Hole Transport in DNA Hairpins via Base Mismatches and Strand Crossings: Efficiency and Dynamics

Arun K. Thazhathveetil, Michelle A. Harris, Ryan M. Young, Michael R. Wasielewski,* and Frederick D. Lewis*

Department of Chemistry and Argonne-Northwestern Solar Energy Research (ANSER) Center, Northwestern University, Evanston, Illinois 60208-3113

*Corresponding authors email: mwasielewski@northwestern.edu (Michael R. Wasielewski); fdl@northwestern.edu (Frederick D. Lewis)

ABSTRACT

The efficiency and dynamics of hole transport via base mismatches and strand crossings have been determined for 10 DNA hairpins by means of femtosecond transient absorption spectroscopy. The hairpins possess a stilbenedicarboxamide hole donor and stilbenediether hole acceptor separated by seven base pairs. Hole transport occurs via an intra- or interstrand A₃G₄ diblock purine sequence that contains either a single base pair mismatch or strand crossing. Pyrimidine-pyrimidine mismatches (TT or CC) effectively block intrastrand hole transport; whereas purine-purine mismatches (AA or GG) permit efficient interstrand hole transport. Purine-to-purine intrastrand hole transport between adjacent matched base pairs is also moderately efficient (AG > AA > GG). Based upon these results AA mismatches are being employed in the design of three-way junctions for incorporation in prototype charge splitting and charge combining devices.

Key words: Donor-acceptor systems, DNA, Hole transport, base-pair mismatch, Transient absorption spectroscopy

INTRODUCTION

Knowledge of the base-sequence dependence of the efficiency and dynamics of charge transport in DNA is of fundamental importance to understanding DNA damage and repair mechanisms [1]. It is also essential to the design of DNA-based molecular electronic devices [2]. The initial studies of the dependence of the relative efficiency of hole transport upon base sequence were based on ratios of strand cleavage at guanine-containing sites proximal vs. distal to the locus of hole injection [3,4]. Relatively efficient hole transport over long distance was observed when adenine repeats (A-tracks) and alternating poly(purine) sequences separated the proximal and distal guanine sites [5,6]. The effect of base mismatches was investigated by Giese and Wessely who reported that replacement of a CG base pair by a TG base pair in a $G^{+}TGTTGGG$ duplex hole transport sequence resulted in a ca. 67% reduction in hole transport efficiency but that replacement of one of the TA base pairs by an AA base pair had little effect on hole transport efficiency [7]. Subsequently, Bhattacharya and Barton observed that $GG^{+}CTGTYACACGG$ duplex hole transport sequences having matched or mismatched base pairs with G in either strand had larger distal/proximal ratios of electrochemical oxidative strand cleavage by a Ru^{2+} intercalator than did duplexes with AA or pyrimidine-pyrimidine base pairs [8]. Schlientz and Schuster observed efficient hole transport to distal GG sites in duplexes having contiguous AA mismatches but not TT mismatches [9].

Transient absorption spectroscopy has also been employed to investigate the effects of base mismatches on charge transport dynamics in DNA. We used tandem GG mismatched base pairs inserted between a stilbenediether electron donor and a TA base pair to investigate the distance dependence of electron injection to thymine [10]. G being a poor electron acceptor, the yield of charge separation decreased for one or two intervening GG base pairs. Osakada et al.

employed nanosecond transient absorption spectroscopy to investigate the effect of base mismatches on the kinetics of hole transport in duplexes possessing a naphthalene imide (NI) hole donor in one strand and a phenothiazine (PTZ) hole acceptor in the complementary strand [11]. They observed faster hole transport for one or two sequential AA mismatches than for several other mismatches (GA, GT, TT, and AC). The quantum yields for all of the sequences investigated were similar and quite low (ca. 2%) as a consequence of the duplex design employed.

We recently investigated hole transport in DNA three-way junctions (3WJ) possessing a stilbenedicarboxamide (Sa) hole donor in one arm and a stilbenediether (Sd) electron donor either of the other two arms (Chart 1b) [2,12]. In the course of this investigation, we observed that splitting of charge at a 3WJ could be achieved by using a G-G mismatch between the locus of hole injection and the junction. In order to obtain more extensive data on the effects of base pair mismatches and strand crossings on hole transport dynamics and efficiency we have undertaken an investigation of charge separation in a series of Sa-Sd capped hairpins related to the diblock hairpin, **1**, by the introduction of a pyrimidine-pyrimidine mismatch (**2** and **3**), a purine-purine mismatch (**4** and **5**), a single purine to purine strand crossing (**6-9**), or multiple G-to-G strand crossings (**10**) into the diblock sequence of **1**. The diblock purine sequence in **1** rather than a poly(A) sequence with 7 A-T base pairs was selected for this study because of the much higher quantum yield for charge separation in the diblock sequence which permits measurement of the quantum yield and dynamics for charge separation by means of femtosecond time resolved transient absorption spectroscopy (fsTA) [13].

