Electron Transfer in Tetrads: Adjacent Guanines Are Not Hole Traps in G Quartets

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Multistranded DNA structures were first observed in early crystallography studies of nucleic acids.[1,2] Guanine-rich sequences, which occur in telomeres at the ends of linear chromosomes, can form G quartets where multiple guanines are organized around a central cation in a four-stranded structure (Figure 1A).[3] An understanding of the factors that stabilize G quartets is a potential route to cancer therapeutics and insight into cell immortality.[2,4] An additional feature of DNA sequences containing adjacent guanines is that in the normal B-DNA form, the 5′-guanine of guanine multiplets is highly electron-rich,[5] and guanine multiplets are therefore traps for oxidizing equivalents generated in the DNA duplex.[6−12] We report here studies of electron transfer in an oligonucleotide that contains a repeating GGG sequence and forms a G quartet at the appropriate monovalent cation concentration.[3,13] Our results show that the guanines in G quartets are somewhat more solvent-accessible than in duplex DNA (as expected from NMR structures),[13] but that the increase in electron density seen for duplex GGG sequences is not present for the same sequence in a G quartet structure.

We have developed a method for measuring the rate constants for electron transfer from guanines in DNA based on the electrochemistry of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine).[14−16] In this approach, guanine oxidation is apparent as an enhancement in the oxidative current in the cyclic voltammogram of $Ru(bpy)_3^{2+}$, which can be simulated using DigiSim to obtain the rate constant for electron transfer from guanine to Ru(III).[14−17] Shown in Figure 1B are the cyclic voltammograms of $Ru(bpy)_3^{2+}$ alone and with two different oligonucleotides at the same concentrations of guanine.[18] Sequence **1** comprises a DNA duplex with no adjacent guanines; sequence **2** contains four GGG triplets and forms a G quartet whose structure is known from NMR.[13] As shown in the Figure, the current enhancement is slightly higher for the G quartet than for the duplex containing the same number of guanines. The average rate constants *per guanine* for each of these sequences are $k(\mathbf{1}) = (1.9 \pm 0.4) \times 10^4\ M^{-1}\ s^{-1}$ and $k(\mathbf{2}) = (3.7 \pm 1.2) \times 10^4\ M^{-1}\ s^{-1}$. We have shown previously that faster rate constants are obtained either because of higher solvent accessibility of guanine[14] or because of a decrease in redox potential due to stacking of adjacent guanines.[19] The increase observed between

