Proton-Coupled Electron Transfer in Duplex DNA: Driving Force Dependence and Isotope Effects on Electrocatalytic Oxidation of Guanine

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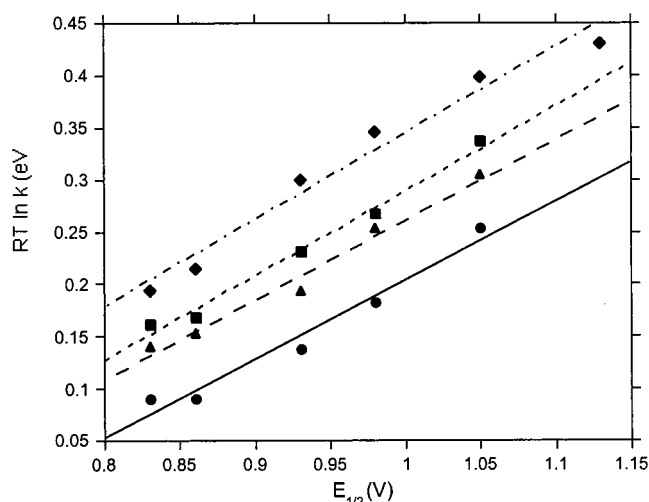*Department of Chemistry, University of North Carolina Chapel Hill, North Carolina 27599-3290*

The electron-transfer reactions of guanine are central to understanding both hole transfer along DNA[1–4] and biological damage to nucleic acids.[5–8] Guanine oxidation has been studied by photochemical methods where the rate of reaction can be monitored in real time,[9,10] recently as fast as the picosecond time scale.[4,11] In addition, guanine oxidation can be followed site-specifically by high-resolution gel electrophoresis where yields of piperidine-labile lesions indirectly provide rates.[1–3,12] A key question in these studies is the role of deprotonation of N1 of the guanine radical cation following electron transfer.[13,14] In the fast photochemical studies, transient spectroscopy shows that the oxidized guanine remains protonated immediately following electron transfer in duplex DNA.[11] In contrast, steady-state absorption spectroscopy shows that the oxidized guanine in mononucleotides is deprotonated at equilibrium in neutral solution and that the guanine radical cation has a pK_a of 3.9.[15] Recent transient spectroscopy studies show an isotope effect on oxidation of guanine mononucleotides, consistent with coupling of the deprotonation to the guanine electron transfer in bimolecular reactions.[9] Here we report on the driving force dependence of the ground-state oxidation of guanine on the millisecond time scale that indicates a proton-coupled electron transfer (PCET) in mononucleotides, single-stranded DNA, and duplexes. This interpretation is supported by a kinetic isotope effect that is also invariant with the guanine secondary structure. The coupling of proton- and electron transfers has profound kinetic implications for many reactions.[16,17]

The rates of electron transfer from guanine to metal complexes based on Ru(bpy)$_3^{3+}$ (bpy = 2,2'-bipyridine) were determined by stopped-flow spectrophotometry and global analysis of the

Figure 1. Plot of $RT \ln k$ for reaction of nucleic acids with metal complexes. Rate constants are shown for reactions with guanosine 5'-triphosphate (◆), single-stranded **1** (■), duplex **1** (▲), and herring testes DNA (●). Metal complexes used are given in Supporting Information.

time-dependent data over a wide range of wavelengths.[18–20] The substrates examined were herring testes DNA (double-stranded), 2'-deoxyguanosine-5'-triphosphate, the oligonucleotide d[5'-GCA GTA GCA TGT GAC GAG TCG] (**1**), and **1** hybridized to its Watson–Crick complement. The data were fit to a model consisting of equal concentrations of two noninterconvertible populations of Ru(bpy)$_3^{3+}$, as we have done previously for this reaction[20] and others have done for many similar reactions with nucleic acids.[10,21] When derivatives of Ru(bpy)$_3^{3+}$ with different $E_{1/2}$ values were used, the rate constant increased with the driving force, as expected. According to Marcus theory, a plot of $RT \ln k$ versus the driving force should be linear with a slope of 0.5;[22,23] however, the variation of $RT \ln k$ for guanine was 0.8 ± 0.1 for all four substrates studied (Figure 1). Thus, there is an abnormally high slope for the guanine–Ru(bpy)$_3^{3+}$ reaction that is observed *in single-stranded, duplex, and mononucleotide forms.* The effect of the driving force on the rate constant was identical for both populations of oxidant used in the fitting; the plots shown in Figure 1 are for the weighted average rate constants.

