Electronic Interactions between π-Stacked DNA Base Pairs and Diphenylacetylene-4,4′-dicarboxamide in Hairpin DNA

Frederick D. Lewis,* Xiaoyang Liu, Scott E. Miller, and Michael R. Wasielewski*

Department of Chemistry, Northwestern University Evanston, Illinois 60208

Received July 15, 1999
Revised Manuscript Received September 7, 1999

The π-stacked base pairs of B-form DNA provide a unique medium for the investigation of electron transfer.[1] Most recent investigations of the dynamics of photoinduced electron transfer in DNA have employed probe chromophores that are π-stacked with an adjacent base pair.[2−4] Our approach has been to use organic chromophores as linkers in hairpin-forming bis(oligonucleotide) conjugates.[2] A hairpin structure in which the organic chromophore is approximately coplanar with the adjacent base pair is supported by spectroscopic studies, molecular modeling, and crystallography.[2,3] Others have employed intercalators or fluorescent nucleobase analogues as probe chromophores.[4] The pronounced effect of intercalation on the spectroscopic properties of some intercalators is generally attributed to a change in solvation from aqueous solution to the hydrophobic interior of duplex DNA. However, π-stacking can also result in charge-transfer[5] or excitonic interactions[6] and might also influence the dynamics of charge separation and recombination.

We report here the results of our investigation of the interactions between the diphenylacetylene-4,4′-dicarboxamide (DPA) chromophore and an adjacent T−A or C−G base pair in several hairpin-forming (bis)oligonucleotide conjugates. Femtosecond time-resolved spectroscopy indicates that charge separation occurs via rapid electron transfer to the DPA-localized singlet state from the neighboring A or G nucleobase to generate a contact radical ion pair, DPA^-A^+ or DPA^-G^+. Charge recombination occurs on a much longer time scale and is strongly energy-gap dependent. Hole hopping from DPA^-A^+ to a nearby G does not compete effectively with charge recombination within the contact ion pair. These results help further elucidate the nature of the electron-transfer processes in DNA.

Diphenylacetylene-4,4′-dicarboxylic acid was prepared from stilbene-4,4′-dicarboxylic acid via bromination−dehydrobromination and converted to the diol **1**, as previously described for the analogous stilbene diol by Letsinger and Wu.[7] The bis(oligonucleotide) conjugates **2**−**5** (Chart 1) were prepared from the monoprotected, monoactivated diol by means of conventional phosphoramidite chemistry using a Millipore Expedite oligonucleotide synthesizer following the procedure developed for related bis(oligonucleotide) conjugates.[7]

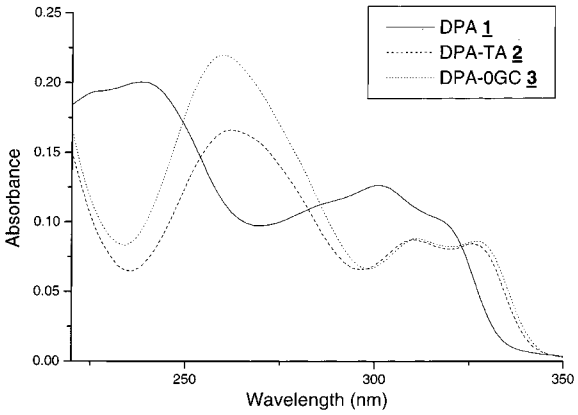
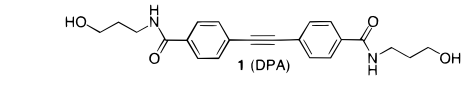


Figure 1. Electronic absorption spectra of diol **1** in aqueous solution and hairpins **2** and **3** in 0.1 M NaCl, 30 mM sodium phosphate, pH 7.2.