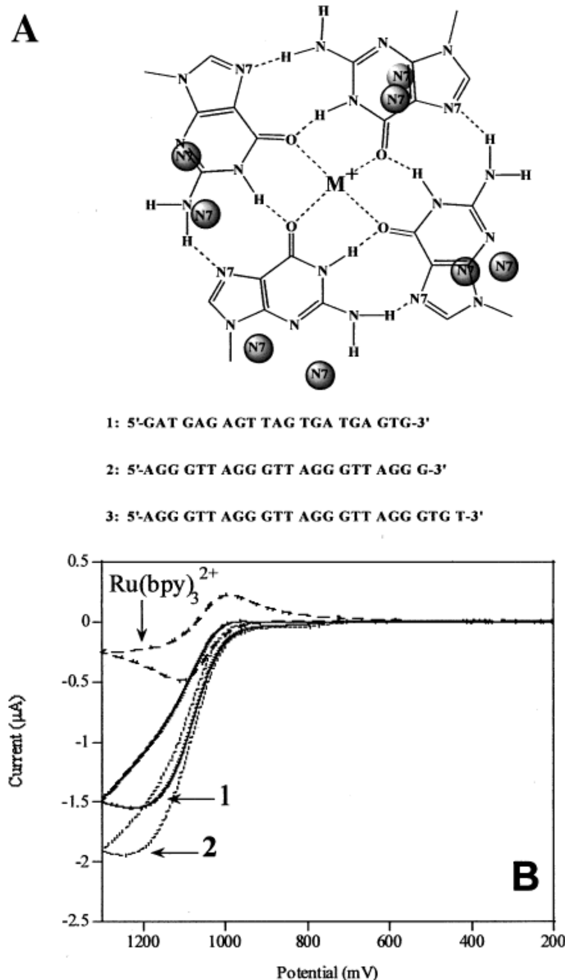

Figure 1. (A) View down the long axis of the top G quartet in **2** (taken from coordinates given in ref 13). The N7 atoms of the lower two quartets are shown as gray spheres. Oligonucleotide sequences used in this study are shown below. (B) Cyclic voltammograms of 25 μM $Ru(bpy)_3^{2+}$ alone (dashed line), and with 12.5 μM **1** (solid line) and 8.3 μM **2** in 50 mM sodium phosphate (pH 7) with 0.8 M added NaCl. Oligonucleotide **1** was annealed to its complement and **2** was folded in the quartet form as shown by native gel electrophoresis (Supporting Information). Oligonucleotide concentrations were chosen to give a final guanine concentration of 100 μM. Scan rate: 25 mV/s. Reference electrode: Ag/AgCl.

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(18) $[Ru(bpy)_3]Cl_2$ and $K_3[Fe(CN)_6]$ were purchased from Aldrich. Oligonucleotides were purchased from the Nucleic Acid Core Facility of the Lineberger Cancer Center at UNC. ITO electrodes were purchased from Delta Technologies, Inc. The Ag/AgCl reference electrode was purchased from Cypress, Inc. Oligonucleotide concentrations were determined spectrophotometrically using the extinction coefficient calculated by the nearest-neighbor approximation. Oligonucleotides for electrochemistry experiments were ethanol precipitated once before use. Formation of the duplex and G quartet forms was confirmed by native gel electrophoresis. Rate constants are given in terms of guanine concentration and were determined by digital simulation as described in ref 14. Radiolabeling and gel electrophoresis are described in detail in the Supporting Information; general procedures were similar to those in Carter, P. J.; Cheng, C.-C.; Thorp, H. H. *J. Am. Chem. Soc.* **1998**, *120*, 632−642.

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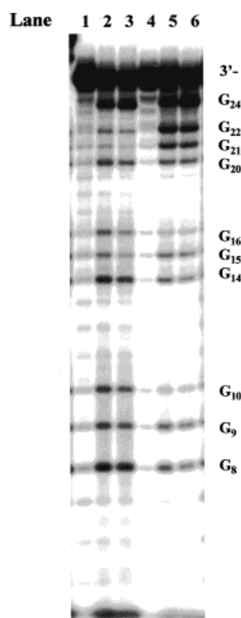

Figure 2. Phosphorimage of a denaturing polyacrylamide gel showing the results of photolytic cleavage of **3** with $Ru(bpy)_3^{2+}$ and $Fe(CN)_6^{3-}$ in 5 mM potassium phosphate and 50 mM added KCl. Lane 1: G quartet **3**, no $Ru(bpy)_3^{2+}$ or $Fe(CN)_6^{3-}$. Lane 2: G quartet **3**, 50 μM $Ru(bpy)_3^{2+}$, 500 μM $Fe(CN)_6^{3-}$. Lane 3: G quartet **3**, 100 μM $Ru(bpy)_3^{2+}$, 1 mM $Fe(CN)_6^{3-}$. Lane 4: duplex **3**, no $Ru(bpy)_3^{2+}$, no $Fe(CN)_6^{3-}$. Lane 5: duplex **3**, 50 μM $Ru(bpy)_3^{2+}$, 500 μM $Fe(CN)_6^{3-}$. Lane 6: duplex **3**, 100 μM $Ru(bpy)_3^{2+}$, 1 mM $Fe(CN)_6^{3-}$. Enhanced reactivity at G_{22} in lanes 5 and 6 is due to partial fraying of the duplex; reaction of **3** hybridized to its full-length complement (i.e., where the TGT overhang was hybridized) gave the same pattern at G_{20}−G_{22} as the other two triplets (Supporting Information). In addition, an oligomer with an 8-base overhang did not form the G quartet and gave very similar reactivity at all guanines for the single-stranded form (Supporting Information).