An analysis published by Ram and Hupp provides an explanation for the large slope observed in Figure 1.[24] Consider the mechanism where electron transfer to form the guanine radical cation precedes the deprotonation of the radical cation to form the neutral radical:

$$G + Ru(bpy)_3^{3+} \rightleftharpoons G^{+\bullet} + Ru(bpy)_3^{2+} \qquad (1)$$

$$G^{+\bullet} \rightleftharpoons G(-H)^{\bullet} + H^+ \qquad (2)$$

According to Ram and Hupp,[24] if eq 1 is rate-limiting, then the slope of the Marcus plot should be the normal value of 0.5, but
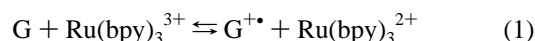
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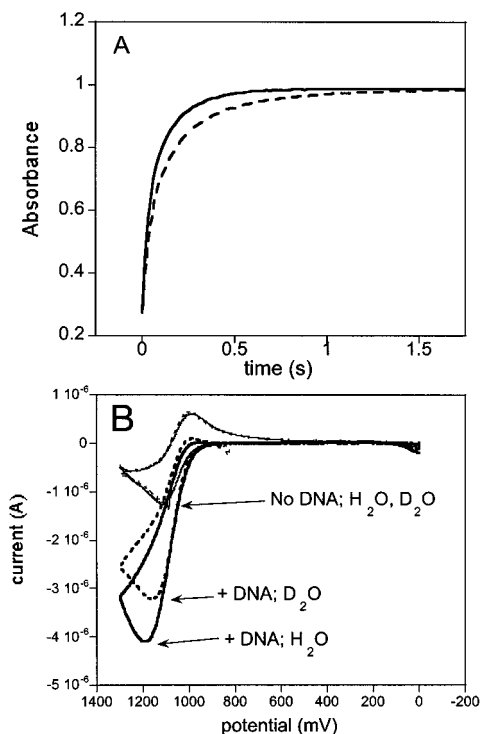
Figure 2. (A) Results of stopped-flow spectrophotometry for the reaction of 780 μM herring testes DNA with 27 μM Ru(bpy)$_3^{3+}$ in 50 mM sodium phosphate, 800 mM sodium chloride in H$_2$O, pH = 7 (solid) and D$_2$O, pD = 7 (dashed). Rate constants obtained from these data were $k_{\text{H}_2\text{O}}$ = (3.0 ± 0.52) × 10^4 M^{-1} s^{-1} and $k_{\text{D}_2\text{O}}$ = (1.6 ± 0.4) × 10^4 M^{-1} s^{-1}; $k_{\text{H}}/k_{\text{D}}$ = 2.1. (B) Cyclic voltammograms (25 mV/s) of 50 μM Ru(bpy)$_3^{2+}$ alone and with 1 mM herring testes DNA in H$_2$O (solid) and D$_2$O (dashed). Voltammograms were performed using an indium tin oxide working electrode with a Pt counter electrode and an Ag/AgCl reference. Digital simulation gave rate constants of $k_{\text{H}_2\text{O}}$ = (1.7 ± 0.34) × 10^4 M^{-1} s^{-1}, $k_{\text{D}_2\text{O}}$ = (8.9 ± 1.3) × 10^3 M^{-1} s^{-1}; $k_{\text{H}}/k_{\text{D}}$ = 2.0.

if eq 2 is rate-limiting, the slope should be 1.0. If the rates of eqs 1 and 2 are comparable, then a value between 0.5 and 1.0 would be observed.[25] Thus, the slope of 0.8 observed in Figure 1 could be due to a proton-coupled electron transfer.