Chart 1. Decay Times for Charge Separation and Recombination



	2	3	4	5
τ_{cs}, ps	2.7	0.4	2.3	1.8
τ_{cr}, ps	1400	19	1350	1500

The ultraviolet absorption spectra of diol **1** and conjugates **2** and **3** are shown in Figure 1. The long-wavelength absorption bands of **2** and **3** are red-shifted with respect to that of **1** and have two maxima of similar intensity. The 260-nm absorption band of **2** and **3** is attributed to overlapping bands of the DPA and nucleobase chromophores. The thermal dissociation profiles for the 260-nm bands provide melting temperatures of 55 and 70 °C for **2** and **3**, respectively. The melting temperature is independent of concentration and substantially higher than that calculated for a hypothetical duplex formed between two molecules of **2** or **3**, in accord with the formation of a hairpin structure.[8] Above the melting temperature, the long-wavelength absorption bands of **2** and **3** resemble that of the diol **1**. The circular dichroism spectra of **2** and **3** exhibit a positive band at 283 nm and a negative band at 248 nm, characteristic of B-form DNA.[9] Molecular modeling confirms that **2** can adopt a hairpin structure in which the DPA chromophore is parallel to the adjacent T−A base pair with a plane-to-plane separation similar to the 3.4 Å separation for π-stacked base pairs in DNA.[10]

The diol **1** is strongly fluorescent in methanol solution (λ_{max} = 351 nm, Φ_f = 0.33); however, the conjugates **2**−**5** are

* Address correspondence to either author. E-mail: lewis@chem.nwu.edu or wasielew@chem.nwu.edu.

(1) For recent reviews, see: (a) Holmlin, R. E.; Dandliker, P. J.; Barton, J. K. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2714−2730. (b) Netzel, T. L. In *Organic and Inorganic Photochemistry, Vol 2*; Ramamurthy, V., Schanze, K. S., Eds.; Dekker: New York, 1998; pp 1−54.

(2) (a) Lewis, F. D.; Wu, T.; Zhang, Y.; Letsinger, R. L.; Greenfield, S. R.; Wasielewski, M. R. *Science* **1997**, *277*, 673−676. (b) Lewis, F. D.; Zhang, Y.; Liu, X.; Xu, N.; Letsinger, R. L. *J. Phys. Chem. B* **1999**, *103*, 2570−2578.

(3) Lewis, F. D.; Liu, X.; Wu, Y.; Miller, S. E.; Wasielewski, M. E.; Letsinger, R. L.; Sanishvili, R.; Joachimiak, A.; Tereshko, V.; Egli, M. *J. Am. Chem. Soc.*, in press (JA991934U).

(4) (a) Fukui K.; Tanaka, K. *Angew. Chem., Int. Ed.* **1998**, *37*, 158−161. (b) Harriman, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 945−949. (c) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014−6019 and references therein. (d) Kelley, S. O.; Barton, J. K. *Science* **1999**, *283*, 375−381.

(5) (a) Asahi, T.; Mataga, N. *J. Phys. Chem.* **1991**, *95*, 1956−1963. (b) Asahi, T.; Ohkohchi, M.; Mataga, N. *J. Phys. Chem.* **1993**, *97*, 13132−13137. (c) Gould, I. R.; Noukakis, D.; Gomez-Jahn, L.; Young, R. H.; Goodman, J. L.; Farid, S. *Chem. Phys.* **1993**, *176*, 439−456. (d) Hubig, S. M.; Bockman, T. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 3842−3851.

(6) Nastasi, M.; Morris, J. M.; Rayner, D. M.; Seligy, V. L.; Szabo, A. G.; Williams, D. F.; Williams, R. E.; Yip, R. W. *J. Am. Chem. Soc.* **1976**, *98*, 3937−3986.

(7) (a) Salunkhe, M.; Wu, T.; Letsinger, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8768−8772. (b) Letsinger, R. L.; Wu, T. *J. Am. Chem. Soc.* **1995**, *117*, 7323−7328.

(8) Duplex formation between complementary DPA-linked conjugates results in the observation of DPA excimer fluorescence. The hairpins **2**−**5** are nonfluorescent.

(9) (a) Jin, R.; Gaffney, B. L.; Wang, C.; Jones, R. A.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8832−8836. (b) Lu, M. Guo, Q.; Kallenbach, N. R. *Biochemistry* **1993**, *32*, 598−601.