Chart 1. (a) Structures of the stilbenedicarboxamide (Sa) capping group and stilbenediether (Sd) hairpin linker, (b) structure of a three way junction having Sa and Sd linkers and a G-G strand crossing (X = hexaethylene glycol linker), and (c) structures of the capped hairpins **1-10** employed in this study. Adenines are shown in red and guanines are shown in blue.

MATERIALS AND METHODS

Materials. The Sa capping group and Sd linker were prepared, purified, and characterized as previously described [10,14]. The oligonucleotide conjugates **1-10** were synthesized using standard phosphoramidite chemistry on an Expedite 8909 synthesizer as described previously [13]. They were deprotected using 30% ammonium hydroxide at room temperature for 24 h, purified by RP-HPLC using a Kinetex 5u EVO C18 column (250 x 4.6 mm) on a Dionex HPLC system (P680 HPLC pump, UVD340S detector) and characterized by MALDI-TOF spectroscopy. Mass spectral data are reported in Supporting Information (Table S1).

Methods. UV/Vis spectra (Agilent 8453 UV-visible Spectroscopy System), CD spectra, and thermal denaturation (Jasco-J-815 CD spectrometer) were measured in 10 mM phosphate buffer (pH~7.2) containing 100 mM NaCl using quartz cuvettes with 1 cm path length at room temperature (25 °C).

Transient Absorption Measurement. Details of the femtosecond transient absorption apparatus have been published previously [15,16]. The samples were excited with a 1 μ J pulse at 350 nm (selective excitation of Sa) using a Ti:sapphire based system having a time resolution of ca. 180 fs, a spectral range of 450-750 nm and a time window out to 7 ns. Samples were stirred in a 2 mm quartz cuvette *in situ* to avoid the effects of local heating or the build-up of photo-degradation products which might pollute or obscure the desired transient spectra or their dynamics. Each experiment was repeated at least twice; quantum yields and hole arrival rates are reported as the average of multiple runs [2].

RESULTS AND DISCUSSION

The UV spectra of hairpins **2-9** are similar to that of **1**, consisting of a long wavelength band with maxima near 335 nm, attributed to the overlapping absorption of the Sa and Sd chromophores, and a stronger band near 250 nm (Figure S1, Supporting Information), attributed to absorption of the base pairs and weaker absorption by the stilbenes [13,17]. The CD spectra **1-10** display a broad negative band at long wavelengths ($\lambda > 300$ nm), attributed to induced circular dichroism of the two stilbene chromophores, and stronger positive bands around 220 nm and between 260-290 nm and negative bands between 240-250 nm attributed to the duplex DNA base pairs (Figure S1). The variation of the CD bands in the 200-300 nm spectral region are attributed to differences in the base sequences and duplex structures. The UV thermal dissociation profiles of **1-10** (Figure S1) all have derivatives > 40 °C, indicative of hairpin structures which are stable at and above room temperature (Figure S1). The highest derivatives are observed for hairpin **1** which has no mismatches and hairpins **5** and **9** which have a single GG mismatch, as expected in view of the known stability of a single GG mismatch within a DNA or RNA duplex [18].

Femtosecond transient absorption (fsTA) spectra for hairpins **1**, **2**, **4**, and **9** are shown in Figure 1 and spectra for the other hairpins are provided in Figure S2 (Supporting Information). The transient spectra of **1** have previously been reported [13]. The 575 nm band formed during the ca. 180 fs, 350 nm laser pump pulse is assigned to the locally excited $^1\text{Sa}^*$ state. Time-dependent growth of a 530 nm shoulder at short delays is assigned to the $\text{Sa}^{\cdot-}$ anion radical which is formed upon hole injection to the neighboring purine A (k_{iA} , Scheme 1). At longer delay times the growth of a discrete band at 530 nm is attributed to the formation of the $\text{Sd}^{\cdot+}$ cation radical (k_{Sd}). The assignment of these bands is based on the use of reference dyads for the

