

## Dynamics of Electron Injection in DNA Hairpins

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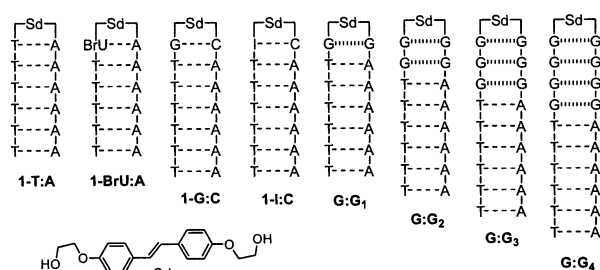
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Photoinduced electron transfer has proven to be a powerful technique for the study of charge injection and migration in duplex DNA.<sup>1–5</sup> Steady state (strand cleavage) methods have been employed to obtain relative efficiencies of charge migration over long distances.<sup>1</sup> The dynamics of charge injection and migration over relatively short distances has been investigated by means of transient absorption spectroscopy on femtosecond-to-microsecond time scales.<sup>4–7</sup> Most of the information obtained to date pertains to hole injection (oxidation of nucleobases). Current knowledge of photoinduced electron injection (reduction of nucleobases) in systems with known donor–acceptor distances is limited to preliminary studies of the efficiency of T–T dimer repair via electron injection from a flavin nucleobase<sup>8</sup> and the picosecond dynamics of electron injection to neighboring T or C bases from singlet donors.<sup>4,9,10</sup> We report here the picosecond dynamics of electron injection in synthetic DNA hairpins from a stilbenediether singlet (<sup>1</sup>Sd\*) electron donor to four different neighboring base pairs which function as electron acceptors and the distance dependence of electron injection from <sup>1</sup>Sd\* to thymine when the donor and acceptor are separated by a variable number of noncanonical G:C base pairs (Figure 1).<sup>11</sup>

The stilbenediether diol **Sd** has a fluorescence quantum yield of 0.32 and a decay time of 350 ps in methanol solution, similar to the values for 4,4'-dimethoxystilbene.<sup>12</sup> Its transient absorption spectrum in methanol displays a broad maximum at 575 nm, a singlet-state decay time of 240 ps. The transient absorption spectra of hairpins **1T:A**, **1BrU:A**, **1G:C**, and **1I:C** display a similar broad maximum at 575 nm at very short decay times. This band decays rapidly and is replaced by a narrower band at 525 nm that grows and then decays. The 525-nm band is similar in shape to that of the cation radical of 4,4'-dimethoxystilbene ( $\lambda_{\text{max}} = 530$  nm in acetonitrile solution) and is assigned to Sd<sup>+</sup>.<sup>13</sup> The decay times of the 575 (<sup>1</sup>Sd\*) and 525 (Sd<sup>+</sup>) transients for these hairpins are reported in Table 1.<sup>14</sup>

The energetics and kinetics of photoinduced electron transfer from <sup>1</sup>Sd\* to a neighboring nucleobase is shown schematically in Figure 2a. According to Weller,<sup>15</sup> the free energy for electron injection is determined by the singlet energy and oxidation potential of the donor stilbene and reduction potential of the acceptor nucleobase [ $\Delta G_{\text{ei}} = -(E_{\text{S}} + E_{\text{ox}}) + E_{\text{rdn}} + C$ ] and the free energy for charge recombination by the sum of the redox potentials [ $\Delta G_{\text{cr}} = E_{\text{rdn}} - E_{\text{ox}}$ ].<sup>16</sup> Literature values of  $E_{\text{rdn}}$  for the single nucleotides C, T, and BrU in nonaqueous solution<sup>17,18</sup> are reported in Table 2 along with calculated values of  $\Delta G_{\text{ei}}$  and  $\Delta G_{\text{cr}}$  and the experimental rate constants for electron injection and charge recombination ( $k_{\text{ei}}$  and  $k_{\text{cr}}$ ).