(10) Hairpin structures were calculated using the molecular mechanics force field (MM^+) within Hyperchem V5.0 (Hypercube, Waterloo, ON, Canada). Local minima were optimized assuming normal B-form DNA geometries.

10.1021/ja9924997 CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/04/1999

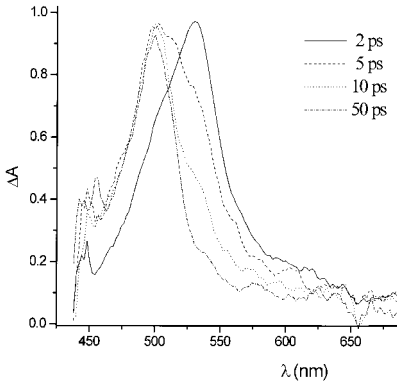


Figure 2. Transient absorption spectra of hairpin **2** obtained after a 0.2-ps, 327-nm excitation pulse at indicated delay times. Spectral intensities are normalized at their maxima.

totally nonfluorescent. The transient absorption spectrum of **1** in methanol solution displays a single narrow band at 535 nm with a decay time of 215 ps.[11] The band shapes determined at 5, 50, and 250 ps delay time following a 0.2-ps, 327-nm laser pulse are identical. The invariant band shape is consistent with the planar and nonpolar nature of the DPA ground and excited states.[12] The transient absorption spectra of conjugate **2** at several delay times are shown in Figure 2. The spectrum obtained for a delay time of 2 ps resembles that of the diol **1**, indicative of a similar electronic structure for the DPA chromophore in diol **1** and hairpin **2**. However, the 535-nm band decays rapidly and is replaced by a new band at 500 nm, which is assigned to the DPA anion radical.[13] Similar time-dependent transient absorption spectra are observed for hairpins **3**−**5**. Decay of both the 535- and 500-nm bands is dominated by a single component, which is assigned to charge separation and charge recombination, respectively. Values of τ_{cs} (k_{cs}^{-1}) and τ_{cr} (k_{cr}^{-1}) are summarized in Chart 1.

The observation of red-shifted DPA absorption for the conjugates **2**−**5** below, but not above, their melting temperatures (Figure 1) suggests that π-stacking of the DPA chromophore with a neighboring base pair is responsible for the spectral shift. The changes in the DPA long-wavelength band are similar to those observed by Nastasi et al.[6] for quinacrine−DNA complexes and attributed to excitonic interaction between the excited dye and adjacent nucleobases, rather than a charge-transfer interaction. Further evidence for relatively weak ground-state interaction between DPA and the adjacent nucleobases is provided by the similarity of the transient absorption spectra of the conjugate **2** at short delay time to that of the diol **1**. The transient absorption spectra of charge-transfer complexes resemble those of the radical ions even at subpicosecond delay times.[5a−c]

The energetics and dynamics of charge separation and charge recombination for conjugates **2** and **3** are summarized in Figure 3. The DPA singlet energy E_S is estimated from the midpoint of the absorption and fluorescence maxima of **1**. The contact ion pair energies are calculated using eq 1,[14]

$$\Delta G_{cip} = (E_d^{ox} - E_a^{red}) + 0.56/\epsilon \qquad (1)$$

using the DPA oxidation potential and A or G deoxynucleoside reduction potential in acetonitrile solution and assuming a dielectric constant $\epsilon \approx 20$, intermediate between those of pyridine and water.[15,16] The charge separation rates for hairpins **2** and **3** are very rapid but not highly dependent upon ΔG_{cs} ($E_s - E_{cip}$). Faster charge separation for hairpin **3** vs **2** is consistent with the larger free energy change for formation of the DPA^-G^+ contact ion pair, assuming that charge separation is in the Marcus normal region.[17] A much larger difference is observed in the charge recombination rates for the DPA^-A^+ vs DPA^-G^+ contact ion pairs. Faster charge recombination for DPA^-G^+ is consistent with the smaller free energy change for electron transfer in the Marcus inverted region. Compared to the results of Mataga, Farid, and Kochi and their co-workers[5] for contact ion pair charge recombination, the rates of charge recombination in Figure 3 are both much larger than expected for the calculated valued of ΔG_{cip} and more strongly dependent upon ΔG_{cip}. This suggests that the values of E_D^{ox} and E_a^{red} obtained for the isolated DPA and nucleosides may be different than the values in the DNA hairpins. Ab initio molecular orbital calculations indicate that the ionization potentials of π-stacked base pairs in B-form DNA are significantly lower than values for the isolated nucleosides and that the stabilization energy is larger for G vs A.[18]