formation of $\text{Sa}^{\bullet-}$ and $\text{Sd}^{+\bullet}$ [19]. The analysis of the transient absorption data is based on the kinetic scheme previously proposed for hairpin **1** (Scheme 1) [13]. The charge separation times (τ_{cs}) reported in Table 1 were determined by means of global analysis of the transient absorption spectra (Figure S3) [2]. This method is considered more accurate than the method based on the ratio of 575/525 nm band intensities used previously and provides a somewhat longer charge separation time (e.g. 1.6 vs. 1.3 ns for **1**) [13]. Relative quantum yields for charge separation (Φ_{cs}) were determined by comparing the integrated areas for the $\text{Sa}^{\bullet-}$ radical anion following hole injection with that for the sum of the $\text{Sa}^{\bullet-}$ and $\text{Sd}^{+\bullet}$ radical ions following charge separation [13]. The relative yields, corrected to the value of $\Phi_{\text{cs}} = 0.27$ previously reported for hairpin **1**, are reported in Table 1.

Figure 1. Femtosecond time resolved absorption spectra of hairpins **1**, **2**, **4**, and **9**, labeled respectively, in 10 mM phosphate buffer (100 mM NaCl, pH ~ 7.2). The spectra are acquired in

semi-logarithmic time steps and the time window is labeled respectively in the graphs. Sa^- absorbs primarily at 575 nm and the $\text{Sd}^{+\bullet}$ at 530 nm.

Scheme 1. Hole transport dynamics in a Sa-Sd hairpins having a diblock purine base sequence. The A- and G-blocks can be in either the same strand or opposite strands. $k_{iA} + k_{tAG} + k_{Sd} = k_{cs}$ and $k = 1/\tau$.

Table 1. Quantum yields for charge separation (Φ_{cs}) and charge separation times (τ_{cs}) to $\text{Sd}^{+\bullet}$ for the capped hairpins. Structures are shown in Chart 1.

Hairpin	Φ_{cs}	τ_{cs} , ns	Hairpin	Φ_{cs}	τ_{cs} , ns
1	0.27 ^a	1.6	6	0.15	1.3
2	0.025	>10	7	0.27	2.0
3	0.015	>10	8	0.13	>10
4	0.27	1.5	9	0.15	>10
5	0.15	4.5	10	0.14	>10

^a Data from ref.13.

According to Scheme 1, the charge separation time (τ_{cs}) is bound by the rate determining step in the hole transport process. Since the rate constants for hole injection into A_m (k_{iA}) and hole trapping by Sd (k_{Sd}) should be the same for all of the hairpins in Chart 1, the variation in τ_{cs}

should be determined by the rate constant for transport from the A-block to the G-block, k_{tAG} (or to the final G in the case of **10**). Similarly, the quantum yield for charge separation should be determined by the competition between hole transport and charge recombination (k_{tAG} vs. k_{rA}) in moving from the A-block to the G-block. The pyrimidine-pyrimidine mismatches CC (**2**) and TT (**3**) are most effective in blocking hole transport, reducing the efficiency by a factor of > 20 and resulting in a hole transport time that is too slow to measure with our fs apparatus (> 10 ns). This result is consistent with previous observations that pyrimidine-pyrimidine mismatches inhibit hole transport to distal GG sites [8] and that a TT mismatch slows hole arrival in a NI-PTZ end-labeled duplex [11]. Both disruption of the base stacking in the vicinity of the mismatch and the higher oxidation potentials of T and C vs. A and G [20] can contribute to the slow hole transport across a pyrimidine-pyrimidine mismatch.

Of greater potential interest to the design of molecular devices including 3WJs are the purine-purine mismatches AA (**4**) and GG (**5**), both of which permit movement of charge from an A-block in one strand to a G-block in the complementary strand. The AA mismatch is particularly effective in this respect, having values of τ_{cs} and Φ_{cs} essentially the same as those for the parent diblock hairpin **1**. This indicates that the AA mismatch does not interfere with hole transport from the A-block to the G-block and is consistent with previous studies of the effect of an AA mismatch on strand cleavage efficiency [9] and ns time-resolved hole transport dynamics [11]. We find that hole transport via a GG mismatch in **5** is somewhat slower and less efficient than hole transport via an AA mismatch in **4**. The opposite order was previously reported by Bhattacharya and Barton for oxidative strand cleavage [8]. However, they were investigating *intrastrand* rather than *interstrand* hole transport. Since GG forms a particularly stable mismatch [18], we suspect the difference in the behavior of GG vs. AA in our hairpins may reflect more

efficient hole trapping by G at the end of an A-block in our hairpins than by A. The more rigid structure of the GG-containing hairpin may also contribute to slower hole transport. Increasing DNA duplex rigidity by the replacement of normal bases with rigid bicyclic bases (“locked nucleic acids”) [21] or using more strongly coordinating Mg^{2+} ions in place of Na^+ [22] also results in slower rates of hole transport.

