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Hole Trapping at N^6-Cyclopropyldeoxyadenosine Suggests a Direct Contribution of Adenine Bases to Hole Transport through DNA

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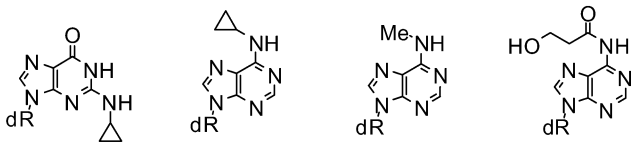
Extensive experimental investigations have revealed that radical cations (holes) migrate through the π-stack of DNA at distances of over 200 Å.[1] It is generally accepted that long-range hole transport (HT) is achieved by a multistep hopping mechanism.[2−6] A typical example is guanine (G) hopping which consists of consecutive hopping between guanine steps.[2,5] The G-hopping mechanism is based on the fact that the oxidation potential of guanine is the lowest among those of the four nucleobases in DNA.[7] According to G-hopping, HT through long A/T sequences is not feasible because only guanines act as a charge carrier. In fact, it has been reported that increasing the number of A/T base pairs between guanines dramatically reduces the HT efficiency. However, recent results indicate that a hole can efficiently migrate through contiguous A/T sequences.[8] To explain these contradictory observations, extended hopping mechanisms have been suggested. In such mechanisms, it is predicted that adenine radical cations ($A^{\bullet+}$) contribute to the HT process through long A/T sequences and exist as a real chemical intermediates.[8−11] However, no experimental evidence for the existence of $A^{\bullet+}$ has been found in DNA-mediated HT reactions thus far.



$d^{CP}G$ $d^{CP}A$ $d^{Me}A$ $d^{HP}A$

We have recently developed a kinetic hole-trapping nucleobase, N^2-cyclopropyldeoxyguanosine ($d^{CP}G$), which possesses a cyclopropyl group on N^2 as a radical-trapping device.[12] When the radical cation is formed in DNA, $d^{CP}G$ effectively traps the radical species by a rapid and irreversible cyclopropane ring opening.[13] We have extended this chemistry to the adenine base and designed a novel hole-trapping nucleobase N^6-cyclopropyldeoxyadenosine ($d^{CP}A$). $d^{CP}A$ can also trap the HT process between two GG sites separated by long A/T sequences. Hole trapping at $d^{CP}A$ strongly supports the possibility of a charge injection into long A/T bridged sequence.

We first examined the one-electron oxidation of the $d^{CP}A$ nucleoside with an anthraquinone derivative (AQ-dC, Figure 2). It was selected for this study since the reduction potential for the triplet state of AQ is adequate to oxidize adenine (2.18 V vs SCE) and AQ-conjugated DNA has been used extensively for HT experiments.[4] AQ-dC is synthesized as a small model of AQ-conjugated DNA and consists of the anthraquinone group linking to the 5′-hydroxyl group of deoxycytidine. $d^{CP}A$ was synthesized from 6-chlorodeoxypurine by substitution of chlorine with cyclopropylamine.[14] The oxidation of $d^{CP}A$ by photoirradiation at 366 nm in the presence of AQ-dC led to a rapid consumption of $d^{CP}A$ and the formation of two major products eluting at shorter retention times after subsequent incubation (Figure 1a). The two major

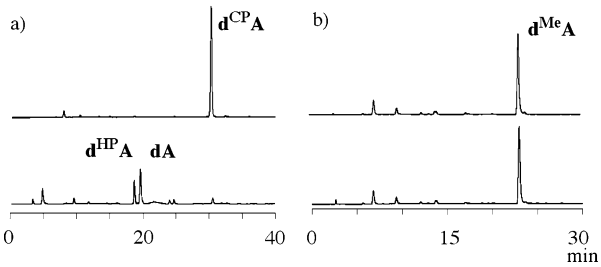


Figure 1. HPLC profiles of AQ-dC-sensitized photooxidation of $d^{CP}A$ (a) and $d^{Me}A$ (b). Dark control (top). After irradiation at 366 nm for 10 min followed by incubation at 37 °C for 2 h (bottom).