The larger value of  $k_{\text{ei}}$  for **1T:A** versus **1G:C** both in our experiments and those of Wan et al.<sup>4</sup> are indicative of a lower

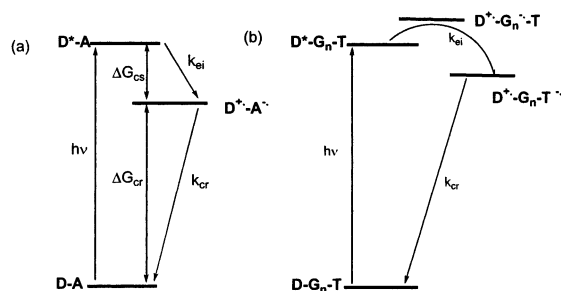


**Figure 1.** Structures of synthetic DNA hairpins and the stilbenediether linker **Sd**.

**Table 1.** Singlet and Cation Radical Decay Times for Sd-Linked Hairpins<sup>a</sup>

hairpin	$\tau_s$ , ps <sup>b</sup>	$\tau_{\text{cat}}$ , ps <sup>c</sup>	hairpin	$\tau_s$ , ps <sup>b,d</sup>
<b>1G:C</b>	3.0	40	<b>G:G<sub>1</sub></b>	140
<b>1I:C</b>	0.7	26	<b>G:G<sub>2</sub></b>	(530)
<b>1T:A</b>	<0.5	32	<b>G:G<sub>3</sub></b>	650, (690)
<b>1BrU:A</b>	<0.5	14	<b>G:G<sub>4</sub></b>	(690)

<sup>a</sup> Data for deoxygenated 10<sup>-6</sup> M solutions of the hairpins in standard buffer excited at 340 nm with the output of a Ti-sapphire laser system with an instrument response function of 150 fs. <sup>b</sup> Decay of the Sd singlet state monitored at 575 nm. <sup>c</sup> Decay of the Sd cation radical monitored at 525 nm. <sup>d</sup> Decay independent of monitoring wavelength. Fluorescence decay times in parentheses.



**Figure 2.** Kinetics and thermodynamics of electron injection and charge recombination. D is the stilbenediether donor. (a) A is the acceptor nucleobase for nearest neighbor quenching. (b) T is the acceptor for G:C-mediated electron injection.

reduction potential for T versus C in  $\pi$ -stacked B-DNA. This result is consistent with the single nucleotide reduction potentials reported by Seidel et al.<sup>17</sup> and single nucleobase electron affinities calculated by Wesolowski et al.<sup>19</sup> However, Voituk et al.<sup>20</sup> predict, on the basis of semiempirical AM1 calculations, that hydrogen bonding should stabilize the anion radical of C more than that of T and that the relative stability of the two anion radicals are similar and may depend on the identity of the neighboring bases.

The larger value of  $k_{\text{ei}}$  for **1I:C** versus **1G:C** may reflect differences in reduction potentials of C resulting from base pairing, the canonical G:C base pair having stronger hydrogen bonding than

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**Table 2.** Energetics and Dynamics of Electron Injection and Charge Recombination

hairpin	$-E_{\text{rdn}}^a$	$-\Delta G_{\text{el}}^b$	$k_{\text{ei}}, \text{s}^{-1}{}^c$	$\Delta G_{\text{cr}}^b$	$k_{\text{cr}}, \text{s}^{-1}{}^d$
<b>1G:C</b>	2.36	0.12	$3.3 \times 10^{11}$	3.28	$2.5 \times 10^{10}$
<b>1I:C</b>	2.36	0.12	$1.4 \times 10^{12}$	3.28	$3.8 \times 10^{10}$
<b>1T:A</b>	2.26	0.22	$> 2 \times 10^{12}$	3.18	$3.1 \times 10^{10}$
<b>1BrU:A</b>	2.20	0.28	$> 2 \times 10^{12}$	3.12	$7.1 \times 10^{10}$

<sup>a</sup> Nucleobase reduction potential in acetonitrile solution, V vs SCE. Data from refs 17,18. <sup>b</sup> Free energies (eV) for charge separation and charge recombination estimated using Weller's equations<sup>15</sup> and the singlet energy (3.45 eV) and oxidation potential of **Sd** (0.92 V vs SCE), and an estimated value of  $C = 0.05$  eV.<sup>16</sup> <sup>c</sup> Rate constant for electron injection ( $k_{\text{ei}} = \tau_{\text{s}}^{-1}$ ). <sup>d</sup> Rate constant for charge recombination ( $k_{\text{cr}} = \tau_{\text{cat}}^{-1}$ ).

