of guanine under conditions where Ru(III) oxidizes guanine effectively. Identical results were obtained when a sequence containing a 5′-GG doublet and a 5′-GGG triplet were used (even when the concentration of GGG was the same as that of 8G in Figure 2b); this oligonucleotide produced a large enhancement in the Ru(III/II) wave but no detectable enhancement in the Os(III/II) couple. When an oligonucleotide containing 8G is used, current enhancement for both waves is observed (Figure 2b). Thus, Os(III) oxidizes only 8G at detectable rates, whereas Ru(III) is an effective oxidant of both 8G and guanine, as expected from the estimated redox potentials of the species involved [14]. Finally, the rate constants for an oligonucleotide containing one 8G and no guanines were compared with those in Table 1, which are for an oligonucleotide containing one 8G and three guanines. The rate constants were identical within experimental error for both oligonucleotides, suggesting that any additional enhancement in the rate from oxidation of guanine by Os(III) could not be detected.

Detection of a physiologically important deletion

Although the results in Figure 1 show that single-base mismatches can be detected in model oligonucleotides,

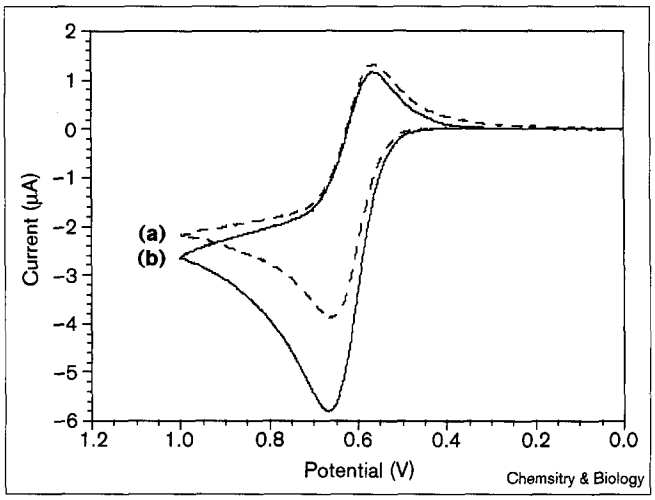
Figure 2



Cyclic voltammograms of $Ru(bpy)_3^{2+}$ and $Os(bpy)_3^{2+}$ with single-stranded oligonucleotides at a scan rate of 25 mV/s in 50 mM sodium phosphate (pH 7.0) with 780 mM NaCl. **(a)** Voltammograms of 50 μM $Ru(bpy)_3^{2+}$ and 50 μM $Os(bpy)_3^{2+}$ alone (dashed line) or in the presence of single-stranded oligonucleotide (5′-AAATATA**G**TATAAAA) containing 100 μM deoxyguanosine (solid line). A similar result with a large enhancement of the $Ru(bpy)_3^{2+}$ wave and no detectable enhancement of the $Os(bpy)_3^{2+}$ wave was also observed with (5′-TC**G** GAT **G**TT **G**TG **GG**TCAG C**G**C CT**G** ATA CT**G** TAC CAT T); no enhancement in the $Os(bpy)_3^{2+}$ wave was observed up to 100 μM oligonucleotide (1.2 mM guanine) with 50 μM $Os(bpy)_3^{2+}$ and 50 μM $Ru(bpy)_3^{2+}$. **(b)** Voltammograms of 50 μM $Ru(bpy)_3^{2+}$ and 50 μM $Os(bpy)_3^{2+}$ alone (dashed line) or in the presence of single-stranded oligonucleotide (5′-AAATATA**8G**TATAAAA) containing 100 μM 8-oxo-deoxyguanosine (solid line).

the ability to detect the secondary-structure dependence for 8G was tested in a physiologically relevant sequence using an oligomer that is complementary to a site of common genetic mutation for cystic fibrosis (CF) [31]. The cyclic voltammograms of $Os(bpy)_3^{2+}$ shown in Figure 3 were generated with an oligonucleotide sequence from the CF gene. Deletion of a phenylalanine codon (TTT) from this gene is responsible for 70% of all cases of CF [30]. The sequence of the probe nucleotide is 5′-ATAGGAAACACC-A8GA-GATGATATTTTC, and

