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Dynamics and Energetics of Hole Trapping in DNA by 7-Deazaguanine**

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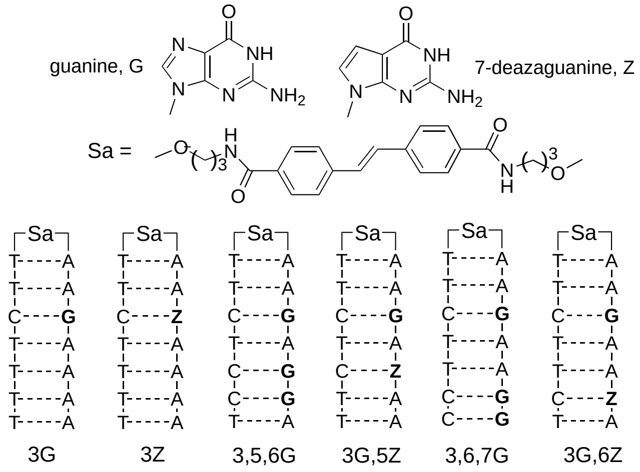
Oxidative cleavage of DNA by photonucleases can occur at sites separated from the nuclease by as many as several dozen base pairs.[1–4] Such long-distance processes are known to occur by a multistep hole-hopping mechanism which is initiated by photoinduced electron transfer between the nuclease (electron acceptor) and a neighboring base (electron donor) resulting in creation of a hole on the base. Migration of the hole over long distances is more rapid than the chemical reactions of the oxidized bases which lead to strand cleavage.[5] Strand cleavage occurs selectively at guanine, the most readily oxidized of the nucleobases, and is sequence selective.[6, 7] Preferential cleavage at GG and GGG sequences has been attributed to stabilization of the hole by delocalization over two or three guanine residues.[8–10]

We recently reported the use of transient absorption spectroscopy with kinetic modeling to obtain the rate constants for reversible hole transports between $G^{\bullet +}$ and GG or GGG sequences separated by a single A:T base pair.[11, 12] The resulting Gibbs energy differences, 52 mV for GG and 77 mV for GGG, are much smaller than those previously estimated from calculations of gas-phase ionization potentials.[8] However, recent theoretical studies by Bixon and Jortner[13, 14] and by Kurnikov et al.[15] have provided results in good agreement with our experimental data. Our kinetic data have also been used by Giese and coworkers[5, 16] to model the results of strand-cleavage studies in duplexes containing G, GG, and GGG sites.

The importance of obtaining experimental values for the dynamics and energetics of hole transport in DNA led us to investigate the use of the modified nucleobase 7-deazaguanine, Z, as a hole trap. The oxidation potential of Z is reported to be 0.3 eV lower than that of G.[17] Z has been employed in investigations of single-step (superexchange) electron transfer in DNA.[18–20] Both Z and 8-oxoguanine, which also has a lower oxidation potential than G, have been used as hole traps in strand cleavage studies.[6, 21] We report here the results of an investigation of the dynamics of forward and return hole transport from G to Z, which establish that Z is a much deeper hole trap than GG or GGG. In addition, the dynamics of the hole transport processes are found to be strongly dependent upon the number of A:T base pairs separating the hole donor and acceptor.

The structures of the synthetic DNA hairpins prepared for the present study are shown in Scheme 1. The stilbene-4,4′-dicarboxamide (Sa) serves as a linker connecting complementary polyT and polyA arms containing one or more C:G or C:Z base pairs.[22] All of these hairpins have high melting temperatures (> 75 °C) and circular dichroism spectra similar to that of a stilbene-linked hairpin known to adopt a normal B-form structure in which the stilbene is parallel to the



Scheme 1. Structures of the synthetic DNA hairpins used in this study.

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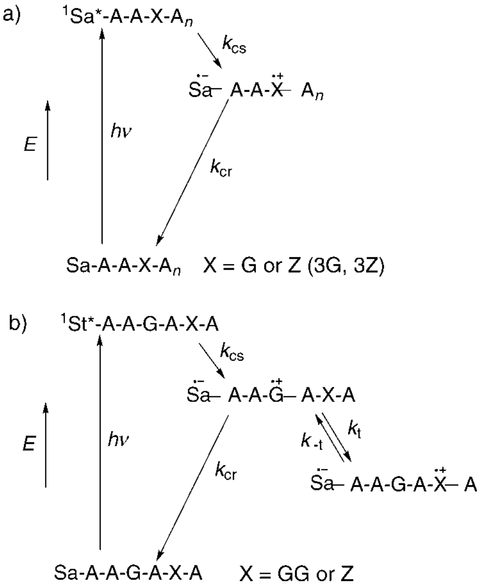
adjacent base pair.[23] In the absence of G or Z, the Sa linker displays strong fluorescence and transient absorption spectra characteristic of the Sa singlet state ($\tau_s = 2.0$ ns). In the presence of G or Z, the fluorescence is quenched and the transient absorption displays a time-dependent change from that of $^1Sa^*$ to that of the radical anion $Sa^{\bullet-}$.[24, 25] These changes are attributed to photoinduced charge separation resulting in the formation of $Sa^{\bullet-}$ and $G^{\bullet+}$ or $Z^{\bullet+}$ followed by charge recombination (Scheme 2a).



