Communications to the Editor

Long-Distance Radical Cation Migration in Z-Form DNA

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The simplicity of DNA's structure revealed its role in biology[1] and inspired investigations of its material properties. The apparent similarity of the π-electron overlap of its aromatic bases with semiconducting organic crystals stimulated examination of its electrical behavior in the solid state.[2−9] Studies of oligomers in solution seemed to support the notion that the stacked bases serve as "π-ways" for rapid one-step electron transfer,[10−13] but this view has been supplanted by charge-transport mechanisms requiring multiple short-range, thermally activated steps (hops).[14−17] Unlike the static regularity of crystals, DNA is structurally dynamic,[18,19] which will affect its electrical properties. The examination of charge transport of DNA has focused on its B-form.[20−23] Although there has been speculation concerning the electronic properties of Z-DNA,[24] there are no experimental investigations of charge transport in these structures. We report here long-distance (ca. 30 Å) radical cation migration in Z-DNA hairpins.

It is clear that the efficiency of radical cation transport in DNA in solution is determined by a competition between the rates for hopping and the irreversible consumption of the radical cation.[20,21,25−27] Both of these processes will depend on the structure of the DNA. Duplex DNA containing an uninterrupted $(CG)_n$ sequence adopts the Z-form at high salt concentrations[28] and in the solid state.[29] Z-DNA differs structurally and dynamically from B-form. Z-DNA forms as a relatively thin, unwound, left-handed helix with two base pairs per repeat.[30] Significantly, the base-pair opening rate of Z-DNA, as measured by proton exchange, is much slower than it is in B-form.[31] These structural and dynamic differences suggest that the mechanism of charge transport may differ between the B- and Z-forms.

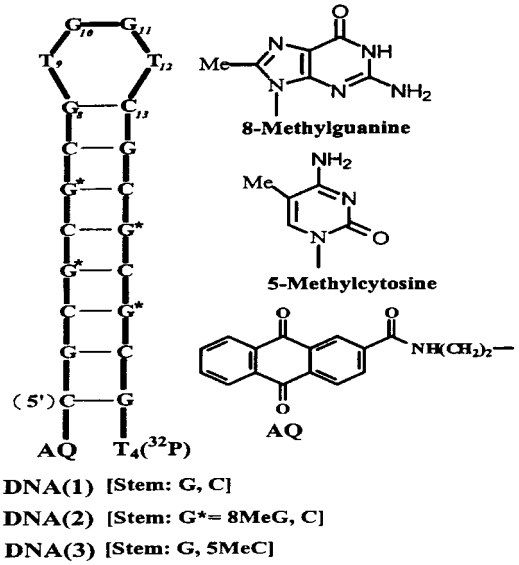

Figure 1. Structures of DNA hairpin assemblies.

Duplexes having base pairs that are out of the repeating $(CG)_n$ order form Z-DNA with increasing difficulty and have B−Z junctions of ambiguous structure.[32] We prepared a series of DNA hairpins that contain a $(CG)_4$ stem capped with a four-base loop, Figure 1. Similar hairpins are known to adopt the Z-DNA structure.[33] Conversion of B-DNA to the Z-form occurs spontaneously for appropriate oligomers in concentrated salt solutions. The substitution of 8-methylguanine (8MeG)[34] or 5-methylcytosine (5MeC)[28] for their unmethylated counterparts lowers the ionic strength at which the B-to-Z conversion occurs.

Radical cations can be injected into duplex DNA by irradiation of an anthraquinone derivative (AQ) covalently attached to a 5′-terminus.[16] They migrate through the DNA and react at GG steps to form products that are revealed as strand breaks by treatment with piperidine.[23,35,36] The DNA hairpin assemblies we studied

