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Self-Repair of Thymine Dimer in Duplex DNA

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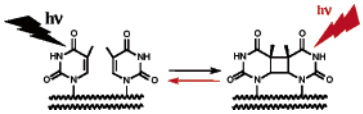
Interest in the photochemistry of DNA is sustained by its central role in mutation and cancer caused by solar irradiation, particularly its UVB component (280−320 nm). Dimerization of thymines was one of the first UV-induced processes to be identified[1] and the resulting cyclobutane dimer (T⟨⟩T) is the major product of direct UV absorption.[2,3] Incidence of T⟨⟩T has been shown to vary with deoxynucleotide (dN) sequence,[4−6] DNA conformation,[7,8] and protein-dependent bending and looping of DNA.[9] While the molecular origins of these observation are not yet known, time-resolved spectroscopy and time-dependent density-functional theory have begun to identify the effect of DNA sequence and structure on its photochemical and photophysical properties.[10] Neighboring bases have been shown to influence the energy and lifetime of singlet and triplet excited states through excimer formation and perhaps delocalization, but their effect on thymine dimerization is not clear.[11,12] Moreover, under common conditions (steady-state irradiation at 254 nm), thymine dimerization is not even favored over its reversion.[2] The observed levels of T⟨⟩T consequently result from competition between the forward and reverse reactions (Scheme 1). Each of which may respond independently to DNA structure. Investigations described below emphasize the importance of the bases adjacent to dipyrimidine sites in controlling the levels of T⟨⟩T. Most importantly, this level is suppressed to the greatest extent by neighboring Gs which may serve as transient electron donors to promote repair of T⟨⟩T as proposed earlier for a deoxyribozyme containing a G-quadruplex.[13]

A series of oligodeoxynucleotides containing a single TT central to the sequences was constructed to measure the influence of the surrounding dN on T⟨⟩T levels. Accumulation of T⟨⟩T from exposure to 254 nm light was monitored by strand scission induced by T4 endonuclease V, an enzyme that is specific for T⟨⟩T in duplex DNA and not influenced by local dN sequence (Figure 1A).[14,15] Rate constants and photostationary (steady-state) levels of T⟨⟩T were calculated as described in Figure 1B.[14,16]

DNA sequences were initially designed to identify why the photostationary level of T⟨⟩T varied by almost 3-fold in two duplexes **DS3** and **DS7** (Chart 1). Efficiency of T⟨⟩T formation could not explain this observation since their dimerization rate constants (k_f) were experimentally indistinguishable. The difference was also not inherent in the individual sequences containing -TT- since the corresponding single strands **SS3** and **SS7** supported similar photostationary levels of T⟨⟩T (7.8 ± 0.3% and 5.8 ± 0.2%, respectively).[14] Consequently, the rate constant of T⟨⟩T repair (k_r) appears to be key and accounts for the 3-fold change on the basis of its value of 15 ± 2 min^{-1} for **DS3** and 4 ± 1 min^{-1} for **DS7**. Individual contributions of dN sequence and composition were dissected by switching the polarity of the sequences (5′ to 3′) without altering the net composition. This change had a profound effect on the photostationary level of T⟨⟩T but not the rate constant

Scheme 1



for T⟨⟩T formation. Reversing the sequence of **DS3** enhanced the T⟨⟩T level by more than 3.5-fold (**DS8**). In a complementary manner, reversing the sequence of **DS7** decreased the T⟨⟩T level by more than 2-fold (**DS5**). Deoxynucleotide sequence is consequently critical to the accumulation of T⟨⟩T in this model system as it was in previous systems based on heterogeneous polydeoxynucleotides.[4,5]

The influence of the dN sequence on the 5′-side of -TT- was found to dominate control of T⟨⟩T levels by comparing a series of chimeric sequences. A relatively low level of T⟨⟩T was maintained when the 5′-sequence of either **DS3** or **DS5** was fused to the 3′-sequence of **DS7** to create **DS2** and **DS1** (Chart 1). Conversely, a relatively high level of T⟨⟩T was maintained when the 5′-sequence of **DS7** was combined with the 3′-sequence of **DS5** to make **DS10**. Regions distal to the -TT- sequence were not responsible for this observation since terminal sequences could be switched as in **DS4** and **DS9** with little influence on T⟨⟩T. Statistical analysis of polydeoxynucleotide reaction previously correlated T⟨⟩T levels with adjacent dNs, but no difference had been noted between 5′-GTTA-

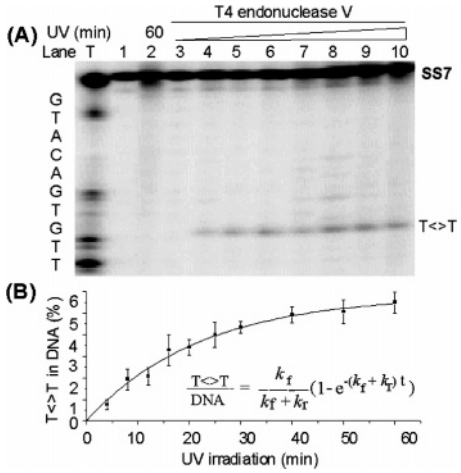


Figure 1. Electrophoretic separation and analysis of T⟨⟩T-containing DNA. (A) A single-stranded DNA (5′-[^{32}P]-**SS7**, 1.6 μM, 50 nCi) with the TT-containing sequence of **DS7** was irradiated at 254 nm (0−60 min) under ambient conditions. The complementary strand was then added, and the resulting duplex was digested by the endonuclease to detect formation of T⟨⟩T (lanes 3−10). A T-sequencing ladder generated by $KMnO_4$ oxidation (lane T) and the untreated parent strand (lane 1) were included. **SS7** was also analyzed directly without digestion after irradiation (lane 2). (B) The fraction of T⟨⟩T-containing DNA was fit to a reversible approach to photostationary levels of T⟨⟩T as described previously[16] in which k_f and k_r are the rate constants for T⟨⟩T formation and repair, respectively, and t is irradiation time.[14] Error bars represent the range of values from two to four independent measurements at each time of irradiation.

