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DNA-Mediated Charge Transport Requires Conformational Motion of the DNA Bases: Elimination of Charge Transport in Rigid Glasses at 77 K

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DNA-mediated charge transport (CT), both in designed assemblies[1] and in biological milieus,[2] cannot simply be described by superexchange or purine hopping models derived from the redox energies of isolated DNA bases.[3] DNA CT is exquisitely sensitive to base stack structure and dynamics.[4] In fact, rapid (ps−ns) conformational motions of an intercalated DNA photooxidant,[5] and bridging DNA bases,[6] modulate the rate constants, yields, and distance dependence of CT. We have suggested a model where DNA CT is gated by base motions,[6] with only certain well-coupled arrangements of the DNA bases being active toward CT.[7] We describe a CT-active conformation as a domain, an extended π-orbital formed transiently as a function of sequence-dependent DNA dynamics.[6b] Here, to establish the significance of such CT-active conformations, we examine the yield of base-base CT at 77 K, where base rearrangement is effectively eliminated.[8,10]

Photoexcited 2-aminopurine (Ap*) probes how base stack structure and dynamics influence CT.[6,11] In DNA, Ap forms a well-stacked base pair with T,[12] providing a nonperturbing fluorescent reporter whose emission is strongly quenched in a sequence- and structure-dependent manner;[13] CT with DNA bases contributes significantly to Ap* reactivity in DNA.[14] We focus here on CT between Ap* ($E_{red} \approx 1.5$ V vs NHE)[11a] and G ($E_{ox} \approx 1.3$ V vs NHE),[15] either directly, or through an intervening $(A)_n$ bridge (Table 1).[16] In DNA, we distinguish this CT by comparing G-containing (redox-active) duplexes to otherwise identical (reference) duplexes where the G hole donor is replaced by 2′-deoxyinosine (I), a G analogue, that, due to its higher oxidation potential ($E_{ox} \approx 1.5$ V vs NHE)[11a] is much less reactive toward CT with Ap*.[11b] The yield of CT between Ap* and G (Fq) is obtained from fluorescence quantum yields as $Fq = 1 - \Phi_G/\Phi_I$, where Φ_G/Φ_I is the relative fraction of G-containing duplexes fluorescing and thus not undergoing CT. For CT through intervening A bridges, localized injection onto A (i.e., hopping) does not contribute to Fq, since this pathway exists in both redox-active and reference duplexes.

To examine low-temperature CT in DNA, we have used 10 M aqueous LiCl since this medium forms a stable and reproducible transparent glass at 77 K (Supporting Information) and is expected to be relatively nonperturbing to DNA structure.[17,18] Our characterization of the local DNA environment using Ap* is consistent with this expectation. In 10 M LiCl at ambient temperatures, the fluorescence quantum yields, anisotropies, and excitation spectra, as well as the yields of CT, are comparable to those observed in 100 mM sodium phosphate buffer (Supporting Information), indicating that the stacking interactions and solvent accessibility of the DNA bases are similar. Upon cooling to 77 K,[19] the fluorescence anisotropies of Ap* in all samples become comparable ($r = 0.35(2)$) and approach the theoretical maximum of 0.4,[20] confirming the expected loss of rotational and translation motion of Ap* at 77 K. The Stokes shift also decreases by ~900 cm^{-1} upon cooling to 77 K, indicating that conformational modes present in DNA at ambient temperatures become kinetically frozen at 77 K.[21]

Table 1. Ap* Fluorescence Yields and Yields of CT with G in 10 M LiCl

	Φ_{rel} 77 K[b]		Φ 77 K/Φ 298 K[c]			
sample[a]	Y = I	Y = G	Y = I	Y = G	Fq 77 K[b]	Fq 298 K
ApY	0.95	0.45	15	25	0.47	0.68
ApAY	0.98	0.95	25	45	0.03	0.47
ApA2Y	1.1	1.1	30	38	0	0.23
ApA3Y	1.1	1.0	30	39	0	0.18
ApA4Y	1.1	1.1	34	38	0	0.14

[a] 25 μM Ap in 35-mer DNA duplexes, 5′-GAT TAT AGA CAT ATT IAp(A)$_n$ YIT ATT AAG TAC ATT AC-3′, Y = I,G, pH 7. [b] All fluorescence yields relative to free Ap in 10 M LiCl at 77 K (λ_{ex} = 325 nm). Evaluated from 2 to 4 replicates, uncertainties ±0.05. [c] For free Ap, (Φ 77 K)/(Φ 298 K) = 1.5.

