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Long-Distance Radical Cation Migration in Duplex DNA: The Effect of Contiguous A·A and T·T Mismatches on Efficiency and Mechanism

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Canonical DNA is composed of the thermodynamically most stable Watson−Crick A·T and G·C base pairs.[1] However, mismatches (A·A or T·T, for example) can be incorporated within duplex DNA, usually with little structural disruption.[2,3] Excess electrons (radical anions) and radical cations (holes) have been observed to migrate tens or hundreds of angstroms in duplex DNA by an adiabatic, thermally activated hopping mechanism.[4−9] Mismatches within DNA change base stacking and hydrogen bonding[10] and may affect long-distance charge transport. There have been a few studies on the effect of mismatches or deletions on charge transport.[11−15] A single C·A mismatch[16,17] seems to reduce radical cation migration efficiency, but an A·A mismatch has no effect.[18] An extensive study using a ruthenium intercalator as sensitizer indicated that radical cation transport is extremely sensitive to single base pair mismatches, which were attributed to local destacking.[19] However, subsequent examination has revealed that ruthenium intercalators promote aggregation of DNA, which will complicate interpretations.[20]

We report here the effect of serial mispairs $(A{\cdot}A)_n$ and $(T{\cdot}T)_n$ (where $n = 2$, 4, or 6) on radical cation transport in duplex DNA. Surprisingly, charge transport through the $(A{\cdot}A)_n$ sequences occurs with comparable or greater efficiency than the corresponding Watson−Crick duplexes, but transport through the $(T{\cdot}T)_n$ sequences depends strongly on their length, which may indicate a change in mechanism.

The DNA constructs examined in this work are shown in Figure 1. One strand contains an anthraquinone group (AQ) linked to its 5′-terminus,[11] and its complementary strand (labeled with ^{32}P at the 5′-terminus) has two GG steps. One GG step is placed before a variable segment ($X_n{\cdot}Y_n$), and the other (GG_2) is after this segment. The variable segments are composed of A·T or T·A pairs or A·A or T·T mismatches.

These DNA duplexes were characterized by their melting behavior (Table 1 and Supporting Information) and by circular dichroism (CD) spectroscopy. In all cases, the melting showed a single reversible transition. For the fully complementary duplexes, T_m is the same for the A·T and T·A series and is approximately independent of n. However, as expected, the T_m decreases with increasing n when the variable segment contains T·T or A·A mismatches. These values are consistent with the expected melting behavior based upon an empirical correlation with structure.[21] In no case is T_m below 45 °C, which indicates that these compounds exist as duplexes under the conditions of the radical cation transport experiments.

The CD spectrum of B-form DNA typically exhibits an exciton band with a positive peak at ca. 275 nm and an equally intense negative peak at ca. 245 nm.[22] The CD spectra (see Supporting Information) show that all of the DNA oligomers investigated here have global structures that are predominantly B-form. This is somewhat unexpected since some of these compounds have as many as six contiguous A·A or T·T mismatches. Nevertheless, both the melting behavior and the CD spectra of these oligomers indicate that the mismatched bases are ordered and that they exist in predominantly intrahelical structures.

AQ-5′ A A A T G C C G G T A C X_n C A T G G C C G T A C G -3′
T T T A C G G_1 C C A T G Y_n G T A C C G G_2 C A T G C* -5′

DNA(1) $X_n = A_2$; $Y_n = T_2$	DNA(5) $X_n = A_4$; $Y_n = T_4$	DNA(9) $X_n = A_6$; $Y_n = T_6$
DNA(2) $X_n = T_2$; $Y_n = A_2$	DNA(6) $X_n = T_4$; $Y_n = A_4$	DNA(10) $X_n = T_6$; $Y_n = A_6$
DNA(3) $X_n = A_2$; $Y_n = A_2$	DNA(7) $X_n = A_4$; $Y_n = A_4$	DNA(11) $X_n = A_6$; $Y_n = A_6$
DNA(4) $X_n = T_2$; $Y_n = T_2$	DNA(8) $X_n = T_4$; $Y_n = T_4$	DNA(12) $X_n = T_6$; $Y_n = T_6$

Figure 1. Structures of AQ-DNA oligomers; * = ^{32}P radiolabel.

Table 1. Melting Temperatures and Proximal to Distal Strand Cleavage Ratios for the AQ-DNA Oligomers

X·Y[a]	DNA ($n = 2$)	T_m[b]	GG_1/GG_2[c]	DNA ($n = 4$)	T_m	GG_1/GG_2	DNA ($n = 6$)	T_m	GG_1/GG_2
A·T	1	62	5	5	60	9	9	62	23
T·A	2	62	9	6	60	14	10	61	16
A·A	3	56	10	7	50	7	11	49	8
T·T	4	56	8	8	48	25	12	47	>40

[a] Base pair (or mismatch) in the variable segment. [b] Melting temperatures (°C) were determined for samples containing 2.5 μM DNA duplex in 10 mM phosphate buffer solution at pH 7 monitored at 260 nm. [c] Ratio of the amount of strand cleavage measured for the proximal (GG_1) step to the distal (GG_2) step.