the I:C base pair. Bromouracil is more readily reduced than T; however, values of  $\tau_{\text{s}}$  for both **1BrU:A** and **1T:A** are too short to resolve with our femtosecond apparatus. The hairpin **1BrU:A** has the least negative value of  $\Delta G_{\text{cr}}$  and largest value of  $k_{\text{cr}}$ , as expected for a charge-transfer process in the Marcus inverted region.<sup>21</sup>

The conjugates **G:G<sub>1</sub>–G:G<sub>4</sub>** have one or more G:G base pairs separating the Sd linker and poly(T:A) hairpin stem. The transient absorption spectra of **G:G<sub>1</sub>** and **G:G<sub>3</sub>** resemble those of the linker **Sd** and do not display a change in band shape on the 1 ps to 10 ns time scale. The transient absorption decay and fluorescence decay times for **G:G<sub>3</sub>** are similar (Table 1), confirming their assignment to <sup>1</sup>Sd\*. The decay times increase for the first three members of this series; however, no further increase is observed for **G:G<sub>4</sub>**. The lifetimes of **G:G<sub>2</sub>–G:G<sub>4</sub>** are longer than that of the **Sd** diol linker. This may reflect restricted singlet-state isomerization in the hairpins.<sup>22</sup> On the basis of the estimated value of  $E_{\text{rdn}}$  for G,<sup>17</sup> photoreduction of G should be endergonic by ca. 0.5 eV, in accord with the apparent absence of electron transfer in **G:G<sub>3</sub>** or **G:G<sub>4</sub>**. Thus, the shorter decay times for **G:G<sub>1</sub>** and **G:G<sub>2</sub>** are attributed to bridge-mediated electron injection from <sup>1</sup>Sd\* to the closest A:T base pair (Figure 2b).

Values of  $k_{\text{ei}}$  for G:G bridge-mediated electron injection in **G:G<sub>1</sub>** and **G:G<sub>2</sub>** calculated from the decrease in singlet decay times for these hairpins versus **G:G<sub>3</sub>** ( $k_{\text{cs}} = \tau_{\text{obs}}^{-1} - \tau_{\text{G:G3}}$ ) are 57 and  $4.4 \times 10^8$  s<sup>-1</sup>, respectively. These rates are ca. 25 times slower than those for hole injection from the singlet stilbenedicarboxamide (Sa) linker to G across one or two T:A base pairs.<sup>6</sup> The slower rates for electron versus hole injection in hairpin systems with similar values of  $\Delta G_{\text{cs}}$  may be a consequence of a longer D–A distance or weaker donor–bridge–acceptor interactions in the Sd- versus Sa-linked hairpins. A single syn–anti (Hoogsteen) G:G base does not disrupt the  $\pi$ -stacking in B-DNA; however, it does distort the deoxyribose conformation and thus destabilize the duplex.<sup>23</sup> A decrease in hairpin melting temperature is observed for the **G:G<sub>n</sub>** hairpins compared to **1T:A**, which has the same number of T:A base pairs.<sup>11</sup> The slow rates for electron injection may limit the steady-state concentration of Sd<sup>+</sup> and thus account for the failure to observe its transient spectrum in the case of **G:G<sub>1</sub>** and **G:G<sub>3</sub>**. This appears to be a common problem in the study of DNA charge separation by means of transient absorption spectroscopy.<sup>4,5</sup>

In conclusion, rate constants for electron injection and charge recombination of Sd with four neighboring base pairs display a driving force dependence similar to that for hole injection in

stilbenedicarboxamide-linked hairpins.<sup>24</sup> The fast rates and shallow driving force dependence for both systems indicate that electron and hole injection occur near the top of the Marcus curve and charge recombination in the inverted region. Rate constants for G:G-mediated electron injection are slower than those determined previously for hole injection, and electron injection is limited to shorter distances than those for hole injection.<sup>25</sup> Despite these limitations, these results suggest that G:G or other mismatched or unnatural base pairs may be used to mediate efficient long-distance electron-transport processes in DNA.

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**Supporting Information Available:** Transient absorption spectra and transient decay profiles for hairpins **1G:C** and **1G:G** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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