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Dynamics and Equilibria for Oxidation of G, GG, and GGG Sequences in DNA Hairpins

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Guanine is the most readily oxidized of the nucleobases and is the primary target of one-electron oxidation of duplex DNA by ionizing radiation, chemical oxidation, or photooxidation.[1,2] Guanine oxidation does not lead directly to strand cleavage but rather results in the formation of alkali-labile base modifications, the presence of which is inferred from gel sequencing experiments following treatment with hot piperidine. Oxidative cleavage at guanine displays base sequence selectivity, guanines with neighboring purine bases being more reactive than those with pyrimidine neighbors and GG sequences being more reactive than GA sequences.[3−5] Even greater selectivity is observed for cleavage at GGG sequences. [6−9] The theoretical basis for this selectivity has been investigated computationally by Sugiyama and Saito.[10] Their results indicate that the ionization potential of guanine is lowered by a neighboring purine, the effect being larger for guanine vs adenine and for a GGG vs a GG sequence (Table 1). Similar results were reported for G vs GG by Pratt et al.[11] In view of the large differences in calculated ionization potential, large differences in the population of holes localized at G vs GG vs GGG sequences would be expected if rapid hole transport leads to equilibrium between neighboring sites. If the rates of the chemical reactions leading to strand cleavage at these sites were similar, then highly selective cleavage should be observed. In fact, the observed selectivities for strand cleavage are comparatively modest. For example, Hickerson et al.[8] recently reported that oxidative cleavage of an 18-mer duplex containing G, GG, and GGG sequences occurred with a cleavage ratio of 1.0:3.7:5.3, respectively (Table 1). Similar low selectivities have been reported by Muller et al.[5] and by Saito and co-workers.[4,6]

The relatively modest selectivities for cleavage at G vs GG vs GGG sequences suggest that either the relative energies of these sites are similar or that the more stable sites are much less reactive. We report here the experimental determination of the dynamics of electron-transfer processes involving G, GG, and GGG sequences. The rate constants for forward and return hole transport provide equilibrium constants for hole transport between G and GG and between G and GGG sequences. These results establish that the difference in thermodynamic stability of holes located at these sequences is in fact quite small and that the chemical reactivity of these sequences decreases with increasing hole stability.

Table 1. Calculated Ionization Potentials, Relative Rate Constants for Strand Cleavage, Charge Separation, and Charge Recombination, Relative Populations of Holes, and Relative Rates of Reaction Leading to Strand Cleavage for G, GG, and GGG Sequences

property of sequence	G	GG	GGG
IP, eV (calcd)[a]	7.51	7.28	7.07
k_{cl}, rel[b]	1.0	3.7	5.3
k_{cs}, rel[c]	1.0	1.7	1.5
k_{cr}, rel[d]	1.0	0.33	0.23
$[G_n^{+\bullet}]$, rel[e]	1.0	7.7	20
k_H^+, rel[f]	1.0	0.48	0.27

[a] Calculated ionization potentials for GA, GG, and GGG sequences from ref 13. [b] Relative yields of oxidative cleavage from ref 11. [c] Relative rate constants for charge separation in hairpins **3C:G**, **3**,**4C:G**, and **3**,**4**,**5C:G** from Figure 1 ($k_{cs} = \tau_s^{-1}$). [d] Relative rate constants for charge recombination in hairpins **3C:G**, **3**,**4C:G**, and **3**,**4**,**5C:G** from Figure 1 ($k_{cr} = \tau_a^{-1}$). [e] Relative equilibrium populations of holes on G, GG, and GGG sequences from eqs 1 and 2. [f] Relative rates of guanine cation radical deprotonation at G, GG, and GGG sequences ($k_H^+ = k_{cl}/[G_n^{+\bullet}]$).

