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Thymine Dimer Photoreversal in Purine-Containing Trinucleotides

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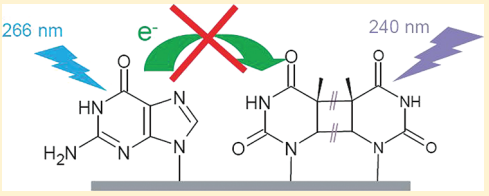
Supporting Information

ABSTRACT: Cyclobutane−pyrimidine dimer yields in UV-irradiated DNA are controlled by the equilibrium between forward and reverse photoreactions. Past studies have shown that dimer yields are suppressed at sites adjacent to a purine base, but the underlying causes are unclear. In order to investigate whether this suppression is the result of repair by electron transfer from a neighboring nucleobase, the yields and dynamics of the reverse reaction were studied using trinucleotides containing a *cis*−*syn* dimer (T<>T) flanked on the 5′ or the 3′ side by adenine or guanine. The probability of forming an excited state on T<>T or on the purine base was varied by tuning the irradiation wavelength between 240 and 280 nm. Cleavage quantum yields decrease by an order of magnitude over this wavelength range and are less than 1% at 280 nm, a wavelength that excites the purine base with more than 95% probability. Conditional quantum yields of cleavage for the trinucleotides given excitation of T<>T are similar in magnitude to the quantum yield of cleavage of unmodified T<>T. These results indicate that within experimental uncertainty all photoreversal in these single-stranded substrates is the result of direct electronic excitation of T<>T. Photolyase-like repair of T<>T due to electron transfer from an adjacent purine is negligible in these substrates. Instead, the observed variation in photoreversal quantum yields for adenine- versus guanine-flanked *cis*−*syn* dimer could be due to uncertainties in absorption cross sections or to a modest quenching effect by the purine on the excited state of T<>T. Pump−probe measurements reveal that the excited-state lifetimes of A or G in the dimer-containing trinucleotides are unperturbed by the neighboring dimer, indicating that electron transfer from purine base to T<>T is not competitive with rapid excited-state deactivation. Pump−probe measurements on unmodified T<>T in aqueous solution indicate that cleavage is most likely complete on a picosecond or subpicosecond time scale.



1. INTRODUCTION

UV irradiation of single strand and duplex DNA possessing thymine−thymine (TT) steps results in the formation of mixtures of photoproducts.[1] The major product formed by the dinucleotide T_pT and longer single strand or duplex systems is the *cis*−*syn* (2 + 2) dimer (T<>T, reaction 1 in Scheme 1), one of a class of cyclobutane pyrimidine dimers (CPDs). CPDs are believed to form by the concerted cycloaddition reaction of adjacent π-stacked thymine bases.[2,3] The low quantum yield for dimerization of single strand and duplex systems possessing only A-T base pairs has been attributed to the low probability that adjacent thymines have ground-state conformations appropriate for dimerization coupled with the very short lifetime of the thymine excited state.[4,5] A growing number of studies reveal significant conformational effects on dimer yields.[2,3,6,7]

Importantly, dimer yields are determined not only by the formation reaction, but also by the light-induced cleavage of a dimer back to the original bases. This process, known as photoreversal, occurs by two mechanisms (Scheme 1, reactions 2 and 3).[8] A dimer can be directly excited by deep UV light, creating an excited state that dissociates with high quantum yield.[9,10] Alternatively, the excited state of a secondary chromophore can sensitize cleavage by one-electron oxidation or reduction of the dimer.

Scheme 1. Thymine Dimer (T<>T) Formation and Cleavage Photoreactions[a]

(1) T + T + hν → T<>T — dimer formation

(2) T<>T + hν → T<>T* → T + T — direct photoreversal

(3) T<>T + S + hν → T<>T + S* → T + T + S — indirect photoreversal

[a] S is a photosensitizer and electronic excitation is denoted by an asterisk.

