Direct Chemical Evidence for Charge Transfer between Photoexcited 2-Aminopurine and Guanine in Duplex DNA

Melanie A. O'Neill, Chikara Dohno, and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received August 7, 2003; E-mail: jkbarton@its.caltech.edu

The adenine analogue, 2-aminopurine (Ap), is among the most extensively exploited fluorescent probes of DNA structure and dynamics.[1] As Ap forms a well-stacked base pair with T,[2] it is a nonperturbing fluorophore that is exquisitely sensitive to the DNA environment. For example, Ap has been used to monitor real-time dynamics of mismatches[3] and to probe interactions of DNA with polymerases,[4] restriction enzymes,[5] and repair proteins.[6] Despite its wide utility, the consequences of photoexcitation of Ap in DNA have not been determined.[7] It is well-known that the fluorescence of photoexcited Ap (Ap*) is markedly quenched in DNA in a manner that depends sensitively on DNA structure and sequence;[2–6,8] such quenching is often attributed to stacking of Ap within DNA. Yet, both experimental[9,10] and theoretical[11] investigations provide compelling evidence that charge transfer (CT) between Ap* and DNA bases is also responsible for quenching. Still, there is no direct evidence, either as transient intermediates or photoproducts, to confirm unequivocally a CT mechanism. Here we present evidence that Ap* initiates hole transfer through duplex DNA, leading to oxidized products at a distance.

Our attempts to establish CT between Ap* and G failed to yield any evidence of oxidative damage.[12] We rationalized that back electron transfer (BET) is significantly faster than trapping of the guanine radical cation (or radical) by water and/or oxygen. Consistent with this explanation is our recent report that photoexcited DNA-bound thionine does not permanently damage G,[13] despite the fact that it is known to undergo ultrafast ($\tau \approx 260$ fs) CT with G in DNA.[14] In that instance fast BET ($\tau \approx 760$ fs)[14] eliminates net CT chemistry. To determine whether fast BET inhibits oxidative damage by Ap*, we have taken advantage of a recently reported hole trap based on the rapid ring opening of the cyclopropylguanine (^{CP}G) radical cation (Scheme 1).[15] While the rate constant for ring opening has not been measured, it is expected[16,17] to be orders of magnitude faster than trapping of guanine radicals.[15,18] Significantly, investigations of ^{CP}G in nucleosides and in DNA confirm that its oxidation potential, base pairing, and stacking properties are similar to those of G.[15,19]

Figure 1 presents HPLC traces of ApAAA^{CP}G duplexes following 325-nm irradiation and enzymatic digestion to the nucleosides.[20] The photoinduced decomposition of ^{CP}G nucleoside is unmistakable. Likewise, light-induced formation of N^2-(3-hydroxypropanoyl)dG (^{HP}G) can also be observed with similar kinetics.[21] Decomposition of Ap was not detected by HPLC or fluorescence.[22,23] Also, no loss of ^{CP}G was observed following 325-nm irradiation of identical duplexes where Ap was replaced by A, nor following digestion of Ap/^{CP}G duplexes that were not irradiated. Single-stranded Ap/^{CP}G samples were likewise unreactive. These results provide the first direct evidence for hole transfer from Ap* to another base, specifically a guanine residue, in duplex DNA.

Decomposition of ^{CP}G indicates that the hole, transferred to ^{CP}G from Ap*, can be trapped before BET. Since the quantum yield[24] for ^{CP}G photodecomposition is on the order of 10^{-3}, it is likely
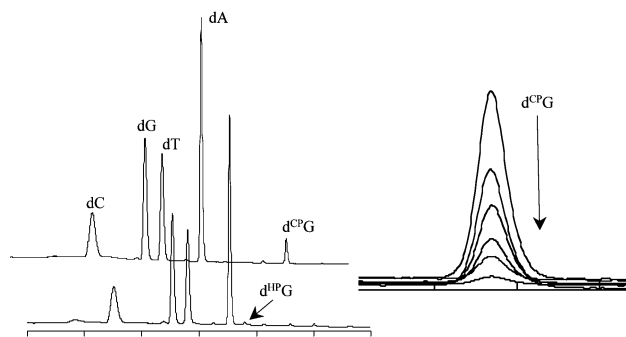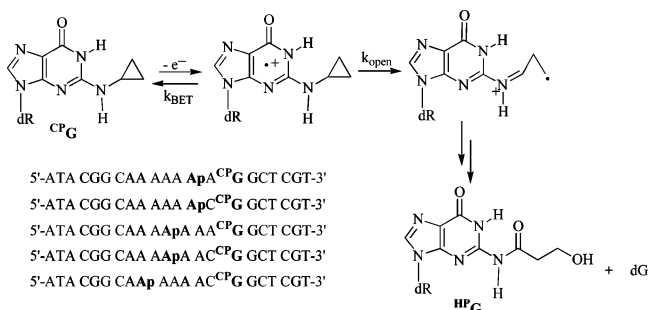

Figure 1. HPLC traces (monitored at 260 nm) before and after irradiation (30 min) of ApAAA^{CP}G duplexes. Expanded region to highlight the loss of ^{CP}G as a function of increasing irradiation time (0, 5, 10, 15, 30, and 60 min).

