VIP DNA Charge Transport

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# Distance-Independent DNA Charge Transport across an Adenine Tract**

*Katherine E. Augustyn, Joseph C. Genereux, and Jacqueline K. Barton**

Long-range charge transport (CT) through the DNA π stack has been extensively studied using a variety of photooxidants and hole traps.[1] With potential applications in nanotechnology and sensing,[2] and biological relevance in pathways such as DNA repair and transcriptional activation,[3] this process is exquisitely sensitive to the intervening bridging bases.[4] A well-coupled π stack facilitates efficient CT with relatively weak distance dependence.[5] We have proposed a conformationally gated mechanism that is governed by base sequence and dynamics.[6] Increasingly, it has become apparent that charge delocalization with a domain length of four bases may occur during this process.[6,7]

Adenine tracts are particularly interesting as a medium for CT because of their resistance to inherent charge trapping,[1] their structural homogeneity, and the established efficiency of the CT.[6,8–14] Yields of CT from sugar radicals to triple guanine sites were found to decrease exponentially with increasing A-tract length up to three adenine base pairs, but yields through longer A tracts followed a weaker distance dependence.[8] A thermally activated localized hopping model was developed to explain this weak distance dependence.[9] A later model allowing delocalized states through A tracts generated yields that were more consistent with the experimental data,[10] and a delocalized polaron model also fit these data.[11] The kinetics of CT through A tracts was examined later by transient absorption of stilbene-capped hairpins; rates with increasingly weak distance dependences were attributed to superexchange, localized hopping, and delocalized hopping with limiting values of $\beta \approx 0.1$ Å$^{-1}$ ($\beta$ = exponential distance decay parameter).[12] Studies to examine injection yields of CT through A tracts have also been performed with phenothiazine as the hole acceptor and naphthaldiimide as the hole donor ($\beta = 0.08$ Å$^{-1}$).[13] With phenothiazine and 8-oxoguanine, a $\beta$ value of 0.2 Å$^{-1}$ is observed. Interestingly, when the A tract is disrupted by insertion of a double guanine site, CT is attenuated. We have investigated charge injection through increasing length A tracts by monitoring the quenching of photoexcited 2-aminopurine by guanine and also observe a shallow distance dependence ($\beta \approx 0.1$ Å$^{-1}$).[6]

Significantly, these studies all incorporate hole acceptors external to the A tract, inherently convoluting transport within the bridge and transport from the bridge to the trap. Herein we report the first study of DNA-mediated CT using a probe interior to the bridge so as to monitor hole occupation at all positions within the tract. Using $N^6$-cyclopropyladenine ($^{CP}$A) as the hole acceptor gives us the unique ability to monitor CT to each position on the bridge itself without modifying the sequence of the duplex.

Cyclopropylamine-substituted nucleosides provide an intrinsic DNA base to monitor CT on the picosecond time scale, at guanine ($N^2$-cyclopropylguanine, $^{CP}$G), adenine, or cytosine ($N^4$-cyclopropylcytosine, $^{CP}$C).[15–17] Model studies show a ring-opening rate of $7.2 \times 10^{11}$ s$^{-1}$.[15] This trap is fast enough to compete with back electron transfer (BET) and charge equilibration over the duplex, allowing events that are suppressed on the slower time scale of trapping at double guanine sites to be revealed. Given the sensitivity of CT to the integrity of the π stack, the cyclopropyl modification allows charge transport to be probed with minimal perturbation to the duplex.[16,18] The $^{CP}$A probe was first used to demonstrate charge occupation on, rather than tunneling through, adenines.[16] Similarly, our $^{CP}$C trap allows observation of hole occupancy on pyrimidines in direct competition with guanine oxidation.[17] These experiments underscore the utility of the kinetic traps in probing preequilibrium CT dynamics.