Scheme 2. Kinetic schemes for charge separation (k_{cs}) and charge recombination (k_{cr}) in (a) hairpins 3G and 3Z containing G or Z 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}) from G to a more distal site containing two G or one Z residue. Only the G-containing arm of the hairpin is shown.

In the case of the hairpins 3G and 3Z, the decay of $^1Sa^*$ and $Sa^{\bullet-}$ (Table 1) can be assigned to the charge separation and charge recombination processes, respectively ($\tau_s^{-1} = k_{cs}$ and $\tau_a^{-1} = k_{cr}$).[26] Decay of the $Sa^{\bullet-}$ transient absorption is essentially complete on the time scale of the picosecond experiment (0–6 ns). More rapid charge separation for Z than for G is attributed to more exergonic electron transfer in the "normal" Marcus region and faster charge recombination for Z than for G to less exergonic electron transfer in the "inverted" Marcus region.[19] Similar results have been reported by Wan et al.[18] and by Hess et al. using other acceptors.[20]

Table 1. Transient decay times and rate constants for hole transport in the DNA hairpins shown in Scheme 1.

Hairpin	τ_s [ps][a]	τ_a [ns][b]	k_t [s^{-1}][c]	k_{-t} [s^{-1}][d]
3G[e]	31 (29)	1.9 (71)		
3Z	16 (38)	1.0 (62)		
3,5,6G[f]	39 (35)	1.5 (50), 170 (15)	5.6×10^7	7.5×10^6
3G,5Z	37 (27)	0.82 (33), 6000 (40)	6.9×10^8	3.8×10^5
3,6,7G	not measured	< 5[g]	ca. 6×10^6[g]	
3G,6Z	26 (28)	1.2 (64), > 10000 (8)	5×10^7[g]	$< 10^5$[g]

[a] Stilbene singlet state decay time (preexponential). [b] Stilbene anion radical decay time(s) (preexponential). [c] Rate constant for hole transport obtained from kinetic modeling. [d] Rate constant for return transport obtained from kinetic modeling. [e] Data from ref. [25]. [f] Data from ref. [12]. [g] Estimated values, see text.

The hairpins 3,5,6G, 3G,5Z, 3,6,7G, and 3G,6Z possess G as the primary hole acceptor and GG or Z, respectively, as a secondary hole acceptor, separated from G by either one or two A:T base pairs (see Scheme 1). The values of τ_s for 3,5,6G, 3G,5Z, and 3G,6Z are similar to that for 3G (Table 1) in accord with their similar structures and the presence of G as the primary hole acceptor. These hairpins also have similar fluorescence quantum yields ($\Phi_f = 0.05 \pm 0.01$). Their $Sa^{\bullet-}$ transient absorption does not decay completely within the delay time limit (6 ns) of the picosecond experiment. Nanosecond transient absorption confirmed the presence of a long-lived species for each of these hairpins. In the case of 3,5,6G and 3G,5Z single exponential fits of the long-lived transient provided decay times of 170 and 6000 ns, respectively. In the case of 3G,6Z a weak, long-lived transient was observed which did not decay appreciably within 10 μs. No long-lived transient ($\tau > 5$ ns) was observed for 3,6,7G and thus the transient spectra at shorter times were not investigated.

The 170 ns decay component of 3,5,6G is attributed to charge recombination of $Sa^{\bullet-}$ with $GG^{\bullet+}$ formed by reversible hole transport from the primary G donor to the secondary GG donor (Scheme 2b).[11, 12] In the case of 3G,5Z, reversible hole transport from G to Z plausibly is responsible for the 6000 ns decay component. Kinetic modeling of the picosecond and nanosecond transient decays according to the mechanism in Scheme 2b provides rate constants for the forward and return hole transport processes (k_t and k_{-t}). Values of k_t and k_{-t} for 3,5,6G and 3G,5Z are reported in Table 1. Comparison of the data for these hairpins reveals that k_t is significantly larger for hole transport to Z than to GG, whereas k_{-t} is smaller for Z than for GG. The ratio of forward and return hole transport rates provides the equilibrium constant and Gibbs energy difference between holes on G and Z summarized in Table 2, along with our published data for GG and GGG.[12]

Table 2. Hole transport equilibria and thermodynamics.