(1) Watson, J.; Crick, F. *Nature* **1953**, *171*, 737−738.
(2) Eley, D. D.; Spivey, D. I. *Trans. Faraday Soc.* **1962**, *58*, 411−415.
(3) Warman, J. M.; de Haas, M. P.; Rupprecht, A. *Chem. Phys. Lett.* **1996**, *249*, 319−322.
(4) Okahata, Y.; Kobayashi, T.; Tanaka, K.; Shimomura, M. *J. Am. Chem. Soc.* **1998**, *120*, 6165−6166.
(5) Fink, H. W.; Schonenberger, C. *Nature* **1999**, *398*, 407−410.
(6) Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635−638.
(7) Tran, P.; Alavi, B.; Grunner, G. *Phys. Rev. Lett* **2000**, *85*, 1564−1567.
(8) Cai, L. T.; Tabata, H.; Kawai, T. *Appl. Phys. Lett.* **2000**, *77*, 3105−3106.
(9) Park, S. J.; Lazarides, A. A.; Mirkin, C. A.; Brazis, P. W.; Kannewurf, C. R.; Letsinger, R. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3845−3848.
(10) Prugganan, M. D.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Science* **1988**, *241*, 1645−1649.
(11) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossman, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025−1029.
(12) Turro, N. J.; Barton, J. K. *J. Biol. Inorg. Chem.* **1998**, *3*, 201−209.
(13) Wan, C.; Fiebig, T.; Kelly, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014−6019.
(14) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747−8748.
(15) Debije, M. G.; Milano, M. T.; Bernhard, W. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 2752−2756.
(16) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(17) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(18) Beveridge, D. L.; McConnel, K. J. *Curr. Opin. Struct. Biol.* **2000**, *10*, 182−196.
(19) Dickerson, R. E. *Methods Enzymol.* **1992**, *211*, 67−111.
(20) Sartor, V.; Henderson, P. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 11027−11033.
(21) Kan, Y.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *11607*, 7−11614.
(22) Aich, P.; Labiuk, S. L.; Tari, L. W.; Delbaere, L. T. J.; Roesler, W. J.;; Falk, K. J.; Steer, R. P.; Lee, J. S. *J. Mol. Biol.* **1999**, *294*, 477−485.
(23) Meggers, E.; Kusch, D.; Spichty, M.; Wille, U.; Giese, B. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 460−462.
(24) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.
(25) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci., U.S.A.* **1999**, *96*, 8353−8358.
(26) Giese, B.; Spichtly, M. *Chem. Phys. Chem.* **2000**, *1*, 195−198.
(27) Sartor, V.; Schuster, G. B. Preliminary results.
(28) Johnston, B. H. *Methods Enzymol.* **1992**, *211*, 127−159.
(29) Ho, P. S.; Moores, B. H. M. *Biopolymers* **1997**, 65−90.
(30) Long, E. C. In *Fundamentals of Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: New York, 1996; pp 3−35.
(31) Kochoyan, M.; Leroy, J. L.; Gueron, M. *Biochemistry* **1990**, *29*, 4799−4805.
(32) Xodo, L. E.; Manzini, G.; Quadrifoligo, F.; Yathindra, N.; van der Marvel, G.; van Boom, J. H. *J. Biomol. Struct. Dyn.* **1989**, *6*, 1217−1231.
(33) Amaratunga, M.; Pancoska, P.; Paner, T. M.; Benight, A. S. *Nucleic Acids. Res.* **1990**, *18*, 577−582.
(34) Sugiyama, H.; Kawai, K.; Matsunaga, A.; Fujimoto, K.; Saito, I.; Robinson, H.; Wang, A., H.-J. *Nucleic Acids. Res.* **1996**, *24*, 1272−1278.
(35) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221−13227.
(36) Kan, Y.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 10857−10864.

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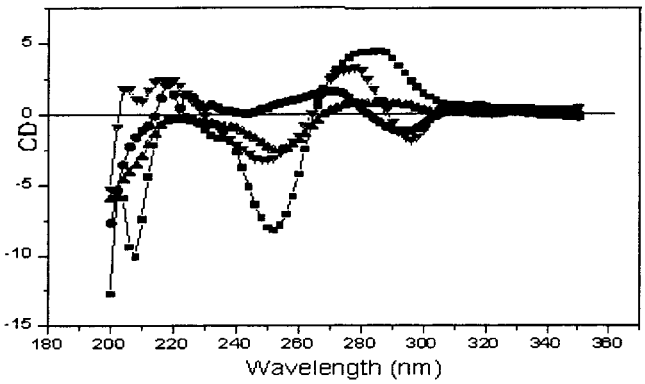

Figure 2. Circular dichroism spectra of DNA assemblies: DNA(1) in 10 mM Na_2PO_4 solution (■); DNA(2) in 10 mM Na_2PO_4 solution (●); DNA(3) in 10 mM Na_2PO_4 solution (▲); DNA(3) in 10 mM $Mg(OAc)_2$ solution (▼).

contain a GG step in the loop, which serves to detect radical cation reactions at that site. Strand cleavage is revealed by PAGE of samples labeled at the 3′-terminus with ^{32}P. The 3′-end of the hairpin was extended with a single-stranded $(T)_4$ sequence to facilitate labeling.

The global structure of DNA is revealed by its circular dichroism (CD) spectrum; in particular, spectra of the B- and Z-forms have opposite signs at ca. 290 nm.[28] The CD spectra of DNA(1) in either 10 mM Na_2PO_4 or $Mg(OAc)_2$ solutions are characteristic of the B-form, Figure 2. DNA(1) is converted to the Z-form in 4 M $NaClO_4$, but this solution is not suitable for study due to a high level of spontaneous guanine oxidation. DNA-(2) is predominantly in the Z-form even in 10 mM Na_2PO_4 solution. In contrast, DNA(3) adopts the B-form in 10 mM Na_2-PO_4 and a Z-like form in 10 mM $Mg(OAc)_2$ solutions. Thus, we are able to switch the structure of the hairpin assemblies by controlling the solution composition.