Scheme 1. Formation and Reaction of ^{CP}G Radical Cation and DNA Sequences Employed (Complements not Shown)

5'-ATA CGG CAA AAA **ApA^{CP}G** GCT CGT-3'
5'-ATA CGG CAA AAA **ApC^{CP}G** GCT CGT-3'
5'-ATA CGG CAA A**ApA** AA^{CP}**G** GCT CGT-3'
5'-ATA CGG CAA A**ApA** AC^{CP}**G** GCT CGT-3'
5'-ATA CGG CA**Ap** AAA AC^{CP}**G** GCT CGT-3'

that BET from ^{CP}G radical cation to Ap radical anion remains competitive with ring opening. This is consistent with the notion that DNA CT between well-coupled donors and acceptors is fast relative to trapping,[25,26] even when trapping is accelerated, presumably to the ns–ps time scale.[27]

Additional evidence for rapid BET comes from the fact that both ApC^{CP}G and ApA^{CP}G duplexes are essentially inert to photoinduced decomposition (Figure 2). Since a single intervening base pair, either A–T or C–G, drastically diminishes trapping efficiency (relative to longer donor–acceptor separations), it is difficult to argue that poor stacking or alternative mechanisms of Ap* quenching are responsible. It is more likely that the close proximity of ^{CP}G and Ap accelerates BET such that it is much faster than ring opening of the ^{CP}G radical cation.[28] Therefore, the distance dependence of the rate of BET is steeper than the distance dependence of the rate of forward CT. This "inverted" distance dependence clearly demonstrates the defining role of BET in oxidative damage via DNA-mediated CT; rapid charge separation needs not be associated with high product yield.

We would also not expect the distance dependence of charge injection, or the forward CT step, to parallel the distance dependence of the product yield. For instance, the yield of ^{CP}G oxidation is not
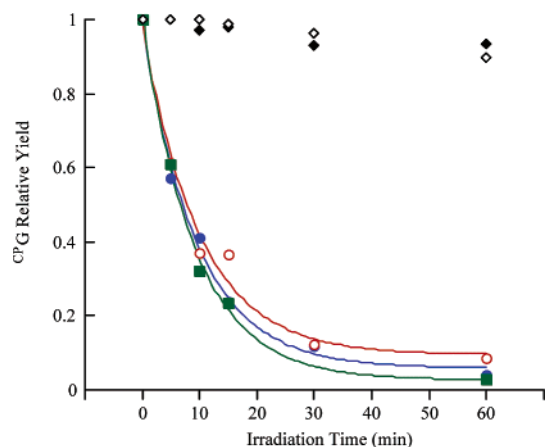
Figure 2. Relative yield of ^{CP}G in Ap-containing duplexes as a function of irradiation time: ApAAA^{CP}G (●), ApAAC^{CP}G (○), ApAAAAC^{CP}G (■), ApA^{CP}G (◆), ApC^{CP}G (◇). The relative yield was determined by HPLC analysis using 2′-deoxyadenosine as an internal standard. Lines represent fits of the data to a single-exponential decay.

significantly influenced when the distance between Ap and ^{CP}G is increased by ∼7 Å (Figure 2). In contrast, CT quenching of Ap* by G is relatively sensitive to distance.[9] In fact, we do not detect quenching at distances ≳14 Å at ambient temperature. These results are clearly a consequence of the fact that the competition between BET and trapping modulates the observed product yield. Significantly, the relative insensitivity of ^{CP}G photodecomposition to distance may suggest that this faster trapping reaction is still slow on the time scale of charge equilibration. Hence, at longer distances trapping becomes more competitive with BET.

The fundamental result presented here is that Ap* undergoes CT with modified guanine residues in duplex DNA to generate oxidative damage at a distance through DNA-mediated CT. CT between Ap* and G must therefore be included in the interpretation of quenching of Ap* in DNA. Correlation of CT rate constants, derived from donor decay, with yields of CT products is only possible with knowledge of the time scales of charge injection, BET, and trapping. These essential features, revealed by investigation of CT between DNA bases, apply to mechanistic descriptions of all DNA-mediated CT reactions.

Supporting Information Available: HPLC traces profiling photodecomposition of ^{CP}G in Ap-containing DNA duplexes, duplex melting temperatures, and emission and excitation spectra. This information is available free of charge via the Internet at http://pubs.acs.org.

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(19) Thermal denaturation and fluorescence of Ap duplexes find no evidence for perturbation by ^{CP}G nucleoside (Supporting Information).
(20) DNA containing ^{CP}G was prepared as described elsewhere,[15] twice purified by HPLC, and analyzed by ESI mass spectrometry. Duplexes (5 μM in 100 mM sodium phosphate, pH 7) were irradiated with a mercury–xenon lamp (3 mW) at 325 nm (320 nm LP filter) and digested by 37 °C incubation with phosphodiesterase, P1 endonuclease, and alkaline phosphatase for 2 h. The nucleosides were separated by reverse phase HPLC and identified with authentic standards (Supporting Information).
(21) The products of oxidative ring opening of ^{CP}G in solution and in DNA have previously been shown to be ^{HP}G and dG (Scheme 1).[15]
(22) Ap 2′-deoxyribonucleoside is observed following enzymatic digestion of DNA duplexes and HPLC monitored at 315 nm. Photoinduced decomposition of this nucleoside is not observed by HPLC. No loss of Ap emission was observed after irradiation (∼40% conversion of ^{CP}G). In fact, the emission intensity increased ∼35%. This may be attributed to loss of CT quenching due to decomposition of ^{CP}G.
(23) Similarly, photodecomposition of ^{CP}G using cyanobenzophenone-modified uridine (CNBPU) did not lead to significant loss.[15]
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(26) The time scale of forward CT over this distance is ∼200 ps[9b]
(27) In DNA the rate constant for ring opening will also be governed by the extent of hole delocalization on the ^{cp}GG doublet, see sequences Scheme 1.
(28) Importantly quenching of Ap* in ApA^{CP}G is comparable to that in ApAG, indicating that the forward CT does occur.

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