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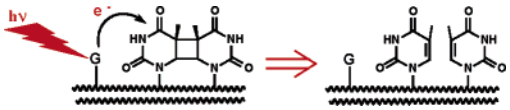
Chart 1. T⟨⟩T Formation and Accumulation[a]

	DNA Sequence	T<>T Formation (k_f, min^{-1})	T<>T Photostationary levels (%)
DS1	GTACAGTGTTGTGACATG CATGTCACAACACTGTAC	0.14 ± 0.01	1.9 ± 0.04
DS2	ATGCAGTGTTGTGACATG TACGTCACAACACTGTAC	0.19 ± 0.03	2.4 ± 0.1
DS3	ATGCAGTGTTACAGTGCA TACGTCACAATGTCACGT	0.36 ± 0.05	2.5 ± 0.06
DS4	GCAGTGTGTTACAGCATG CGTCACACAATGTCGTAC	0.25 ± 0.04	3.3 ± 0.1
DS5	GTACAGTGTTACATGACG CATGTCACAATGTACTGC	0.25 ± 0.03	3.5 ± 0.1
DS6	GCAGTACGTTATGACATG CGTCATGCAATACTGTAC	0.33 ± 0.04	4.0 ± 0.1
DS7	GCAGTACATTGTGACATG CGTCATGTAACACTGTAC	0.32 ± 0.07	7.2 ± 0.5
DS8	ACGTGACATTGTGACGTA TGCACTGTAACACTGCAT	0.31 ± 0.1	9.7 ± 1
DS9	GTACTACATTGTGTGACG CATGATGTAACACACTGC	0.41 ± 0.05	10 ± 0.4
DS10	GCAGTACATTACATGACG CGTCATGTAATGTACTGC	0.73 ± 0.1	13 ± 0.2

[a] Each duplex was characterized as illustrated in Figure 1.[14]

Scheme 2



3′ and 5′-ATTG-3′, perhaps due to the heterogeneity of these systems.[5] In contrast, lower levels of T⟨⟩T in the current study correlate with 5′-GTTA-3′ (**DS3**−**DS6**) and higher levels correlate with 5′-ATTG-3′ (**DS7**−**DS9**). This trend was confirmed by the nearly 50% decrease in the level of T⟨⟩T after switching the flanking A/G of **DS7** to form **DS6**.

The ability of a 5′-G directly preceding -TT- to influence levels of T⟨⟩T may in part reflect perturbations to excitation transfer or excited-state delocalization,[3,12,17] although how these might differentially influence dimerization or repair to alter photostationary levels of T⟨⟩T is far from apparent. The most satisfying explanation derives from a preferential ability of G to repair T⟨⟩T through charge-transfer (Scheme 2). Participation of a G-quadruplex in such repair of a proximal T⟨⟩T has already been observed in a deoxyribozyme,[13] and similar charge-transfer has been invoked to explain the structural dependence of DNA photooxidation at sites containing bromodeoxyuridine (BrdU).[18] Equivalent reactions have also been induced by charge-transfer from electron donors held at distal sites.[19,20]

The apparent ability of a neighboring dN to promote T⟨⟩T repair may also contribute to the conformational dependence of T⟨⟩T distribution. Efficient charge-transfer requires base stacking, and, accordingly, its influence should increase from a single-stranded to duplex structure. The levels of T⟨⟩T decrease as expected for enhanced repair from **SS3** to **DS3** as described above and also from **SS5** (5.4 ± 0.3%) and **SS6** (7.7 ± 0.2%) to their corresponding duplex structures.[14] However, such a decrease in T⟨⟩T is not common after duplex formation in heterogeneous sequences of DNA.[8] Indeed, the level of T⟨⟩T increases from **SS7** to **DS7** in the absence of a G on the 5′-side of TT (see above).

The extent to which a nucleobase within duplex DNA may promote repair of a neighboring T⟨⟩T might logically be connected to its oxidation potential. The lower levels of T⟨⟩T when surrounded by G versus A above (**DS1** and **DS2** vs **DS10**) are consistent with this assumption as are previous observations that levels of T⟨⟩T were statistically lower when surrounded by purines versus pyrimidines.[4] When 7-deazaG (E_{ox} = 1.0 V)[21] was substituted for G (E_{ox} = 1.3 V)[21] on either the 5′- or 3′-side of -TT- in **DS1**, the already low level of T⟨⟩T was suppressed even further. T⟨⟩T accumulated to less than <0.4% after 60 min in these substituted duplexes.[14] While self-repair may not be the only variable affecting T⟨⟩T levels, it certainly helps to explain many observations of native DNA. In the future, its role in protein-dependent perturbation of T⟨⟩T levels may become evident on the basis of the ability of certain proteins to alter the charge-transfer properties of duplex DNA.[22] Distinct contributions from the 5′- and 3′-side of TT also merit further investigation.

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Supporting Information Available: General material and methods, T⟨⟩T formation versus UV irradiation for all single- and double-stranded oligodeoxynucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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