Photosensitized dimer reversal is the mechanism used by the enzyme photolyase to repair CPDs. This enzyme, which is found in all three kingdoms of life, uses visible and near UV light to initiate electron transfer from a flavin cofactor to a dimer.[11−13] Cleavage of the dimer radical anion ensues, followed by back electron transfer to the oxidized electron donor.[11] Photosensitized cleavage has also been studied in photolyase-inspired model systems in which an excited-state electron donor molecule is covalently tethered to a CPD.[14−19]

Although indirect photoreversal is initiated in the model systems by excitation of a non-nucleic acid chromophore, recent

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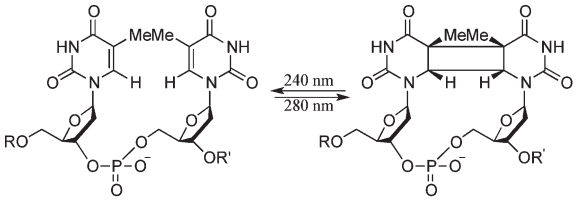
Scheme 2. Structure and Nomenclature of the Trinucleotides Investigated



(2+2) *cis-syn* thymine dimer

TT: R = H, R' = H
ATT: R = dAMP, R' = H
TTA: R = H, R' = dAMP
GTT: R = dGMP, R' = H
TTG: R = H, R' = dGMP

T<>T: R = H, R' = H
AT<>T: R = dAMP, R' = H
T<>TA: R = H, R' = dAMP
GT<>T: R = dGMP, R' = H
T<>TG: R = H, R' = dGMP

studies have fueled interest in whether the DNA bases can themselves photosensitize CPD repair.[20−22] Chinnapen and Sen discovered a DNA sequence by in vitro selection that catalytically repairs an incorporated thymine dimer when exposed to 305 nm light.[20] This self-repairing deoxyribozyme was proposed to function in its folded state by donating one or more electrons from a guanine quadruplex to a nearby thymine dimer in a mechanism analogous to that used by the photolyase repair enzymes. Rokita and co-workers later observed that the amount of thymine dimers induced in double-stranded DNA 18-mers by 254 nm light is reduced when the dimer site is adjacent to a guanine.[21] They suggested that electron transfer from a proximal guanine to the thymine dimer reduces photoproduct yields under photostationary conditions. The efficiency of duplex TT dimerization depends upon the identity of the flanking base pairs, and is lower for purine versus pyrimidine flanking bases, for guanine versus adenine bases, and for 5′ versus 3′-guanine.[21,23−25]

Guanine is the most easily oxidized nucleobase,[26] and it has been suggested that singlet excitations in DNA can decay via interbase electron transfer.[27,28] These facts make electron transfer repair by photoexcited guanine a plausible hypothesis, but direct evidence is lacking. Here we report a steady-state and time-resolved study of thymine dimer reversal in trinucleotide model systems containing a single *cis−syn* dimer that is adjacent to a purine base (A or G) on the 3′ or the 5′ position (Scheme 2). The results indicate that photoreversal in these single-stranded model systems proceeds overwhelmingly via direct electronic excitation of T<>T with negligible repair upon electronic excitation of the neighboring purine base.

2. EXPERIMENTAL METHODS

The synthesis, isolation, and characterization of the (2 + 2) *cis−syn* dimer T<>T, and the *cis−syn* dimer-containing compounds AT<>T, T<>TA, GT<>T, and T<>TG (Scheme 2) were described in an earlier study.[7] Irradiated solutions were analyzed by HPLC using a detection wavelength of 260 nm, and HPLC peak areas were corrected for the absorption of dimers and trinucleotides.

For femtosecond transient absorption measurements, an optical chopper that passes every third pump pulse in the original 1 kHz pulse train modulated the pump beam. The pulse energy of each pump pulse was ∼1.0 μJ. Individual probe pulses were at least 100 times weaker in energy. The pump spot size ($1/e^2$ radius) was 0.48 mm, and the probe spot size was 0.16 mm at the sample position as measured by a knife-edge scan.

