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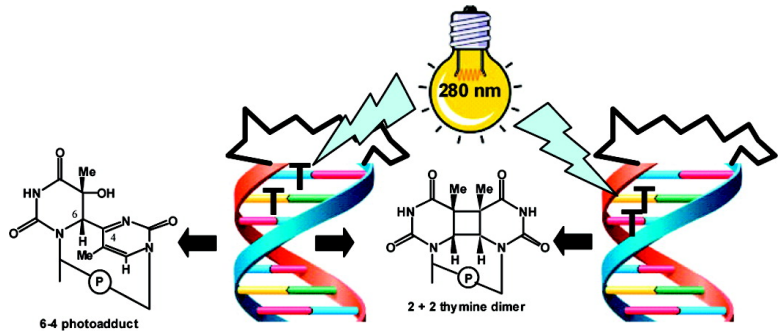

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Context-Dependent Photodimerization in Isolated Thymine−Thymine Steps in DNA

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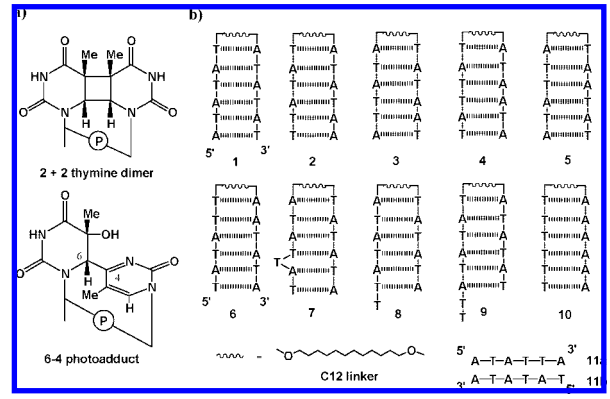
Dimerization of thymine has long been recognized as a leading cause of cellular photodamage by ultraviolet light.[1,2] A revival of interest in T−T dimerization has been driven by advances in fast spectroscopy,[3,4] studies of ground-state conformational dynamics[5,6] and excited-state potential energy surfaces,[7] and synthetic nucleotide chemistry.[8,9] The cis−syn cyclobutane dimer formed in a [2+2] cycloaddition reaction between adjacent thymines is the major product of irradiation of duplex DNA, accompanied in some cases by lesser amounts of the 6−4 adduct, which is thought to arise from a Paterno−Büchi cycloaddition followed by thermal ring opening of the oxetane intermediate (Chart 1a).[10] Both the efficiency of product formation and the product ratio in duplex DNA are dependent upon the base sequence, lower yields being observed for flanking purines vs pyrimidines.[11,12] Dimerization efficiency has also been related to conformational flexibility.[2,12,13] Recently attention has focused on the importance of ground-state conformational populations in determining T−T dimerization efficiency.[4,5]

We report here an investigation of T−T photodimerization in a family of minihairpins possessing only A−T base pairs and a single T−T step connected by an alkane linker (C12, Chart 1b). Minihairpins having short, well-defined base pair sequences with chromophores located at one or both ends have been employed in our studies of electron transfer, energy transfer, and exciton coupling in DNA.[14] The use of hairpins with tetranucleoside loops for the study of the base-sequence dependent formation of UV-induced lesions has been reported by Carell et al.[9] However, the context dependence of the photodimerization of hairpins possessing a single T−T step as a function of its location has not been explored. We find that both the efficiency and products of photodimerization are remarkably dependent upon the location of the T−T step within a duplex domain and in conjugates possessing a T bulge or overhang.

Oligonucleotide conjugates **1**−**10** and single strand sequences **11a,b** (Chart 1) were prepared using standard phosphoramidite chemistry, purified using reversed-phase HPLC, and characterized by MALDI-TOF mass spectrometry (Table S1). Conjugates **1**−**10** exhibit UV and CD spectra (Figures S1, S2) characteristic of poly(A−T) duplexes or hairpins.[15] Conjugates **1**−**6** have melting temperature of 45.5−49.5 °C (Table S2) obtained from thermal dissociation profiles in 10 mM phosphate buffer (pH 7.2) containing 100 mM sodium chloride (standard buffer). Conjugate **7** (single T bulge) has a lower T_M (40.1 °C), and **8** and **9** (single and double T overhang) and **10** (continuous A-tract) have higher T_M values (Table S2). Solutions containing ca. 1−1.2 μM hairpin in standard buffer were irradiated with monochromatic 280 nm light at 10 °C in 1 cm path length quartz cuvettes. The progress of irradiation was monitored at 260 nm by high temperature HPLC with a column temperature of 60 °C, a procedure similar to that employed by Barton and co-workers in their studies of thymine dimer repair.[16] The use of high temperature HPLC to achieve thermal rather than chemical denaturation permits analysis of small aliquots of irradiated solutions with excellent resolution.

Chart 1[a]



[a] (a) Structures of possible thymine−thymine photoproducts. (b) Structures of C12-linked hairpins **1**−**10** and single strand oligos **11a** and **11b**.

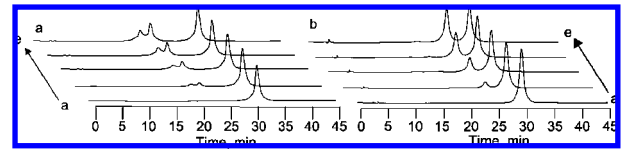


Figure 1. HPLC traces a−e for irradiation of hairpins (a) **6** and (b) **7** at 280 nm with increasing irradiation times 0, 10, 20, 30, and 40 min, respectively. Solutions were analyzed by high temperature reversed-phase HPLC on a Microsorb MV C_{18} reversed-phase column maintained at 60 °C using a gradient of 20 mM ammonium acetate containing 7% acetonitrile to 12% acetonitrile.