In the present study we constructed three sets of duplexes containing either a 14-, 6-, or 4-base-pair A tract and a covalently attached $[Rh(phi)_2(bpy')]^{3+}$ moiety serving as the photooxidant (Scheme 1; phi = phenanthrenequinone-9,10-diimine; bpy′ = 4-(4′-methyl-2,2′-bipyridyl)valerate). $^{CP}$A was serially substituted at each site of the A tract by treating the commercially available $O^6$-phenylinosine precursor with aqueous cyclopropylamine. The duplexes were subsequently irradiated at 365 nm for 30 seconds to induce hole injection into the DNA. Following enzymatic digestion with phosphodiesterase I and alkaline phosphatase, the resulting deoxynucleosides were analyzed by reverse-phase HPLC to quantify the amount of $^{CP}$A decomposition relative to a non-irradiated standard (Figure 1; see also the Experimental Section).[7,16] Irradiation time courses confirm that $^{CP}$A decomposition is not saturated at 30 seconds. Prior experiments with both $^{CP}$C and $^{CP}$G showed variation in decomposition as a function of sequence, indicating that ring opening is not rate-limiting.[7]

Remarkably, over the 14-base-pair A tract, we find essentially no change in degree of decomposition ($\beta = 0.0013(3)$ Å$^{-1}$; Figure 2). This result contrasts with the larger values found with acceptors external to the bridge.[8–13] The flatness of the slope implies that all holes reach the A-tract

[*] K. E. Augustyn, J. C. Genereux, Prof. J. K. Barton
Department of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125 (USA)
Fax: (+1) 626-577-4976
E-mail: jkbarton@caltech.edu

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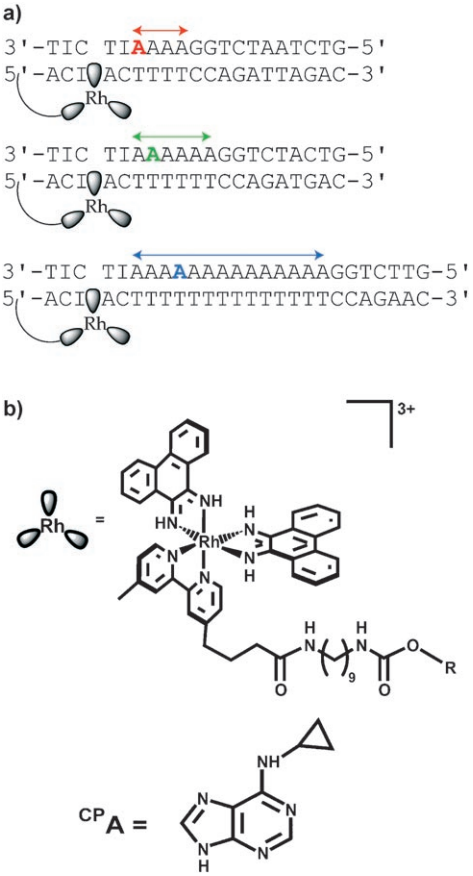


***Scheme 1.*** a) Examples of duplexes used in this study for the $^{CP}$A-4, -6, and -14 base-pair series. One position of $^{CP}$A is indicated in color and an arrow spans the A tract indicating all employed positions of substitution. b) Structures of the rhodium photooxidant $[Rh(phi)_2(bpy')]^{3+}$ and the $^{CP}$A nucleoside.

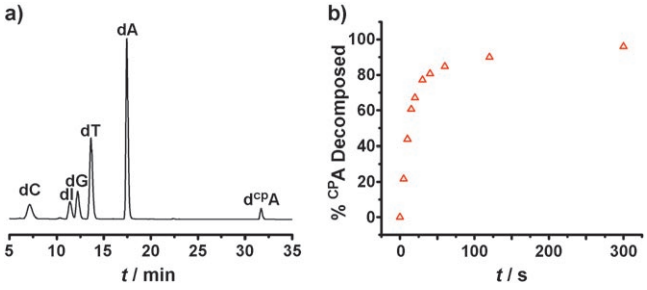


***Figure 1.*** a) Representative HPLC trace monitoring at 260 nm showing the relative retention times of the various deoxynucleosides. b) Plot of $^{CP}$A decomposition as a function of irradiation time as determined by HPLC for the 14-base-pair adenine tract in which $^{CP}$A is substituted at the first position.