The hairpins **6-10** all have one or more strand crossing in their polypurine pathways connecting Sa and Sd (Chart 1). Hairpins **6** and **7** which have A A and A-to-G strand crossings have values of τ_{cs} and Φ_{cs} which are similar to those for the parent diblock hairpin **1**. The higher value of Φ_{cs} for **7** may reflect the more favorable A-to-G vs. A-to-A charge gradient for hole transport in this hairpin. Hairpins **8-10** all have moderately high values Φ_{cs} but slow values for τ_{cs} . The values of Φ_{cs} are the same as that reported for a Sa-Sd hairpin with an A_4G_2 base sequence [13], which may reflect moderately efficient arrival at the first base in the G-block. However, τ_{cs} may be relatively slow due to the large number of bases that must be traversed prior to reaching the G-block. Slower inter- vs. intrastrand G-to-G hole transport in adjacent G-C base pairs is consistent with the previous observations of Majima and co-workers [23]. However efficiencies of hole transport were not measured by these workers.

CONCLUSIONS

In summary, we have investigated the effects of base pair mismatches and strand crossings on both the efficiency and dynamics of intra- and interstrand photoinduced hole transport in DNA hairpins. In previous studies, either relative efficiencies or rate constants were measured but never both in the same study. fsTA measurements have been employed to determine the quantum yields and time constants for charge separation between a stilbenedicarboxamide hole

donor and stilbenediether hole acceptor. Pyrimidine-pyrimidine mismatches TT and CC inhibit hole transport resulting in low values of Φ_{cs} (≤ 0.025) and time constants for charge separation too long to measure with our fs apparatus ($\tau_{cs} > 10$ ns). We attribute the effect of the pyrimidine-pyrimidine mismatches to a combination of poor base stacking in the vicinity of the mismatch and the introduction of a tunneling barrier for hole transport by the high-lying HOMO levels of the pyrimidine bases [24].

Both the AA mismatch in **4** and the A-to-A strand crossing in **6** have values of τ_{cs} similar to those of the base-paired diblock hairpin **1**, in accord with prior reports of rapid intrastrand hole transport via AA mismatches [11]. The A-to-G strand crossing in **7** also provides for efficient and rapid intrastrand hole transport. The GG mismatch in **5** and G-to-G strand crossings in **8-10** have less efficient and slower charge separation than their A-to-A counterparts. The lower efficiency of GG vs. AA hole transport in **5** vs. **4** may be a consequence of the increased rigidity of the GG mismatch or differences in hole delocalization. The information obtained in the current study is being utilized in the design of 3WJs that provide more efficient charge splitting and charge combination [2,12].

Acknowledgments. This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Chemical Sciences, Geosciences, and Biosciences Division under Award DE-FG02-96ER14604 (F.D.L.) and the U.S. Office of Naval Research MURI grant no. N00014-11-1-0729 (M.R.W.).

REFERENCES

- [1] M. A. Grodick, N. B. Muren, J. K. Barton, DNA charge transport within the cell, *Biochemistry* 54 (2015) 962-973.
- [2] R. M. Young, A. P. N. Singh, A. K. Thazhathveetil, V. Y. Cho, Y. Zhang, N. Renaud, F. C. Grozema, D. N. Beratan, M. A. Ratner, G. C. Schatz, Y. A. Berlin, F. D. Lewis, M. R. Wasielewski, Charge transport across DNA-based three-way junctions, *J. Am. Chem. Soc.* 137 (2015) 5113-5122.
- [3] P. T. Henderson, D. Johes, G. Hampikian, Y. Kan, G. B. Schuster, Long-distance charge transport in duplex DNA: The phonon-assisted polaron-like hopping mechanism, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 8353-8358.
- [4] B. Giese, J. Amaudrut, A.-K. Köhler, M. Spormann, S. Wessely, Direct observation of hole transfer through DNA by hopping between adenine bases and by tunnelling, *Nature* 412 (2001) 318-320.
- [5] C.-S. Liu, G. B. Schuster, Base sequence effects in radical cation migration in duplex DNA: Support for the polaron-like hopping model, *J. Am. Chem. Soc.* 125 (2003) 6098-6102.
- [6] B. Giese, Long-distance charge transport in DNA: The hopping mechanism, *Acc. Chem. Res.* 33 (2000) 631-636.
- [7] B. Giese, S. Wessely, Influence of mismatches on long-distance charge transport through DNA, *Angew. Chem. Internat. Ed.* 39 (2000) 3490-3491.
- [8] P. K. Bhattacharya, J. K. Barton, Influence of intervening mismatches on long-range guanine oxidation in DNA duplexes, *J. Am. Chem. Soc.* 123 (2001) 8649-8656.