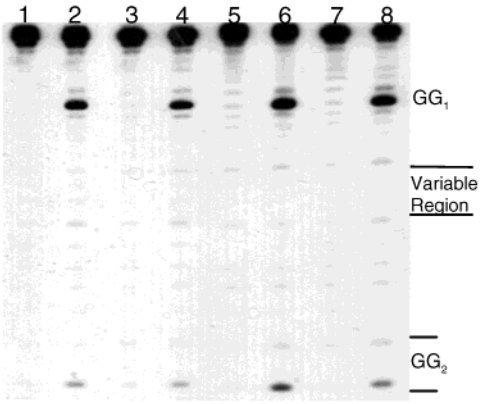

Figure 2. Autoradiogram showing strand cleavage of DNA samples following irradiation and piperidine treatment. Lanes 1(2), 3(4), 5(6), 7(8) correspond to 0(3) min irradiations of DNA(2), (3), (1), and (4), respectively.

We examined the effect of the A·A and T·T mismatches on radical cation transport by irradiation of the AQ-DNA oligomers in buffer solution (ca. 30 °C, pH 7) at 350 nm where only the AQ absorbs. The irradiated samples were treated with piperidine to reveal base damage and then were analyzed by polyacrylamide gel electrophoresis. A typical gel is shown in Figure 2 (see Supporting Information for the others); the data are summarized in Table 1. Clearly, as expected, irradiation leads to one-electron oxidation of the DNA to form a radical cation and consequential strand cleavage at the proximal (GG_1) and distal (GG_2) steps.[5] As has been

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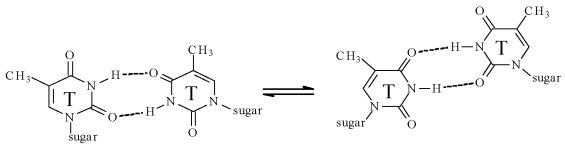

Figure 3. T·T wobble base pair mismatch structures.

previously observed in base-paired DNA, when A_n segments separate the GG steps (as in DNA 2, 6, 10), the efficiency of charge transport becomes independent of n when $n > 3$, and when T_n segments separate the GG steps (as in DNA 1, 5, 9), transport efficiency from GG to GG is less efficient.[9,23] Surprisingly, radical cation transport through the contiguous A·A mismatch segments (DNA 3, 7, 11) is at least as efficient as for the A_n segments and is similarly independent of length. In contrast, when there are more than two T·T mismatches between the GG steps, the efficiency of radical cation transport decreases dramatically.

The effect of the contiguous A·A and T·T mismatch segments on radical cation transport is likely to be related to their structures. A T·T mismatch can form well-stacked asymmetric (2-carbonyl-*N*3, *N*3-4-carbonyl) wobble base pairs (Figure 3) with modest distortion of the helix.[21,24] The oxidation potential of purines is considerably lower than for pyrimidines;[25] of course, the T·T mismatches contain no purines. Two mechanisms for long-distance charge transport in DNA are currently under consideration. The first is phonon-assisted polaron hopping where the radical cation moves adiabatically onto "bridges" of A·T base pairs between GG steps. The second postulates quantum mechanical tunneling from G to G through short bridges (1, 2, or 3 base pairs) and a change to an adiabatic mechanism for longer bridges.[26,27] However, an analysis based on variable transition-state theory shows that tunneling is not necessary to account for charge transfer even for short adenine-containing bridges.[9] Significantly, we find that radical cation transport through contiguous $(T·T)_n$ mismatches depends very strongly on distance, which may indicate that the tunneling mechanism is operating in this special circumstance.

It is clear that radical cation transport is not inhibited by contiguous $(A·A)_n$ mismatches. In fact, the barrier when $n = 2$ is no greater than for fully matched DNA, and no additional barrier is created by increasing n from two to six. A·A mismatches can form wobble base pairs, but that requires considerable helical distortion.[22] Shepard and co-workers[28] determined the structure of an $(A·A)_2$ mismatched segment by X-ray crystallography of duplex d(GCGAAAGCT). They found that the A·A mismatches form an interdigitated "zipper-like" motif having a well-aligned stack of adenines that are separated by ca. 3.3 Å. We suggest that the A·A mismatched segments examined here form the zipper-like structure shown schematically in Figure 4, and this motif facilitates radical cation transport by phonon-assisted polaron hopping. A central postulate of this mechanism is that oxidation of a base to its radical cation results in a stabilizing local distortion of the DNA structure. Adenines that form the zipper are not restricted in their motions by association with a complementary base, and this may enhance charge transport by easing polaron formation and motion.[5]

In conclusion, we observed radical cation transport in DNA containing contiguous T·T and A·A mismatches. In fully complementary DNA, radical cations migrate adiabatically. This mechanism is sufficient to account for charge transport through the A·A

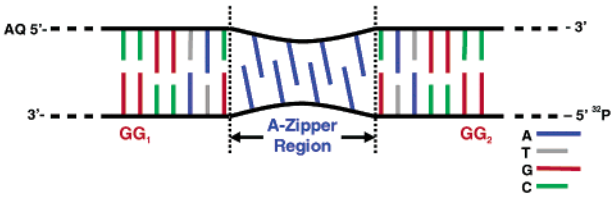

Figure 4. Schematic representation of the zipper-like region within A·A mismatched DNA duplexes.

mismatches. However, T·T mismatches show a strong distance dependence, and nonadiabatic tunneling may account for the observed charge transport in this case. These properties of mismatched bases may complicate development of DNA hybridization sensors that depend on charge transport and are expected to be sensitive to single base mutations.[29]

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Supporting Information Available: Autoradiographs, melting behavior, and CD spectra of the DNA oligomers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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