Table 1 presents the relative quantum yields (Φ_{rel}) of Ap at 77 K. As expected, Φ_{rel} is enhanced upon cooling, due at least in part to decreased thermal deactivation of Ap*. For free Ap, the enhancement in Φ_{rel} is rather modest, about 1.5-fold. In duplex DNA, however, Φ_{rel} increases by 15−45-fold (Table 1, Figure 1a). This increased emission is not due to a structural perturbation, such as extrusion of Ap from the duplex, associated with cooling. At 77 K the fluorescence excitation spectrum reveals significant energy transfer from the natural DNA bases to Ap (Figure 1b) indicating that Ap is well stacked within the duplex.[22] Instead, the dramatic enhancement in emission intensity reflects the loss of Ap* quenching in duplex DNA upon cooling to 77 K. The fluorescence enhancement is remarkable: The quenching of Ap* in DNA that has been so extensively exploited[13] is eliminated in rigidified duplexes, unless Ap* is directly adjacent to G.

Various mechanisms have been proposed to account for quenching of Ap* in DNA, including stacking interactions, hydrogen bonding, collisional deactivation,[23] electron transfer,[6,11,14] and enhanced population of a nonfluorescent dark state.[24] Here, the simple observation of suppressed reactivity upon cooling suggests that, irrespective of mechanism, quenching of Ap* in duplex DNA is largely a dynamic process. Static quenching is not significant. Instead, mechanisms involving conformational motion of the DNA bases dominate Ap* reactivity in these duplexes.

The yield of CT is also dramatically altered upon cooling to 77 K (Table 1, Figure 1, c and d). Significantly, no DNA-mediated CT is detected at 77 K. Only in the ApG assembly, where Ap* is in direct contact with G, does CT persist at 77 K, albeit at a lower efficiency than at ambient temperatures. As Fq is determined in 10 M LiCl at both ambient and low temperature (Table 1), the vanishing yield of CT at 77 K cannot be attributed to LiCl. These results indicate that CT between DNA bases involves more than simple tunneling. They are also not rationalized by a loss of thermally induced hopping, since localized hopping is not included in our CT yield (*vide supra*). These data suggest instead that CT,

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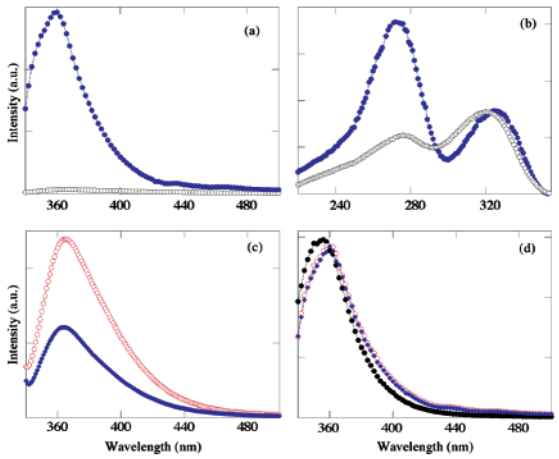

Figure 1. Steady-state fluorescence of Ap* in 10 M LiCl pH 7. Emission (a, $\lambda_{ex} = 325$ nm, 25 μM) and normalized excitation (b, $\lambda_{em} = 370$ nm, 2.5 μM) spectra of ApAG and ApA_4G duplexes, respectively, at 298 K (open black circles) and 77 K (closed blue circles). (c) Emission spectra of ApAG (closed blue diamonds), and ApAI (open red circles) at 298 K. (d) Emission spectra of the same samples as in (b), along with free Ap (black circles), obtained at 77 K.