Figure 3



Detection of the most common deletion (–TTT) in the cystic fibrosis transmembrane conductance receptor (CFTR) gene by cyclic voltammetry. **(a)** Hybridization of the wild-type CFTR oligonucleotide with its complement containing 8-oxo-deoxyguanosine (5′-GAAAATATCATC-**TTT**-GGTGTTTCCTAT)•(5′-ATAGGAAACACC-A**8G**A-GATGATATTTTC). **(b)** Hybridization of the mutant CFTR oligonucleotide with its complement containing 8-oxo-deoxyguanosine (5′-GAAAATATCATC---GGTGTTTCCTAT)•(5′-ATAGGAAACACC-A**8G**A-GATGATATTTTC). Hybridizations of oligonucleotides (100 μM in 8-oxo-deoxyguanosine nucleotide concentration) were carried out in 50 mM sodium phosphate (pH 7.0) with 780 mM NaCl. Cyclic voltammograms were collected in the presence of 50 μM $Os(bpy)_3^{2+}$ at a scan rate of 250 mV/s.

the wild-type complement contains a 5′-TTT triplet opposite A8GA on the probe. As expected, the A8GA sequence gives a relatively small enhancement when hybridized to the full-length (+TTT) complement (Figure 3a). When hybridized to the –TTT mutant, however, the 8G is in a single-stranded A8GA bulge, which gives a significantly higher current enhancement (Figure 3b). As shown in Figure 1 and Table 1, single base mismatches also produce readily detectable changes in current enhancements, although these changes are not as large as that seen for the hybridization event in Figure 3, which forces 8G into a single-stranded bulge.

The rate constants for the oligonucleotides in Figure 3 were also determined by digital simulation. For the A8GA oligonucleotide hybridized to the wild-type (+TTT) complement, the rate constant was 3.0×10^4 M^{-1} s^{-1}, which, as expected, is similar to the value in Table 1 for an 8G•T mismatch. For the A8GA probe hybridized to the –TTT mutant, the rate constant is 3.7×10^5 M^{-1} s^{-1}, which is over an order of magnitude higher. The rate constant for the single-stranded form of the A8GA probe is 4.9×10^5 M^{-1} s^{-1}, suggesting that the 8G included in the three-base bulge is nearly as reactive as the fully single-stranded form.

Discussion

Electron-transfer kinetics and potential

We have shown previously for Ru(III) that the rate constant for guanine oxidation depends on the driving force in the manner expected from Marcus theory [8,22]:

$$k = \upsilon \exp[\beta(r - r_0)]\exp[-(\Delta G^0 + \lambda)^2/4\lambda RT] \quad (5)$$

where υ is the rate constant in the diffusion-controlled limit, r is the distance between reactant and product in the activated complex, r_0 is the distance of closest approach of the reactant and product, and β describes the influence of the intervening medium [32,33]. Using this analysis for the reaction of guanine with Ru(III) (equation 2), we have previously demonstrated that the plot of RT ln k versus ΔG° at low driving forces (i.e. $\Delta G^\circ << \lambda$) is linear with a slope of 0.5, as expected [8]. Assuming a λ of 1–1.5 eV, which is typical for reactions similar to equation 2, this analysis suggested that the potential for the $G^{+/0}$ couple was 1.1 ± 0.1 V, which was in good agreement with the value later determined using equilibrium titration [7]. If we now assume that the difference in the rate constants for the Ru(III)/guanine (equation 2) and Os(III)/8G (equation 4) reactions is solely due to a change in the driving force, we estimate the redox potential for $8G^{+/0}$ in hybridized DNA to be 0.5 ± 0.1 V.

Because osmium and ruthenium are nearly identical in size, the reorganizational energies of the two couples can be considered identical for our purposes. If the reorganizational energy for oxidation of 8G is lower than for oxidation of guanine, however, then the thermodynamic potential could be somewhat higher than our estimated value of 0.5 V, according to equation 5. Nevertheless, the ability to observe the 8G oxidation as a catalytic enhancement in the Os(III/II) couple suggests that the potential cannot be significantly higher than 0.62 V, which is the potential for the Os(III/II) couple. The rate constants for the quenching of C_{60} by the 8G and guanine nucleosides in nonaqueous solution are 1.1×10^8 M^{-1} s^{-1} and 3.3×10^6 M^{-1} s^{-1}, respectively [14]; this difference can be accounted for solely by the driving force dependence predicted by the Rehm–Weller equation, which assumes no change in λ for the two reactions. The estimate for the redox potential of the free 8G nucleoside in nonaqueous solvent is about +0.85 V, compared with 1.26 V for the guanosine nucleoside under the same conditions [14]. This difference of 0.4 V is somewhat lower than our difference of 0.5–0.6 V, which may result from differences in the degree to which the oxidized forms are stabilized by incorporation in the double helix. This idea is consistent with the somewhat smaller change in rate constant observed upon complete hybridization of single-stranded 8G (factor of 60) than for guanine (factor of 150). Deprotonation at N1 probably plays a role in the oxidation of both nucleotides, and differences in the pK_a values of the two