Hole transport sequence	K_{ht}[a]	ΔG_{ht} [eV] ([kJ mol^{-1}])
$\overset{\cdot+}{G}AZ \rightleftharpoons GA\overset{\cdot+}{Z}$	1800 ± 200	0.19 ± 0.03 (18.4)
$\overset{\cdot+}{G}AGG \rightleftharpoons GAG\overset{\cdot+}{G}$[b]	7.7 ± 1	0.052 ± 0.006 (5.0)
$\overset{\cdot+}{G}AGGG \rightleftharpoons GAGG\overset{\cdot+}{G}$[b]	20 ± 1	0.077 ± 0.005 (7.5)

[a] Calculated from k_t/k_{-t}. [b] Data from ref. [12].

The effect of adding a second A:T base pair between the primary and secondary donors has been investigated for the hairpins 3,6,7G and 3G,6Z. The picosecond transient absorption spectrum of 3G,6Z displays a 1.2 ns component, similar in decay time to that of hairpin 3G, and a long-lived component of lower amplitude (ca. 10% of the 1.2 ns component). The nanosecond transient absorption displays only a low-amplitude component which does not decay appreciably within

10 µs. Based on the amplitude of the long-lived component, we estimate a value of $k_t \sim 5 \times 10^7\,s^{-1}$ for 3G,6Z, a factor of 10 smaller than that for 3G,5Z (Table 1). Similar values for the attenuation of hole transport from G to GGG across multiple A:T base pairs have been reported by Meggers et al.[27] and by Kawai et al.[28] for hole transport from G to pyrene across multiple A:T base pairs. In the case of 3,6,7G no long-lived component is detected by means of nanosecond transient absorption. Assuming that k_t for 3,6,7G is a factor of 10 smaller than that for 3,5,6G, the resulting value of $k_t \sim 6 \times 10^6\,s^{-1}$ would be too small for the trapping to compete with charge recombination of the $Sa^{\bullet -}/G^{\bullet +}$ primary radical ion pair ($k_{cr} = 5.3 \times 10^8\,s^{-1}$). The value of k_{-t} for 3G,6Z can be estimated either from the absence of µs decay ($k_{-t} < 10^5\,s^{-1}$) or from the value of k_t and the equilibrium constant for hole transport between G and Z in 3G,5Z ($k_{-t} \sim 3 \times 10^4\,s^{-1}$).

Comparison of our results for hole transport involving Z, GG, and GGG sites (Table 2) indicates that Z is a much deeper hole trap than either GG or GGG. The value of $\Delta G^{\ominus}$ for hole transport between G and Z in 3G,5Z, (-0.19 ± 0.03) eV, is smaller than either the value of -0.3 eV estimated from the irreversible oxidation potentials of dG and dZ as single nucleotides[17] or the value of -0.39 obtained from the calculated gas-phase ionization potentials of G and Z.[6] Base stacking is expected to lower the oxidation potentials of both dG and dZ and to exert a leveling effect on effective oxidation potentials.[13, 14] In fact, Jortner[29] estimates a value of $\Delta G^{\ominus} = (-0.14 \pm 0.03)$ eV for hole transport from G to Z, in good agreement with our experimental result.

Additional experimental data for hole trapping by Z is provided in a recent study by Nakatani et al.[6] of the photooxidative strand cleavage reactions of duplexes containing the partial sequence 5′-GGGTTXTTGGG-3′, where X = A, G, or Z. When X = A, photooxidation at the 5′-GGG site results in strand cleavage predominantly at that site. However, when X = G, cleavage occurs at both the 5′- and 3′-GGG sites with a ratio of 1.7:1. Little cleavage occurs at the bridging A or G. When X = Z, cleavage occurs predominantly at Z with some cleavage at the 5′-GGG site and essentially no cleavage at the 3′-GGG site. These results are consistent with hole transport rates that are smaller than that of the reaction of the 5′-GGG site with water, k_w, in the case of X = A, but larger than k_w in the case of X = G or Z. Selective cleavage at GGG versus G when X = G is consistent with equilibria which favor GGG (Table 2) and similar values of k_w for GGG and G.[12] In the case of X = Z the equilibrium between 5′-GGG and Z favors Z by a factor of 90. Furthermore, the rate constant for hole transport from Z to 3′-GGG is expected to be $< 10^5\,s^{-1}$, the upper limit for k_{-t} across two A:T base pairs in 3G,6Z (see Table 1). This value is similar to that estimated by Giese and Spichty for the pseudo-first-order reaction of $G^{\bullet +}$ with water, $k_w \sim 6 \times 10^4\,s^{-1}$.[5] Thus it is likely that the reaction of Z with water is faster than hole transport to G, GG, or GGG sites across two base pairs. This would account both for the absence of cleavage at the 3′-GGG site in Nakatani's investigation[6], [30] and for the absence of charge recombination in the transient absorption spectra of 3G,6Z.

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