Irradiation (350 nm) of DNA(1) in 10 mM Na_2PO_4 or Mg-$(OAc)_2$ solution leads to selective cleavage at the GG step in the loop of the hairpin (Figure 3). The cleavage efficiency is not affected when the solvent is changed from H_2O to D_2O in this case or for experiments with DNA(2) and DNA(3), which rules out a role for $^1O_2$.[37] Irradiation of DNA(2) in either Na_2PO_4 or $Mg(OAc)_2$ solutions, where it exists in the Z-form, shows reaction at each 8MeG in addition to the GG step in the loop. This finding reveals an unanticipated complication. Methyl substitution lowers the oxidation potential of guanine,[38] which causes an increase in reactivity that is unrelated to the DNA global structure. This complication is absent in DNA(3), which contains only normal guanines. Irradiation of DNA(3) in its B-form (Na_2PO_4 solution) or its Z-form ($Mg(OAc)_2$ solution) gives similar yields of cleavage at the loop GG step, which demonstrates long-distance radical cation transport in Z-DNA.

We expected that radical cation migration would be more efficient in Z-DNA than in the B-form because the latter's slower base-pair opening rate suggests a more hydrophobic core. However, comparison of the relative efficiency of radical cation migration rates in these hairpins is confounded by different geometries of the GG step in the loops.[39,40] Molecular mechanics calculations for DNA(1) in the B- and Z-forms reveal that the

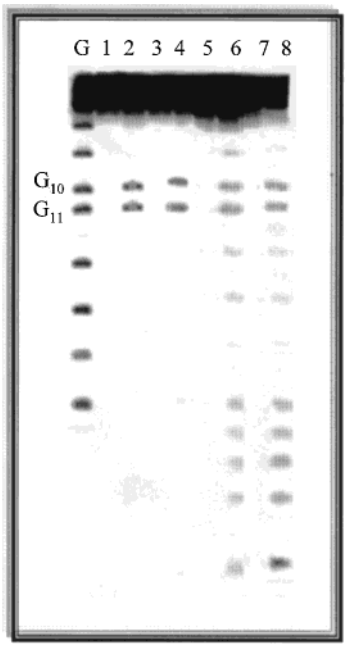

Figure 3. Autoradiograms of PAGE gels from irradiation of the DNA hairpin assemblies. Lanes 1, 3, 5, and 7 are dark controls for the experiments illustrated in the adjacent even-numbered lane. The control samples were treated identically with the experimental samples except that they were not exposed to UV light. The lane labeled G is the Maxam−Gilbert G-sequencing lane for DNA(1). All experimental samples were irradiated for 120 min in a Rayonet photoreactor, worked up in the usual way[25] and subjected to piperidine treatment before electrophoresis. Lane 2: DNA(1) irradiated in 10 mM $Mg(OAc)_2$ solution. Lane 4: DNA-(1) irradiated in 10 mM Na_2PO_4 solution. Lane 6: DNA(3) irradiated in 10 mM Na_2PO_4 solution. Lane 8: DNA(3) irradiated in 10 mM Mg-$(OAc)_2$ solution. As has been seen previously,[36] the 3′- and 5′-guanines of GG steps are approximately equally reactive in single-strand regions.

5′-link to the loop originates from the major groove side of the B-form helix and the 3′-link comes from the minor groove side. In this structure, the guanines of the GG step are not stacked on each other. In contrast, both the 5′- and 3′-links to the loop come from the minor groove side in Z-DNA, and the guanines are stacked.[41] This change in GG geometry may affect the rate for charge hopping to the GG step and the rate for trapping of the radical cation with H_2O; either change could result in different strand cleavage efficiency at the GG step for B- and Z-form DNA.

In summary, these experiments clearly show that radical cation transport through Z-form DNA occurs over distances greater than 30 Å. These findings are consistent with the current view that radical cations hop in duplex DNA from purine to purine (an all-guanine path in this case) in competition with their irreversible consumption by reaction with H_2O.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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(37) Rogers, M. A. J.; Snowden, P. T. *J. Am. Chem. Soc.* **1982**, *104*, 5541−5543.
(38) Sheu, C.; Foote, C. S. *J. Org. Chem.* **1995**, *60*, 4498−4503.
(39) Wolk, S. K.; Hardin, C. C.; Germann, M. W.; van de Sande, J. H.; Tinoco Jr., I. *Biochemistry* **1988**, *27*, 6960−6967.
(40) Chattopadhyaya, R.; Grzeskowiak, K.; Dickerson, R. E. *J. Mol. Biol.* **1990**, *211*, 189−210.
(41) The structures are shown in Supporting Information.