species may also contribute to the apparent difference in the kinetics for equations 2 and 4 [9].

Guanine multiplets are particularly good donors, and numerous studies have shown that the 5′-G of GG or GGG sequences is significantly easier to oxidize than isolated guanines [5,11–13,34]. We show in Figure 2, however, that although a sequence with GG and GGG sequences is readily oxidized by Ru(III), there is still no detectable current enhancement with Os(III). The potential for 8G must therefore be significantly lower than that of the 5′-G of a guanine multiplet. As our estimate for the potential of (isolated) 8G is 0.5 ± 0.1 V, the guanine multiplet must have a potential of 0.8–0.9 V. This result shows that 8G can be used to probe secondary structure in diverse sequences containing many guanines, including guanine multiplets. Using equation 5, we can calculate an expected rate constant for the reaction of Os(III) with isolated guanine on the basis of only the driving force difference, which gives an upper limit for the Os(III)–guanine rate constant for duplex DNA of 1 M^{-1} s^{-1}. The difference in redox potential of guanine and 8G is therefore sufficient to give > 10^4-fold greater selectivity for 8G, according to equation 5.

Relationship to other guanine electron-transfer studies

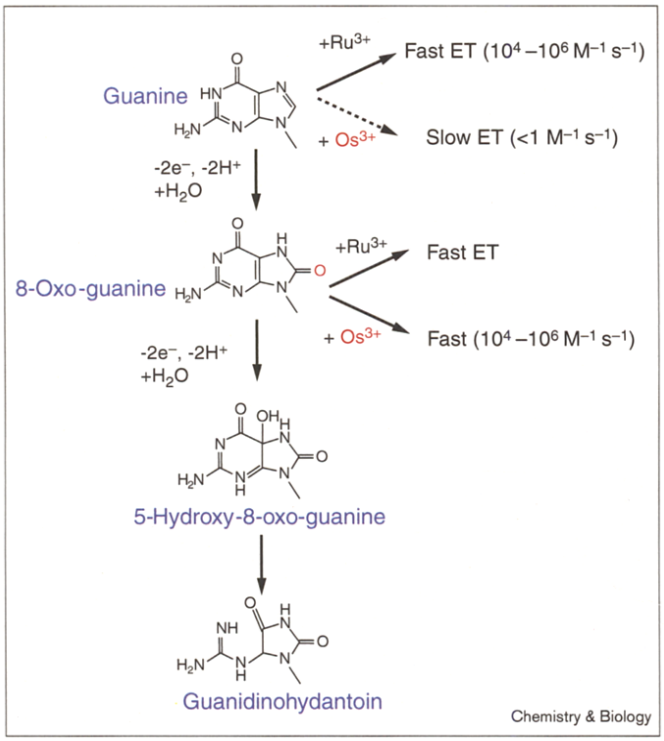
The other studies that provide absolute rate constants for guanine electron transfer generally involve photochemically excited oxidants [3–6] or a high-energy acceptor generated by an initial photoinduced electron transfer with an exogenous quencher [35]. These oxidants are either intercalated in DNA or covalently attached at a site that is remote from the guanine donor, so these studies are generally aimed at understanding the electronic coupling of the photochemical oxidant and the guanine donor along the long axis of the DNA duplex. In contrast, our studies involve thermal electron transfer by exogenous oxidants that, because the ionic strength is high, are not bound to DNA upon electrochemical activation. The reaction therefore involves a collision between the electrochemically activated catalyst and the DNA. We have therefore assumed that the mismatch dependence arises primarily from differences in the donor–acceptor distance (i.e. r in equation 5) in the activated complex that gives rise to electron transfer [8]. This analysis is oversimplified in neglecting changes in driving force and reorganizational energy as a function of sequence context and secondary structure. In fact, G•A mismatches exhibit a higher reactivity than G–C pairs [36], as discussed below, which must also contribute to the enhancement for 8G•A seen in Figure 1. Such an analysis also assumes that the number of productive collisions is the same for different mismatches. The rate constants given here are all in terms of 8G concentration, which in these cases is the same as the DNA strand or duplex concentrations, because there is only a single 8G.