Representative HPLC traces obtained at 10 min irradiation intervals for hairpins **6** and **7** are shown in Figure 1. The growth of a single dominant product peak at a shorter retention time than the starting material is observed for **2**−**5**, **7**−**9**, and the single strand **11a** (Figure 1b). However, in the case of **6** two product peaks are formed in a ca. 45:55 ratio (Figure 1a). No new product is observed upon irradiation of hairpin **1** or single strand **11b** which lack a TT step, and multiple product peaks are observed for hairpin **10** which has a polyT sequence. All of the products obtained from hairpins **2**−**9** have the same mass as the starting material. The isolated product from hairpin **5** has a value of T_M = 30 °C, significantly lower than that of **5** (47.5 °C), as expected for the formation of a photolesion.[17] All of the product peaks except for the minor product from **6** have UV spectra similar to those of the starting materials (Figure S3a) and undergo reversion to starting materials upon irradiation at 240[18] or 254 nm,[19] as expected for 2+2 thymine dimers. The minor product from **6** is tentatively assigned to the 6−4 adduct based on its weak absorption band at 320 nm (Figure S3b) and stability upon irradiation at 240 nm.[20]

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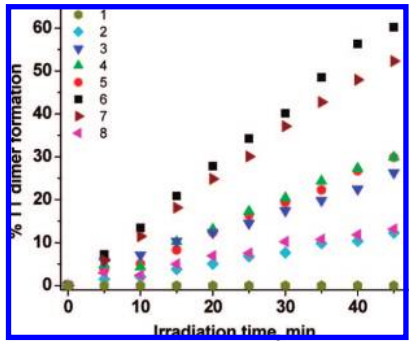

Figure 2. Relative percentage dimer formation of hairpin sequences **1**−**8** as determined using HPLC with increasing time of irradiation at 280 nm. Data for **6** are the sum of both products.

The time-dependent conversion of hairpins **1**−**8** to photoproducts upon irradiation is shown in Figure 2. The linearity of these plots to high conversions is a consequence of the low absorbance of the photoproducts at the 280 nm irradiation wavelength. Hairpins **3**−**5** which have a TT step within the interior of their A−T base pair domains display similar conversions of ca. 30% after 45 min of irradiation. Using their conversions as a basis for comparison, significantly lower conversions are observed for hairpins **2**, **8**, and **9** (13−14% conversion) and higher conversions for hairpins **6** (60% total conversion), **7** (53% conversion), and **10** (95% total conversion). Single strand **11a** was irradiated in a 1:1 mixture of the noncomplementary strand **11b** to provide a ratio of reactive and nonreactive T and A chromophores similar to that for the hairpins. The conversion observed for **11a** is 14%, similar to that for hairpins **2**, **8**, and **9**.

Our results for hairpins **1**−**5** and single strands **11a,b** are consistent with previous reports that the 2+2 dimer is the major product obtained from TT steps in either duplex and single strand DNA but is not formed from alternating AT sequences.[9] Unprecedented are the observations of (a) identical reactivity for the interior TT steps in **3**−**5**, (b) diminished dimerization efficiency for TT steps at the 5′−3′ hairpin terminus of **2** and the single and double T and TT overhangs in **8** and **9**, as well as in the single strand **11a**, (c) enhanced 2+2 dimerization at the site of a single T bulge in **7**, and (d) formation of both 2+2 and 6−4 products adjacent to the alkane linker in **6**. These observations can be understood in terms of the topochemical principles of Fischer for solid state photodimerization[21] as applied by Kohler and others to T−T dimerization in DNA.[4] That is, dimerization occurs only in a subset of ground-state conformations in which the adjacent thymines have their chromophores properly aligned so that bonding can compete with ultrafast nonradiative decay or energy transfer.

Diminished reactivity in hairpins **2**, **8**, and **9** suggests that TT steps at the hairpin terminus and the overhangs in **8** and **9** (like the single strand **11a**) are less likely to adopt reactive conformations than are TT steps in the B-DNA interior of **3**−**5**. Solution NMR studies of duplexes having terminal A−T base pairs or T overhangs display line broadening indicative of a high degree of conformational mobility or end-fraying.[22] Thus conformational mobility is not a good predictor of high dimerization efficiency. The enhanced reactivity of hairpin **7** is indicative of a "stacked-in" rather than a "looped-out" conformation for the "extra" T.[23] The formation of a 6−4 adduct from hairpin **6** finds analogy in reports by Clivio and co-workers for TpT dinucleotides having C3′-endo sugar conformations.[8] The enhanced reactivity of **6** and **7** suggests that the presence of reactive conformations rather than flexibility is the best indicator of T−T dimerization efficiency, as suggested by recent molecular dynamics simulations for single strand poly(T).[5] Solution NMR studies and molecular dynamic simulations of the C12-linked hairpins **6** and **7** are in progress as are further studies of the context-dependent photodimerization in isolated TT steps.

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Supporting Information Available: MALDI-TOF analysis of the hairpin sequences **1**−**10** and the single strand sequences **11a** and **11b**, melting points of sequences **1**−**10**, representative UV and circular dichoism spectra of sequence **1**, change in absorption spectra of **2** and **6** with increasing irradiation time. This material is available free of charge via the Internet at http://www.pubs.acs.org.

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