If deprotonation contributes to the rate-determining step in the oxidation of guanine, an isotope effect on the reaction rate would be expected. When the reaction was carried out in D$_2$O, a kinetic isotope effect of 2.1 was observed by fitting the stopped-flow spectrophotometry data (Figure 2A).[26] This isotope effect was the same for all of the guanine substrates examined (Table 1). The isotope effect was also apparent in cyclic voltammograms of Ru(bpy)$_3^{2+}$ in the presence of guanine where lower catalytic currents were observed in D$_2$O compared to H$_2$O (Figure 2B).[26] Analysis of the catalytic currents using digital simulation as described previously gave isotope effects on the oxidation rate constants identical to those from stopped-flow; the value for herring testes DNA shown in Figure 2 was 2.0. A similar isotope effect was observed in the bimolecular reaction of photoexcited 2-aminopurine with guanine mononucleotide.[9] We therefore attribute the high slope in Figure 1 to PCET.

These observations bring a number of important points to the understanding of guanine electron transfer. First, it should be noted that the reactions of Ru(bpy)$_3^{3+}$ and related complexes with guanine occur at low driving forces, close to $\Delta G° = 0$. According to Steenken and Jovanovic,[15] the steady-state potential for PCET

Table 1. Driving Force Dependence and Isotope Effects on Electron Transfer from Guanine to Polypyridyl Complexes

substrate	slope[a]	$k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$[b]	$k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$[c]
mononucleotide[d]	0.83	1.4	1.8
single-stranded **1**	0.81		
duplex **1**	0.77		
herring testes DNA	0.76	2.1	2.0

[a] Taken from Figure 1. [b] From stopped-flow spectrophotometry. [c] From digital simulation of cyclic voltammetry data. [d] 2′-Deoxyguanosine-5′-triphosphate was used for driving force studies, and 2′-deoxyguanosine-5′-monophosphate was used for isotope effect studies.

from guanine to G(−H)$^{\bullet}$ at neutral pH is 1.05 V, while the potential for oxidation to the radical cation (i.e., without deprotonation) is 1.34 V. Thus, the proton-coupled reaction of guanine with Ru(bpy)$_3^{3+}$ ($E_{1/2}$(III/II) = 1.04 V) is roughly thermoneutral, while the reaction of Ru(bpy)$_3^{3+}$ with guanine to form the protonated guanine radical cation is uphill by 300 mV. In contrast, the stilbene-4,4′-dicarboxamide chromophore of Lewis et al. has a redox potential of 1.5 V and is therefore able to oxidize guanine to the radical cation without deprotonation in a thermodynamically favorable reaction;[4,11] photoexcited 2-aminopurine is also a stronger oxidant than Ru(bpy)$_3^{3+}$.[10] Thus, the rapid electron transfer observed by Lewis et al.[4,11] does not require deprotonation. The observation of PCET as described here should be limited to low driving forces where the energy for the formation of the radical cation is not available.

The next point of note is that these results now demonstrate PCET for guanine *in duplex DNA*. Previous observations suggesting PCET for guanine have been limited to mononucleotides and single-stranded DNA.[9,27] The escape of the proton from the base pair could occur via the "breathing" reaction, which is identical to imino proton exchange and well-known by NMR to occur on the 1−100 ms time scale.[28] This reaction time is similar to those observed here for oxidation by Ru(bpy)$_3^{3+}$. This observation suggests that oxidized guanines that are in duplex DNA can deprotonate before any other follow-up chemistry could occur. This idea is supported by recent studies showing that the primary fate of guanine that has undergone one-electron oxidation is an oxazolone lesion,[5,29−31] which forms following deprotonation of the guanine radical cation. There has been a suggestion that greater yields of 8-oxo-7,8-dihydroguanine in duplex DNA result from trapping of the N1 proton inside the double helix;[7,32] our findings suggest that changes in the product distribution would arise from effects of the secondary structure on the follow-up chemistry and not on the primary PCET event. In addition to these points, the large slope observed in Figure 1 may be a general diagnostic for PCET.

Supporting Information Available: Experimental procedures and tables of rate constants (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) The precise dependence expected would be a curved plot with a limiting slope of 0.5 at low driving forces and a limiting slope of 1.0 at high driving forces. The range of driving forces investigated here likely does not allow this dependence to be resolved, resulting in a linear plot with an overall slope of 0.8.
(26) Electrochemistry experiments were performed as described in ref 20 and in the Supporting Information.

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