Another example of mismatch-sensitive electron transfer has been obtained from normal guanine to Ru(III) that is tethered to the DNA helix at long range [36]. The reaction was monitored by cleavage of the guanine on a gel following base work-up. This reaction is faster for G•A mismatches but not other mismatches at guanine. As the reaction is long range along the DNA long axis, the origin of this effect is likely to be a somewhat more favorable electron transfer for G•A mismatches than for other guanine environments. This effect probably contributes to our faster electrochemical rate constants for G•A mismatches with Ru(III) and 8G•A mismatches with Os(III) given in Table 1.

Other examples of selective electron transfer in the presence of normal guanine include the use of guanine multiplets, which are significantly easier to oxidize at the 5′-guanine than isolated guanines [5,11–13,34]: 7-deazaguanine, which is a better electron donor than guanine [4,37]; thymine dimers, which are apparently easily oxidized [38]; and 8G [3,14]. In the majority of these cases, the selectivity was apparent in the higher cleavage yields of these sites on high-resolution sequencing gels, and in all of these cases, the goal was to determine the electronic coupling along the long axis of DNA rather than to understand the perpendicular escape of the electron to an exogenous catalyst. Some of the issues surrounding electronic coupling along the DNA long axis remain somewhat controversial [39–41]; however, there seems to be agreement on the idea that authentic oxidizing equivalents on guanines can migrate rapidly along the DNA helix and become localized on low-energy traps such as those listed above [3,5,13,39,40,42–44]. One relevant study not aimed at understanding the long-axis coupling is that of selective Ir(IV) oxidation of 8G, which provides a convenient gel electrophoretic assay for one-electron guanine oxidation [15]. Further studies by the same group on selective 8G oxidation show new types of base misincorporations via the 8G oxidation products 5-hydroxy-8-oxoguanine and guanidinohydantoin [18].

Oxidative charge migration in DNA provides a means for the redistribution of damage once the initial oxidation has occurred [3,5,13]. In contrast, our studies seek to understand the parameters that describe how the electron is removed in the first place, because much of the initial biological DNA oxidation probably occurs through reaction with exogenous small molecules [1,2]. In this case, a low-energy donor provides a means for the selective formation of oxidizing equivalents upon reaction with an exogenous oxidant. We had suggested earlier that 7-deazaguanine should be an inert site that is not oxidized, on the basis of our observation of a slow reaction in solution between 7-deazaguanosine triphosphate and $Ru(bpy)_3^{3+}$ [45]. Recent studies, however, clearly show that this modified base is an excellent electron donor when incorporated in DNA [4,37]. We are presently attempting to determine whether this difference arises from a large effect of incorporating the 7-deazaguanine in the double helix or

Figure 4



Oxidation pathway for guanine based on [18] showing the electron-transfer reactivity reported here. Guanine is oxidized by two electrons to 8-oxo-guanine and undergoes rapid electron transfer to $Ru(bpy)_3^{3+}$ but not to $Os(bpy)_3^{3+}$. The 8-oxo-guanine derivative can be further oxidized to 5-hydroxy-8-oxo-guanine and undergoes rapid electron transfer to both $Ru(bpy)_3^{3+}$ and $Os(bpy)_3^{3+}$. The 5-hydroxy-8-oxo-guanine product undergoes further reaction to guanidinohydantoin. An alternative mechanism has been suggested in [16]. ET, electron transfer.

from an artifact that masks the reaction of the free triphosphate with catalyst.