1 and **2** is much smaller than that observed for duplex GG sequences, which show an increase of a factor of 12 for the 5′-G.[19]

The increase in rate constant for **2** could be due either to higher exposure of the guanines in the G quartet to $Ru(bpy)_3^{3+}$ or to higher reactivity as a result of guanine stacking. To determine which of these factors was contributing, we examined the extent of oxidation at each guanine using gel electrophoresis on sequence **3**, which is identical to **2** except for the addition of a single-stranded TGT triplet to the 3′ end. The oligonucleotide was oxidized by photolyzing $Ru(bpy)_3^{2+}$ in the presence of $Fe(CN)_6^{3-}$. This "flash-quench" technique leads to one-electron oxidation of guanine by $Ru(bpy)_3^{3+}$, the same oxidant used in our electrochemical measurements.[20] Shown in Figure 2 are the results for photolysis of $Ru(bpy)_3^{2+}$ in the presence of **3** that is either hybridized to its Watson−Crick complement to form a duplex (with a TGT overhang) or in the G quartet form. Proper formation of the duplex or G quartet was confirmed by using native gel electrophoresis (gel given in Supporting Information). As shown in lanes 5 and 6, the duplex form gives the characteristic pattern for electron transfer from GGG triplets, where the 5′-guanines are enhanced in reactivity compared to the 3′-guanine. In contrast, the G quartet form (lanes 2 and 3) shows a pattern of 5′ > 3′ > central where the reactivity of the 3′-guanine compared to the other two guanines is much greater than for the typical reaction of duplex guanine triplets. The overall reactivity of the guanines in the G quartet is somewhat enhanced relative to the duplex form, as expected for a more solvent-accessible structure. The comparison of intensities between the two forms is enabled by the presence of the TGT overhang, which shows similar reactivity for both forms at the single-stranded G. Quantitative comparison of individual intensities is given in Table 1, which gives averages over four different gels for each nucleotide. The middle guanine in the GGG triplets is twice as reactive as the 3′-G for the duplex form, but there is clearly less selectivity in the G quartet. Schuster et al. have shown that selectivity for the 5′-G in a guanine doublet is reduced in A-form DNA:RNA hybrids,[9] further supporting the idea that the increase in electron density due to guanine stacking is specific to B-form DNA.

Table 1. Cleavage Intensities for Oxidation of Oligonucleotide *3* by Ru(III)

sample	G_{16}[a]	G_{15}	G_{14}	G_{10}	G_9	G_8
G quartet **3**	34 ± 5	29 ± 4	55 ± 5	45 ± 8	40 ± 5	57 ± 5
duplex **3**	12 ± 4	20 ± 3	17 ± 1	10 ± 3	21 ± 7	21 ± 5

[a] Values determined by phosphorimagery of four independent gel experiments performed as in Figure 2 with 50 μM $Ru(bpy)_3^{2+}$. Intensities are percentages normalized to G_{24}, which is in the single-stranded TGT overhang in both forms.

The combined results from electrochemistry and gel electrophoresis show that guanines in G quartets are slightly more accessible to the Ru(III) oxidant than in B-form duplexes but that *an increase in reactivity due to stacking of adjacent guanines does not occur in the G quartet*. The modest increase in solvent accessibility for G quartets apparent in Figure 1B is supported by available structures.[2,13,21] In particular, the average solvent accessible surface areas for guanines in duplex, G quartet, and single strands (148, 164, and 253 Å^2, respectively) follow the same trend as our measured rate constants (1.9×10^4 M^{-1} s^{-1}, 3.7×10^4 M^{-1} s^{-1}, and 2×10^5 M^{-1} s^{-1}, respectively).[22] The lack of a guanine-stacking effect in G quartets is consistent with the hypothesis that the increase in electron density of the 5′-guanine in B-DNA is due to fortuitous positioning of the N7 of the 3′-guanine relative to the π-system of the 5′-guanine.[23] As shown in Figure 1A, there is no similar alignment of any of the adjacent N7 atoms in the G quartet structure. Thus, although sequences that form G quartets contain guanine multiplets, the three-dimensional structure does not provide for the increase in reactivity observed due to guanine stacking in duplex DNA. Thus, adjacent guanines in G quartets are not effective hole traps, and formation of the G quartet structure might therefore protect guanine multiplets from oxidation in vivo due to remote charge migration.[10,24]

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Supporting Information Available: Additional experimental information, native gels showing formation of the G quartets, and a sequencing gel showing cleavage of **3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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