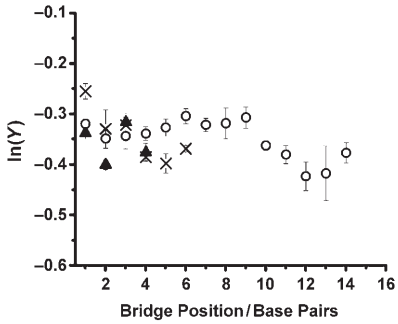


***Figure 2.*** Decomposition ($Y$) as a function of bridge position for $^{CP}$A-4 (▲), -6 (×), and -14 (○) after irradiation for 30 s at 365 nm. Decomposition was determined by integrating the $^{CP}$A peak in the HPLC trace of an irradiated sample relative to that of a nonirradiated sample. Each HPLC trace was normalized to an internal inosine standard. The bars correspond to 2 standard errors for a 95% confidence level (see the Experimental Section).

terminus after injection. Thus, the time scale for transport over the entire 48-Å A tract must be faster than BET from the first bridge position.[15,19] A range of experiments have found that BET over several base pairs can occur on the picosecond time scale.[20] Note that the inclusion of inosines near the Rh site also retards competing electron-transfer processes. These data cannot be explained by a localized-hopping mechanism through the 14 bases of the A tract.

There is consensus in the current literature that the distance dependence for hole acceptors external to the bridge is characterized by a $\beta$ value of around 0.1–0.2 Å$^{-1}$. Guanine-damage experiments[5,8] result also in a shallow distance dependence, but with a guanine trap there is charge equilibration prior to the millisecond trapping event.[21] In this case, the cyclopropylamine ring opening occurs faster than charge equilibration.

We previously found that the stacking of the donor and acceptor with the DNA bases has a dramatic effect on the distance dependence of CT through A tracts.[5] With ethenoadenine, a poorly stacked adenine analogue, as the photooxidant, a steeper $\beta$ value of 1.0 Å$^{-1}$ is found, which is consistent with poorly coupled superexchange. This is a characteristic value found for purely σ-bonded systems.[22] With the well-stacked adenine analogue 2-aminopurine as photooxidant, the distance dependence is that expected in well-stacked systems. In this context, the present results are not surprising. Interestingly, when a G residue intervenes within an A tract, CT is attenuated.[5,7,14]

Thus, a well-coupled trap incorporated into an A-tract bridge can be oxidized through DNA-mediated CT without significant attenuation over 5 nm. These results are completely consistent with a fully delocalized transport model.

## Experimental Section

Strands containing covalently tethered $[Rh(phi)_2(bpy')]^{3+}$ were synthesized as described.[7] Strands containing $^{CP}$A were synthesized by using standard phosphoramidite chemistry by placing $O^6$-phenylinosine at the target $^{CP}$A site. After incubation overnight at 60 °C in aqueous 6 M cyclopropylamine resulting in simultaneous cyclopropyl substitution, cleavage, and deprotection, the strands were purified by reverse-phase HPLC and characterized by MALDI mass spectrometry. Duplexes (30-μL aliquots, 10 μM) were irradiated for 30 s at 365 nm and were subsequently digested into deoxynucleosides using phosphodiesterase I and alkaline phosphatase overnight at 37 °C. The resulting deoxynucleosides were analyzed by HPLC. The amount of $^{CP}$A decomposition (Y) was determined by subtracting the ratio of the area under the $^{CP}$A peak in an irradiated sample over that in a nonirradiated sample from one with inosine as an internal standard

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for all HPLC traces. Irradiations were repeated three times and the results averaged. Data are reported with 2 standard errors for a 95% confidence level.[7]

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