- [9] N. W. Schlientz, G. B. Schuster, Long-distance radical cation migration in duplex DNA: The effect of contiguous A·A and T·T mismatches on efficiency and mechanism, *J. Am. Chem. Soc.* 125 (2003) 15732-15733.
- [10] F. D. Lewis, Y. Wu, X. Liu, Synthesis, structure, and photochemistry of exceptionally stable synthetic DNA hairpins with stilbene diether linkers, *J. Am. Chem. Soc.* 124 (2002) 12165-12173.
- [11] Y. Osakada, K. Kawai, M. Fujitsuka, T. Majima, Kinetics of charge transfer in DNA containing a mismatch, *Nucleic Acids Res.* 36 (2008) 5562-5570.
- [12] Y. Zhang, R. M. Young, A. K. Thazhathveetil, A. P. N. Singh, C. Liu, Y. A. Berlin, F. C. Grozema, F. D. Lewis, M. A. Ratner, N. Renaud, K. Siriwong, A. A. Voityuk, M. R. Wasielewski, D. N. Beratan, Conformationally gated charge transfer in DNA three-way junctions, *J. Phys. Chem. Lett.* 6 (2015) 2434-2438.
- [13] J. Vura-Weis, M. R. Wasielewski, A. K. Thazhathveetil, F. D. Lewis, Efficient charge transport in DNA diblock oligomers *J. Am. Chem. Soc.* 131 (2009) 9722-9727.
- [14] R. L. Letsinger, T. Wu, Use of a stilbenedicarboxamide bridge in stabilizing, monitoring, and photochemically altering folded conformations of oligonucleotides, *J. Am. Chem. Soc.* 117 (1995) 7323-7328.
- [15] R. M. Young, S. M. Dyar, J. C. Barnes, M. Juriček, J. F. Stoddart, D. T. Co, M. R. Wasielewski, Ultrafast conformational dynamics of electron transfer in exbox4+⊂perylene, *J. Phys. Chem. A* 117 (2013) 12438-12448.
- [16] K. E. Brown, B. S. Veldkamp, D. T. Co, M. R. Wasielewski, Vibrational dynamics of a perylene–peryleneimide donor–acceptor dyad probed with femtosecond stimulated raman spectroscopy, *J. Phys. Chem. Lett.* 3 (2012) 2362-2366.

- [17] F. D. Lewis, Y. Wu, L. Zhang, X. Zuo, R. T. Hayes, M. R. Wasielewski, DNA-mediated exciton coupling and electron transfer between donor and acceptor stilbenes separated by a variable number of base pairs, *J. Am. Chem. Soc.* 126 (2004) 8206-8215.
- [18] M. E. Burkard, D. H. Turner, NMR structures of r(GCAGGCGUGC)₂ and determinants of stability for single guanosine–guanosine base pairs, *Biochemistry* 39 (2000) 11748-11762.
- [19] F. D. Lewis, H. Zhu, P. Daublain, T. Fiebig, M. Raytchev, Q. Wang, V. Shafirovich, Crossover from superexchange to hopping as the mechanism for photoinduced charge transfer in DNA hairpin conjugates, *J. Am. Chem. Soc.* 128 (2006) 791-800.
- [20] C. A. M. Seidel, A. Schulz, M. H. M. Sauer, Nucleobase-specific quenching of fluorescent dyes. 1. Nucleobase one-electron redox potentials and their correlation with static and dynamic quenching efficiencies., *J. Phys. Chem.* 100 (1996) 5541-5553.
- [21] A. K. Thazhathveetil, J. Vura-Weis, A. A. Trifonov, M. R. Wasielewski, F. D. Lewis, Dynamics and efficiency of hole transport in LNA:DNA hybrid diblock oligomers, *J. Am. Chem. Soc.* 134 (2012) 16434.
- [22] A. Kalliat Thazhathveetil, A. A. Trifonov, M. R. Wasielewski, F. D. Lewis: The effect of Mg²⁺ cations on the dynamics and efficiency of hole transport in DNA. In *J. Phys. Chem. A*; American Chemical Society, 2014.
- [23] K. Kawai, T. Majima, Hole transfer kinetics of DNA, *Acc. Chem. Res.* 46 (2013) 2616-2625.
- [24] F. D. Lewis, J. Liu, W. Weigel, W. Rettig, I. V. Kurnikov, D. N. Beratan, Donor-bridge-acceptor energetics determine the distance dependence of electron transfer in DNA, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 12536-12541.