Hole Trapping at N6-Cyclopropyldeoxyadenosine Suggests a Direct Contribution of Adenine Bases to Hole Transport through DNA

Chikara Dohno,* Atsushi Ogawa, Kazuhiko Nakatani, and Isao Saito*

Contribution from the Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, SORST, Japan Science and Technology Corporation (JST), Kyoto 606-8501, Japan

Received April 30, 2003; E-mail: cdohno@caltech.edu; saito@sbchem.kyoto-u.ac.jp

Extensive experimental investigations have revealed that radical cations (holes) migrate through the π-stack of DNA at distances of over 200 Å. It is generally accepted that long-range hole transport (HT) is achieved by a multistep hopping mechanism. A typical example is guanine (G) hopping which consists of consecutive hopping between guanine steps. 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. 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. To explain these contradictory observations, extended hopping mechanisms have been suggested. In such mechanisms, it is predicted that adenine radical cations (A+) contribute to the HT process through long A/T sequences and exist as a real chemical intermediates. However, no experimental evidence for the existence of A+ has been found in DNA-mediated HT reactions thus far.

We have recently developed a kinetic hole-trapping nucleobase, N6-cyclopropyldeoxyguanosine (dCPG), which possesses a cyclopropyl group on N6 as a radical-trapping device. When the radical cation is formed in DNA, dCPG effectively traps the radical species by a rapid and irreversible cyclopropane ring opening. We have extended this chemistry to the adenine base and designed a novel hole-trapping nucleobase N6-cyclopropyldeoxyadenosine (dCPA). dCPA can also trap the HT process between two GG sites separated by long A/T sequences. Hole trapping at dCPA strongly supports the possibility of a charge injection into long A/T bridged sequence.

We first examined the one-electron oxidation of the dCPA 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. 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 deoxyctydine. dCPA was synthesized from 6-chlorodeoxyxpurine by substitution of chloride with cyclopropylamine. The oxidation of dCPA by phototirradiation at 366 nm in the presence of AQ-dC led to a rapid consumption of dCPA and formation of two major products eluting at shorter retention times after subsequent incubation (Figure 1a). The two major products were identified as dA and N6-(3-hydroxypropanoyl)da (dHPA) by 1H NMR and high resolution FAB-MS and were found to be structurally similar to the oxidation products of dCPG. In marked contrast to the one-electron oxidation of dCPA, oxidation of dMeA resulted in a complete recovery under the same reaction conditions (Figure 1b). The oxidation potential of dCPA and dMeA measured in water containing 0.1 M LiClO4 is 1.25 and 1.26 V (vs SCE), respectively. Taking into account the oxidation potentials, it is plausible that the radical cation of dCPA 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 dCPA indicates that the unique reactivity of dCPA is attributed to the essential feature of the N-cyclopropyl group which undergoes a rapid ring opening upon generation of the radical cation.

Having established that the dCPA radical cation undergoes a rapid cyclopropane ring opening, we then examined the capability of hole trapping by dCPA in HT processes through long A/T sequences. Oligodeoxynucleotides (ODN) containing dCPA were prepared according to standard phosphoramidite chemistry. We used 22-mer probe ODNs possessing a sequence of 5′-GGTTTTTGG-3′ (Table 1). The intervening bridging sequence between the two GG sites, TTXTT, contained A (A22), MeA (MeA22), or CTPA (CPA22) as the base X. The complementary strand contains a covalently attached anthraquinone chromophore (AQ, AQ22) 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′-32P-labeled probe ODNs at hole trapping GG sites following hot piperidine treatment. To end up to the GG site, the hole must go through the long A/T bridge, TTXTT. If the hole can reside in the A/T base pairs, CPA 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 GGd with much weaker band intensity (Figure 2, lane 2). With the duplex MeA22/AQ22, the major strand cleavage was again observed at GGp, but the remote oxidative cleavage was
Figure 2. (a) Autoradiogram of a denaturing sequencing gel for photoreactions of duplexes of dT.P-S′-end-labeled A₂₂, MeA₂₂, and CP₄₂₂ hybridized to AQ₄. Duplex ODNs were irradiated at 366 nm in Naphosphate 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, MeA₂₂; lanes 4–6, CP₄₂₂. (b) Time course of the amount of dG₄, MeA, and CP obtained from HPLC analysis.

Table 1. Sequences of Oligomers Used for HT Experiments

<table>
<thead>
<tr>
<th>ODN</th>
<th>G₄</th>
<th>G₄p</th>
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<tbody>
<tr>
<td>A₂₂</td>
<td>5′-ATA TAG GGG GGA GAA AAA A-3′</td>
<td></td>
</tr>
<tr>
<td>MeA₂₂</td>
<td>5′-ATA TAG GGG GGA GAA AAA A-3′</td>
<td></td>
</tr>
<tr>
<td>CP₂₂₂</td>
<td>5′-ATA TAG GGG GGA GAA AAA A-3′</td>
<td></td>
</tr>
<tr>
<td>AQ₂₂</td>
<td>3′-TAT ATC CAA TAA CCT CTG TTT T-AQ</td>
<td></td>
</tr>
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also apparent at GG₃ (lane 3, GG₄/GG₅ = 0.23). The enhanced HT between GG₄ to GG₅ is rationalized by the lower oxidation potential of the bridging base d₈₆A compared to that of d₈A. The pattern of strand cleavage was drastically changed when CP₄ was 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 d₈G, such as d₈A, are also able to participate directly in the multistep hopping mechanism. 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 in Figure 2; possible mechanism of one electron oxidation of d₈A; HPLC and PAGE analysis of d₈CNBP-containing DNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

15. Detailed analysis by HPLC and mass spectrometry suggests the conversion of thymine to 5-formyluracil after photoradiation. This explains the occurrence of strand cleavage at the 5′-side of d₈A, since 5-formyluracil is alkane labile. The formation of 5-formyluracil is rationalized by a tautomer of d₈G₆. The pattern of strand cleavage bands at the 5′-side of d₈A (Figure S2) were scarcely observed under the same conditions. The consumption rate of d₈A is much slower than in the AQ system. This slow consumption is reasonable since our recent investigations with d₈CNBP₄ provided a strongly sequence-dependent HT and the contribution of A₄⁺ is considered to be negligible. The more A₄⁺ contributes to the HT process, the lower the sequence and distance dependency. The hole trapping at CP₄ 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 CP₄ 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 d₈G, such as d₈A, are also able to participate directly in the multistep hopping mechanism. Moreover, the efficiency of charge injection into such bases significantly depends on the employed HT system.