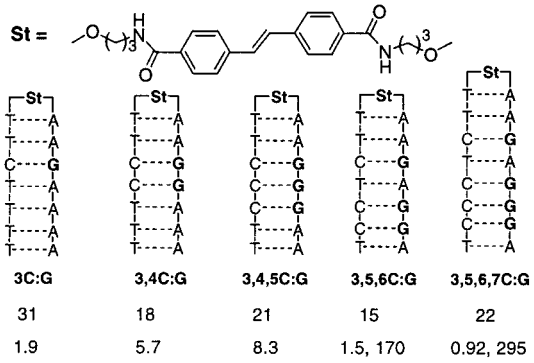

Figure 1. Structures of the stilbene linker and synthetic hairpins and the decay times of the stilbene singlet state (τ_s) and anion radical (τ_a). T- - -A and C- - -G are thymine:adenine and cytosine:guanine base pairs, respectively.

We have previously reported the synthesis, purification, and characterization of several bis(oligonucleotide) conjugates possessing a stilbene dicarboxamide linker (St) and complementary polyT and polyA "arms" which contain one or more G:C base pairs, including the hairpins **3C:G**, **3**,**4C:G**, and **3**,**5**,**6C:G** (Figure 1).[12,13] Selective charge-transfer quenching of the stilbene singlet state by guanine, but not by the other three common nucleobases, has permitted investigation of the distance and driving force dependence of contact and bridge-mediated charge-transfer processes in DNA.[12,13] Hairpins **3**,**4**,**5C:G** and **3**,**5**,**6**,**7C:G** containing a GGG sequence were prepared for the present study. Molecular modeling and X-ray crystallography indicate that stilbene-linked hairpins can adopt B-form structures in which the stilbene is approximately parallel to the adjacent base pair.[14] The transient absorption spectra of the hairpins in Figure 1 recorded at short delay times (1−2 ps) following 340 nm pulsed laser excitation resemble those of the stilbene linker and are assigned

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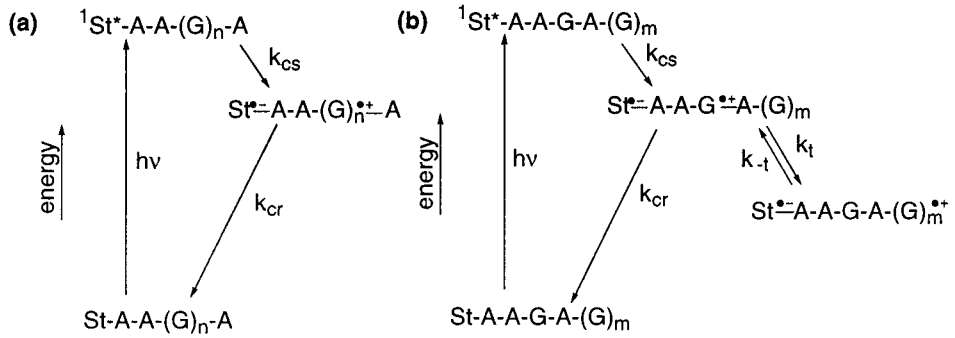

Figure 2. Kinetic scheme for charge separation (k_{cs}) and charge recombination (k_{cr}) in (a) hairpins containing one or more guanines separated from the stilbene linker by two T:A base pairs and (b) hairpins which can also undergo hole transport (k_t and k_{-t}) to a more distal site containing multiple guanines. Only the guanine-containing arm of the hairpin is shown.

to the stilbene singlet state, ^{1}St*. The band shape changes with time to that of the stilbene anion radical, St$^{-\bullet}$. Analysis of the transient decays at 575 nm provides the decay times for ^{1}S* (τ_s) and S$^{-\bullet}$ (τ_a) reported in Figure 1.[15]

The decay times for the hairpins **3C:G**, **3**,**4C:G**, and **3**,**4**,**5C:G** are assigned to the charge separation ($\tau_s^{-1} = k_{cs}$) and charge recombination ($\tau_a^{-1} = k_{cr}$) processes, respectively (Figure 2a). Values of k_{cs} and k_{cr} relative to that for **3C:G** are reported in Table 1. The modest increase in k_{cs}(rel) for a GGG vs G sequence is similar to that observed by Meggers et al. in their study of radical-induced strand cleavage.[16] According to Marcus theory for electron transfer, the values of k_{cs} and k_{cr} should be dependent upon the free energy of the electron-transfer process.[17] The small differences in these values for hairpins containing G, GG, and GGG sequences are seemingly incompatible with the large differences in the calculated ionization potentials of these sequences (Table 1).