products were identified as dA and N^6-(3-hydroxypropanoyl)dA ($d^{HP}A$) by ^{1}H NMR and high resolution FAB-MS and were found to be structurally similar to the oxidation products of $d^{CP}G$.[12]

In marked contrast to the one-electron oxidation of $d^{CP}A$, oxidation of $d^{Me}A$ resulted in a complete recovery under the same reaction conditions (Figure 1b). The oxidation potential of $d^{CP}A$ and $d^{Me}A$ measured in water containing 0.1 M $LiClO_4$ are 1.25 and 1.26 V (vs SCE), respectively. Taking into account the oxidation potentials, it is plausible that the radical cation of $d^{Me}A$ is formed but reverts rapidly to its ground state via back-electron transfer prior to the trapping reaction. Quite different behavior in the one-electron oxidation of $d^{Me}A$ indicates that the unique reactivity of $d^{CP}A$ is attributed to the essential feature of the *N*-cyclopropyl group which undergoes a rapid ring opening upon generation of the radical cation.[12,13]

Having established that the $d^{CP}A$ radical cation undergoes a rapid cyclopropane ring opening, we then examined the capability of hole trapping by $d^{CP}A$ in HT processes through long A/T sequences. Oligodeoxynucleotides (ODN) containing $d^{CP}A$ were prepared according to standard phosphoramidite chemistry. We used 22-mer probe ODNs possessing a sequence of 5′-GGTTXTTGG-3′ (Table 1). The intervening bridging sequence between the two GG sites, TTXTT, contained A (**A22**), ^{Me}A (**^{Me}A22**), or ^{CP}A (**^{CP}A22**) as the base X. The complementary strand contains a covalently attached anthraquinone chromophore (AQ, **AQ22**)[4] at the 5′-end, which can inject a radical cation into the duplex DNA after photoirradiation. Hole migration through the duplex DNA is revealed as the cleavage bands of 5′-^{32}P-labeled probe ODNs at hole trapping GG sites following hot piperidine treatment. To end up to the GG_d site, the hole must go through the long A/T bridge, TTXTT. If the hole can reside in the A/T base pairs, ^{CP}A in the TTXTT sequence is expected to capture the hole by a rapid cyclopropane ring opening.

ODN duplex **A22**/**AQ22** was photoirradiated at 366 nm in sodium phosphate buffer (10 mM, pH 7.0) at room temperature. Most of the oxidative cleavage occurred at GGp and the cleavage was also detectable at GG_d with much weaker band intensity (Figure 2, lane 2). With the duplex **^{Me}A22**/**AQ22**, the major strand cleavage was again observed at GG_p, but the remote oxidative cleavage was

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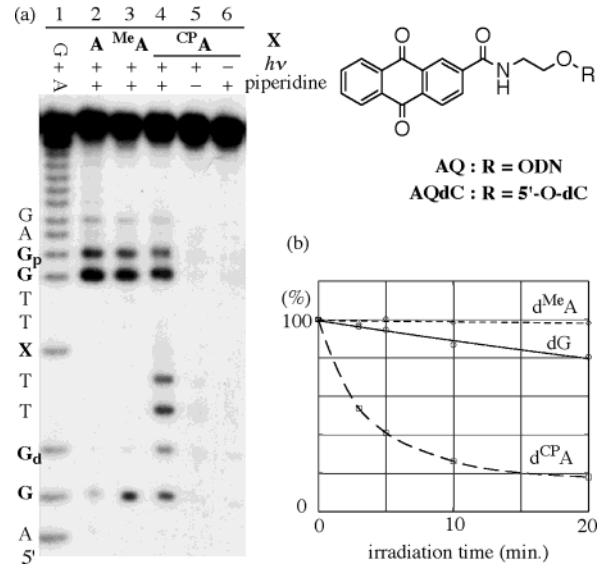

Figure 2. (a) Autoradiogram of a denaturing sequencing gel for photoreactions of duplexes of ^{32}P-5′-end-labeled **A22**, ^{Me}**A22**, and ^{CP}**A22** hybridized to **AQ22**. Duplex ODNs were irradiated at 366 nm in Na·phosphate buffer (pH 7.0). After piperidine treatment (90 °C, 20 min), the samples were electrophoresed using a denaturing polyacrylamide (15%)/urea gel (7 M). Lane 1, Maxam−Gilbert G+A sequencing reactions; lane 2, **A22**; lane 3, ^{Me}**A22**; lanes 4−6, ^{CP}**A22**. (b) Time course of the amount of dG, ^{Me}A, and ^{CP}A obtained from HPLC analysis.