like quenching of Ap*, requires conformational motion. Contrary to the notion that dynamic disorder hinders the ability of DNA to transport charge,[25] these observations are consistent with conformationally gated CT.[26] It is noteworthy that structural fluctuations are also key elements in mechanisms for DNA CT involving polaron formation.[27] Interestingly, the requirement of base dynamics for CT may, in addition, explain the much slower CT in stilbene−DNA systems, where the stilbene linker imposes an unnatural rigidity onto the double helix.[28]

In our model, the yield of conformationally gated CT reflects the sum of two probabilities: the probability of being in a CT-active conformation upon excitation, and the probability of accessing a CT-active conformation, via base rearrangement, in the lifetime of Ap*. At 77 K, the latter probability is close to zero, and we can address the likelihood that the static structures adopted upon cooling are CT-active. For DNA-mediated CT, even through a single A, this likelihood appears to be very small, and it is not altered by lengthening the bridge up to four intervening A's.[29] While the stack of heterocyclic aromatic base pairs is requisite for DNA CT, our static picture of B-DNA may not represent an optimum CT-active conformation.

We have thus demonstrated that quenching of Ap* in DNA is strongly suppressed at 77 K, and we propose that this suppression is due to restricted conformational motion of the DNA bases. Through base motion, CT-active conformations, delocalized domains, rapidly form and break up in the DNA duplex, both facilitating and limiting CT. Just as all biological functions of DNA depend on its rich dynamics, so too does its ability to transport charge.