Relationship to natural DNA damage

A likely pathway for natural guanine oxidation is shown in Figure 4 [1,18]. One-electron oxidation of guanine leads ultimately to 8G, which requires addition of a water molecule, loss of an additional electron and double deprotonation [16,36]. These events must occur on a much slower time scale following initial electron transfer. The 8G base is directly mutagenic and leads to G→T transversions, because 8G preferentially pairs with adenine [1]. Further oxidation of 8G leads ultimately to 5-hydroxy-8G and guanidinohydantoin [18], although other studies suggest an alternative pathway involving rupture of the glycosidic bond followed by oxidation of the sugar to a ribonolactone [16]. The reactions producing 5-hydroxy-8G and guanidinohydantoin are initiated by single electron transfer and therefore are probably most relevant to the work described here. At least one of these latter products leads to G→T and G→C transversions via misincorporation of dAMP and dGMP across from the oxidized species [18]. The results here parallel the natural pathway in that the native guanine base is oxidized only by the more strongly oxidizing Ru(III), whereas the more reactive 8G can be oxidized by both Ru(III) and Os(III). Continued description of the initial electron transfers should lead to a more detailed understanding of the parameters by which mutagenic reactions such as those shown in Figure 4 are realized via single electron transfers from nucleobases to exogenous small molecules.

Significance

The issues surrounding one-electron oxidative damage of DNA are of increasing importance in understanding the fundamental steps in mutagenesis, especially G→T transversions that occur via 8-oxo-guanine (8G). The potential for oxidative hole migration is of importance in understanding the localization of damage, but an equally pertinent question is the means by which electrons are abstracted by exogenous small molecules initially to create the migrating holes. We have previously shown that the kinetics of one-electron guanine oxidation by $Ru(bpy)_3^{3+}$ (bpy = 2,2′-bipyridine) can be determined conveniently from catalytic current enhancements in cyclic voltammetry. Here we show that 8-oxo-guanine can be selectively oxidized by $Os(bpy)_3^{3+}$ in the presence of guanine; addition of normal guanine to $Os(bpy)_3^{2+}$ gives no detectable current enhancement, whereas addition of 8-oxo-guanine gives current enhancements indicative of one-electron oxidation by Os(III). These rate constants are highest for single-stranded 8-oxo-guanine, lowest for hybridization to C or T, and intermediate for 8G–purine mismatches. In addition to providing kinetic and thermodynamic parameters on the 8-oxo-guanine oxidation, this approach provides a method for selectively detecting distortions at 8-oxo-guanine in the presence of guanine. In particular, we show that a –TTT deletion responsible for cystic fibrosis can be detected by probing the deletion site with an 8-oxo-guanine selectively placed on the opposite strand.

Materials and methods

Reagents and DNA

Synthetic oligonucleotides were synthesized by the University of North Carolina Lineberger Comprehensive Cancer Nucleic Acids Core Facility. Oligonucleotides were purified by two successive rounds of ethanol precipitation, resuspended in dH_2O, and stored at −20°C. Oligonucleotide concentrations were determined spectrophotometrically. $Os(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$ were purchased from Aldrich Chemical Company. All other reagents were of analytical grade.

Cyclic voltammetry

Oligonucleotides were hybridized as follows: 10 nmol of either 8-oxo-deoxyguanosine-containing or deoxyguanosine-containing oligonucleotides in 90 µl 50 mM sodium phosphate (pH 7.0, 780 mM NaCl) were combined with a slight excess of their complements, heated to > 95°C for 5 min, and then slow cooled to room temperature (25°C) over 2.5 h. An aliquot (10 µl) of 500 µM stock solutions of $Ru(bpy)_3^{2+}$ or $Os(bpy)_3^{2+}$ was then added to the cooled hybridized solutions to give a final concentration of 50 µM. The final concentration of guanine-containing oligonucleotide was 100 µM in all experiments. Cyclic voltammograms were collected using a BAS100B electrochemical

analyzer with a single-compartment voltammetric cell equipped with an indium tin oxide (ITO) working electrode (area = 0.11 cm^2), a Pt-wire counter electrode, and an Ag/AgCl reference electrode. For solutions containing $Os(bpy)_3^{2+}$, cyclic voltammograms from 0–1.0 V were taken at two different scan rates (0.25 mV/s and 250 mV/s). For solutions containing both $Ru(bpy)_3^{2+}$ and $Os(bpy)_3^{2+}$, cyclic voltammograms from 0–1.4 V were collected at similar scan rates. Background scans of buffer alone and metal complexes in buffer alone were collected and subtracted from scans of hybridized oligonucleotides. Guanine oxidation rate constants were determined by digital simulation of cyclic voltammetric data to a two-step mechanism using the DigiSim software package (Bioanalytical Systems, Inc.) as described in [8].

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