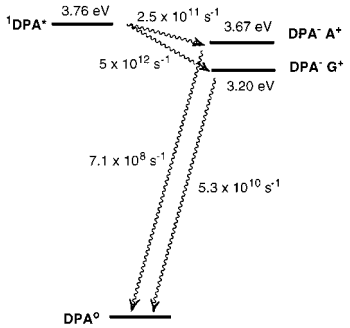


Figure 3. Energetics and dynamics of photoinduced electron transfer to singlet DPA from the nucleobases A or G.

The relatively slow charge recombination rate for the DPA^-A^+ contact ion pair in hairpin **2** suggested that it might be possible to observe hole transfer from the initially oxidized A^+ to a G located farther from the DPA acceptor. Increasing the distance between the reduced acceptor and oxidized donor is expected to result in slower charge recombination.[2a] Hairpins **4** and **5** were synthesized in order to test this possibility. Their charge recombination rates are the same as that for hairpin **2**. Thus, we conclude that hole hopping from A^+ to G does not compete effectively with charge recombination of the DPA^-A^+ contact ion pair ($k_{cr} = 7 \times 10^8\ s^{-1}$). Since a 10% component of slow charge recombination would have been detected, an upper limit of $1 \times 10^8\ s^{-1}$ can be estimated for hole hopping. This result is consistent with a report by Melvin et al.[19] that hole migration in photoionized duplex DNA occurs on a microsecond time scale at room temperature. Thus, it may be necessary to create a long-lived, isolated nucleobase cation radical in order to observe efficient hole hopping. Both the absolute quantum yields and dynamics of hole hopping in DNA remain to be established.[20]

Acknowledgment. We thank R. L. Letsinger for helpful discussions. This research is supported by grants from the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy, under contracts DE-FG02-96ER14604 (F.D.L.) and W-31-109-Eng-38 (M.R.W.).

JA9924997

(11) For a description of the laser system, see ref 2a and the following: Greenfield, S. R.: Švec, W. A.; Gosztola, D.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 6767−6777.

(12) Abramenkov, A. V.; Almenningen, A.; Cyvin, B. N.; Cyvin, S. J.; Jonvik, T.; Khaikin, L. S.; Rømming, C.; Vikov, L. V. *Acta Chem. Scand. A* **1988**, *42*, 674−684.

(13) The unsubstituted diphenylacetylene anion radical has a similar band shape with a maximum at 446 in a methyltetrahydrofuran glass at 77 K: Shida, T. *Electronic Absorption Spectra of Radical Ions*; Elsevier: Amsterdam, 1988; p 109.

(14) Arnold, B. R.; Farid, S.; Goodman, J. L.; Gould, I. R. *J. Am. Chem. Soc.* **1996**, *118*, 5482−5483.

(15) The reduction potential of diol **1** in DMF solution is −1.98 V vs SCE. Nucleoside oxidaton potentials in DMF solution reported by Seidel et al.[14] for dA and dG are 1.22 and 1.69 V vs SCE, respectively.

(16) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553.

(17) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966−978.

(18) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.

(19) Melvin, T.; Plumb, M. A.; Botchway, S. W.; O'Neill, P. O.; Parter, A. W. *Photochem. Photobiol.* **1995**, *61*, 584−591.

(20) For a recent investigation of hole hopping in DNA, see: Giese, B.; Wessley, S.; Spormann, M.; Lindemann, U.; Meggers, E.; Michel-beyerle, M. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 996−99.