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Supporting Information Available: Emission profiles of Ap* in 10 M LiCl glasses, comparison of Ap-DNA in 10 M LiCl and 100 mM sodium phosphate at ambient temperature. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For recent reviews see *Topics in Current Chemistry: Long-Range Electron Transfer in DNA: 1*, Schuster, G. B., Ed.; Springer, **2004**, *236*.
(2) (a) Núñez, M. E.; Noyes, K. T.; Barton, J. K. *Chem. Biol.* **2002**, *9*, 403−415. (b) Núñez, M. E., Holmquist, G. P.; Barton, J. K. *Biochemistry* **2001**, *40*, 12465-12471. (c) Dandliker, P. J.; Holmlin, R. E.; Barton, J. K. *Science* **1997**, *275*, 1465−1468. (d) Boon, E. M.; Livingston, A. L.; Chmiel, N. H.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12543−12547.
(3) (a) Bixon, M.; Giese, B.; Wessely, S.; Langenbacher, T.; Michel-Beyerle, M. E.; Jortner, J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11713−11716. (b) Berlin, Y. A.; Burin, A. L.; Ratner, M. A. *Chem. Phys.* **2002**, *275*, 61−74.
(4) Bhattacharya, P.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 8649−8656.
(5) Wan, C. Z.; 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.
(6) (a) O'Neill, M. A.; Becker, H. C.; Wan, C.; Barton, J. K.; Zewail, A. H. *Angew. Chem.-Int. Edit.* **2003**, *42*, 5896−5900. (b) O'Neill, M. A.; Barton, J. K.; *J. Am. Chem. Soc.* **2004**, *126*, 11471−11483.
(7) While conformational substates are perhaps the paradigm of protein function,[8] analogous models for DNA CT are surprisingly limited.[9]
(8) Frauenfelder, H.; Sligar, S. G.; Wolynes, P. G. *Science* **1991**, *254*, 1598.
(9) Bruinsma, R.; Gruner, G.; D'Orsogna, M. R.; Rudnick, J. *Phys. Rev. Lett.* **2000**, *85*, 4393−4396.
(10) Brauns, E. B.; Murphy, C. J.; Berg, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 2449−2456.
(11) (a) Kelley, S. O.; Barton, J. K. *Science* **1999**, *283*, 375−381. (b) Wan, C. Z.; Fiebig, T.; Schiemann, O.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14052−14055. (c) O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 13053−13066. (d) O'Neill, M. A.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16543−16550.
(12) (a) Nordlund, T. M.; Andersson, S.; Nilsson, L.; Rigler, R.; Graslund, A.; McLaughlin, L. W. *Biochemistry* **1989**, *28*, 9095−9103. (b) Xu, D. G.; Evans, K. O.; Nordlund, T. M. *Biochemistry* **1994**, *33*, 9592−9599.
(13) see e.g. (a) Guest, C. R.; Hochstrasser, R. A.; Sowers, L. C.; Millar, D. P. *Biochemistry* **1991**, *30*, 3271−3279. (b) Allan, W. B.; Reich, N. O. *Biochemistry* **1999**, *38*, 5308−5314.
(14) (a) O'Neill, M. A.; Dohno, C.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 1316−1317. (b) Fiebig, T.; Wan, C. Z.; Zewail, A. H. *Chem. Phys. Chem.* **2002**, *3*, 781−788. (c) Jean, J. M.; Hall, K. B. *Biochemistry* **2002**, *41*, 13152−13161.
(15) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.
(16) Oligonucleotides were prepared on an ABI synthesizer, HPLC purified, and analyzed by mass spectrometry. Duplexes (25 μM) were annealed in 100 mM NaCl pH 7, dried and resuspended in 10 M LiCl, pH 7.
(17) There have been conflicting reports about Li^+ promoting formation of C-DNA. See van Dam, L.; Levitt, M. H. *J. Mol. Biol.* **2000**, *304*, 541−561.
(18) Electron tunneling in B-DNA has been investigated in LiCl glasses: e.g Cai, Z.; Li, X.; Sevilla, M. D. *J. Phys. Chem. B* **2002**, *106*, 2755−2762.
(19) Samples in sealed quartz cuvettes (5 mm path length) were immersed in an optical dewar (ISS) filled with liquid N_2 yielding stable, uniform glasses.
(20) The maximum anisotropy of $r = 0.4$ is observed only for collinear absorption and emission dipoles. At ambient temperatures, $r \sim 0$ and $r \sim 0.2-0.3$ for Ap free and in duplex DNA, respectively.
(21) Stokes shifts at ambient temperature and at 77 K are ~ 3930 and ~ 2990 cm^{-1}, respectively. If the DNA dynamics were purely vibrational, one would expect no change in Stokes shift upon cooling.[10]
(22) Xu, D.; Nordlund, T. M. *Biophys. J.* **2000**, *78*, 1042−1058.
(23) see e.g. Rachofsky, E. L.; Osman, R.; Ross, J. B. A. *Biochemistry* **2001**, *40*, 946−956.
(24) Larsen, O. F. A.; van Stokkum, I. H. M.; de Weerd, F. L.; Vengris, M.; Aravindakumar, C. T.; van Grondelle, R.; Geacintov, N. E.; van Amerongen, H. *Phys. Chem. Chem. Phys.* **2004**, *6*, 154−160.
(25) Dynamic disorder has been suggested to decrease electronic coupling, e.g. (a) Grozema, F. C.; Siebbeles, L. D. A.; Berlin, Y. A.; Ratner, M. A. *ChemPhysChem.* **2002**, *6*, 536−539, and to localize charge, e.g. (b) Hjort, M.; Stafström, S. *Phys. Rev. Lett.* **2001**, *87*, 228101.
(26) Conformational flexibility and gating are significant to DNA CT in other conjugated donor-bridge-acceptor systems. See Davis, W. B.; Ratner, M. A.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2001**, *123*, 7877.
(27) Although these data do not distinguish this mechanism, the yield of CT via a phonon-assisted polaron hopping is expected to decrease at 77 K. See Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(28) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159−170.
(29) The yield of CT need not always decrease as the number of bridging bases increases (see ref 6b); we examined longer bridges to determine if certain bridges may inherently favor CT-active conformations.

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