Table 1. Sequences of Oligomers Used for HT Experiments

ODN :	G G_d X GG_p
A22 :	5'- ATA TAG GTT **A**TT GGA GAA AAA A -3'
^{Me}**A22** :	5'- ATA TAG GTT ^{Me}**A**TT GGA GAA AAA A -3'
^{CP}**A22** :	5'- ATA TAG GTT ^{CP}**A**TT GGA GAA AAA A -3'
AQ22 :	3'- TAT ATC CAA TAA CCT CTT TTT T -**AQ**

also apparent at GG_d (lane 3, $GG_d/GG_p = 0.23$). The enhanced HT between GG_p to GG_d is rationalized by the lower oxidation potential of the bridging base $d^{Me}A$ compared to that of dA. The pattern of strand cleavage was drastically changed when ^{CP}A was incorporated as the bridging base. Photolysis of ^{CP}**A22**/**AQ22** revealed the oxidative damage not only at GG_p but also at 5′-side of ^{CP}A (lane 4).[15] HPLC analysis of photoirradiated ^{CP}**A22**/**AQ22** provides further evidence for the consumption of ^{CP}A. The quantities of nucleosides obtained from the enzymatic digestion were monitored by HPLC.[16] It can be easily seen from Figure 2b that the amount of $d^{CP}A$ rapidly diminishes with increasing irradiation time. In contrast, $d^{Me}A$ is stable under the reaction conditions used for the PAGE analysis. These results suggest that the ^{CP}A radical cation exists as a real intermediate in the HT process and the hole injected into TT^{CP}ATT bridge is effectively trapped by ^{CP}A. We also performed separate experiments using cyanobenzophenone-substituted uridine ($d^{CNBP}U$)[5] in place of AQ. In this case, the additional cleavage bands at the 5′-side of ^{CP}A (Figure S2) were scarcely observed under the same conditions. The consumption rate of $d^{CP}A$ is much slower than in the AQ system. This slow consumption is reasonable since our recent investigations with $d^{CNBP}U$ provided a strongly sequence-dependent HT[5] and the contribution of $A^{\bullet+}$ is considered to be negligible. The more $A^{\bullet+}$ contributes to the HT process, the lower the sequence and distance dependency. The hole trapping at ^{CP}A depends highly on the HT system used, probably because the charge injection efficiency into the adenine varies in each system.

We have demonstrated that the migrating hole is trapped at ^{CP}A incorporated into long A/T bridge between two GG sites. Cyclopropyl-substituted nucleosides are promising tools to prove the existence of the transient radical species in DNA. The present results are in accord with the previous consideration that nucleobases possessing higher ionization potential than dG, such as dA, are also able to participate directly in the multistep hopping mechanism.[9−11] Moreover, the efficiency of charge injection into such bases significantly depends on the employed HT system.

Supporting Information Available: HPLC analysis for the consumption of ^{CP}A in Figure 2; possible mechanism of one electron oxidation of $d^{CP}A$; HPLC and PAGE analysis of $d^{CNBP}U$-containing DNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Detailed analysis by HPLC and mass spectrometry suggests the conversion of thymine to 5-formyluracil after photoirradiation. This explains the occurrence of strand cleavage at the 5′-side of ^{CP}A, since 5-formyluracil is alkaline labile. The formation of 5-formyluracil is rationalized by a hydrogen abstraction of the ring-opened ^{CP}A radical species from the methyl group of thymine. Tandem reactions may contribute to the cleavage bands in Figure 2.
(16) dA is a major oxidation product of $d^{CP}A$ in the duplex DNA, whereas a trace amount of $d^{HP}A$ is detected.

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