The ^{1}S* decay times for the hairpins **3**,**5**,**6C:G** and **3**,**5**,**6**,**7C:G** are similar to that for **3C:G**; however, the S$^{-\bullet}$ decay times are dual exponential with one component having a somewhat shorter decay time than that of **3C:G** and the other substantially longer (Figure 1). In the case of **3**,**5**,**6C:G** this behavior was attributed to the formation of a hole on the G proximal to the stilbene linker followed by hole transport from G to the GG sequence located farther away from the stilbene linker (Figure 2b).[18] Nonlinear fitting of the S$^{-\bullet}$ decay data for **3**,**5**,**6C:G** using the exact analytical solution to this kinetic model provides rate constants for forward and return hole transport of $k_t = 5.6 \pm 0.6 \times 10^7$ s^{-1} and $k_{-t} = 7.5 \pm 0.8 \times 10^6$ s^{-1}. Analysis of the S$^{-\bullet}$ decay for **3**,**5**,**6**,**7C:G** provides values of $k_t = 8.7 \pm 1 \times 10^7$ s^{-1} and $k_{-t} = 4.3 \pm 0.1 \times 10^6$ s^{-1}. These rate constants provide values for the equilibrium constants and free energies of reaction for the hole transport processes shown in eqs 1 and 2. Comparison of eqs 1 and 2 provides a value of $\Delta G° = -0.025 \pm 0.005$ eV for a hole localized on a GG vs GGG sequence. Thus the effect of the first

$$G^{+\bullet} + GG \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} G + GG^{+\bullet} \qquad K = 7.7 \pm 1,\ \Delta G = -0.052 \pm 0.006 \quad (1)$$

$$G^{+\bullet} + GGG \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} G + GGG^{+\bullet} \qquad K = 20 \pm 1,\ \Delta G = -0.077 \pm 0.005 \quad (2)$$

neighboring guanine is larger than that of the second, in accord with experimental results and theoretical predictions based on a tight binding model for charge delocalization in a π-stacked system.[19]

The experimental values of $\Delta G°$ are the first available for hole equilibria in DNA. These values are considerably smaller than the differences predicted by Sugiyama and Saito[10] based on calculated ionization potentials (Table 1). Our values are in better accord with numerous experimental observations. First, the small values of $\Delta G°$ are consistent with the similar values of k_{cs} and k_{cr} for the hairpins **3C:G**, **3**,**4C:G**, and **3**,**4**,**5C:G** (Table 1) and the modest difference in rate constants for oxidation of G vs GG sequences recently reported by Sistare et al.[20] Second, they are consistent with observations that GG sequences are very shallow hole traps and do not block hole migration in DNA,[21,22] and with a recent report by Nakatani et al.[9] that hole migration between two GGG sequences is much more efficient when they are separated by a TTGTT than by a TTATT sequence.[23]

Finally, our values of $\Delta G°$ are consistent with the modest selectivity for oxidative strand cleavage at GGG or GG vs G sequences.[4−6,8,24] Our equilibrium populations, [$G_n^{+\bullet}$], can be used in combination with the data of Hickerson et al.[8] for piperidine-induced strand cleavage (Table 1) to provide relative rate constants for the chemical step (presumably proton transfer[25]) leading to strand cleavage of the guanine cation radical ($k_H^+ = k_{cl}/[G_n^{+\bullet}]$). The resulting values of k_H^+(rel) are reported in Table 1. These are average values for the entire GG or GGG sequence and thus do not reflect the known preference for cleavage at the 5′G of a GG sequence and at the middle or 5′G of a GGG sequence.[7] However, they establish that the average reactivity of a hole on guanine(s) decreases as its stability increases, in accord with the basic tenets of physical organic chemistry.

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