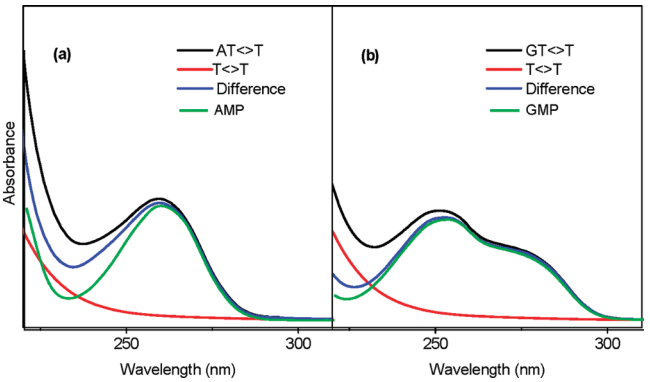


Figure 1. UV absorption spectra recorded in 10 mM phosphate buffer (pH 7.1) containing 100 mM sodium chloride. (a) AMP, AT<>T, T<>T, and the difference spectrum calculated as the AT<>T spectrum minus the T<>T spectrum. (b) GMP, GT<>T, T<>T, and the difference spectrum calculated as the GT<>T spectrum minus the T<>T spectrum.

Transient absorption signals were recorded from a solution of the sample held between two 25.4 mm diameter CaF_2 windows separated by a spacer with a thickness of 1 mm. The pump and probe spots were overlapped 8.0 mm from the center of rotation of the spinning cell, and the cell was spun by an electrical motor at several hundred rpm with the axis of rotation perpendicular to the windows and approximately parallel to the direction of the crossed laser beams. As the cell rotates, the overlapped beams sample a ring of circumference $2\pi \times 8$ mm or ∼50 mm. For a pump spot diameter of ∼1 mm, this means that the longest time needed for previously excited solution to again be located at the position of pump−probe overlap is 0.15 s, which is the time for 50 pump pulses. Because the pump pulses interact with the sample for approximately 5 s at each delay setting, the transient absorption measurements do not probe a solution of pure *cis−syn* dimers, but instead interrogate the induced absorbance change in a solution that is continuously changing in composition. Control experiments showed that the rate of diffusion of unexcited solution into the ring exposed to the pump beam occurred at a slow rate of several tens of minutes. Consequently, the time needed to reach photoequilibrium is determined by the amount of solute in the ring excited by the pump pulse and not by the total solute in the spinning cell.

Transient absorption signals were recorded at delay times between −10 and +500 ps using a step size that was smallest near time zero and increased quadratically with increasing delay time. A single scan consisted of 86 discrete delay settings between pump and probe pulses. A photomultiplier tube in combination with a lock-in amplifier measured the transmission of the probe pulse. At each delay position the lock-in amplifier was allowed to settle fully before 100 readings were made of the voltage measured by the lock-in amplifier from the photomultiplier tube used to detect the probe pulse. These readings were averaged to give the final signal value. Approximately 7.5 min were needed to complete each scan. Immediately following each scan, a UV/vis absorption spectrum of the sample was recorded by placing the sample cell inside a UV/vis absorption spectrophotometer.

3. RESULTS

3.1. Steady-State Cleavage Experiments. The UV absorption spectra of AT<>T and GT<>T and their constituents are

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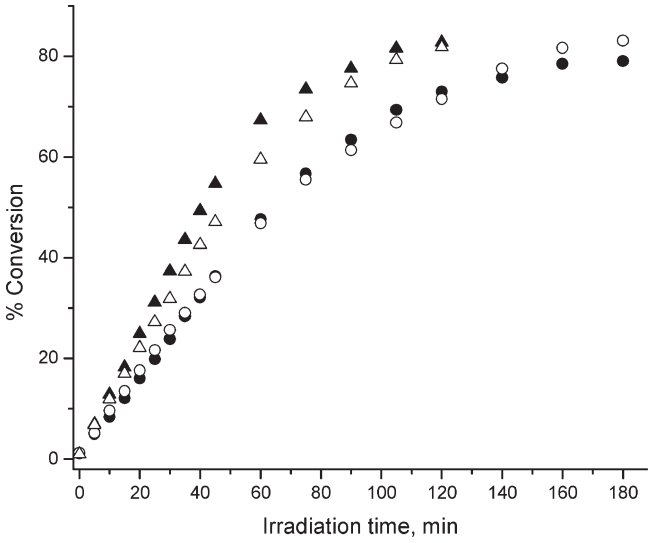

Figure 2. Time dependence of conversion to products for thymine dimers AT<>T (●); T<>TA (○); GT<>T (▲); T<>TG (△) upon monochromatic 240 nm irradiation (3 mW) in 10 mM phosphate buffer (pH 7.1) containing 100 mM sodium chloride.

compared in Figure 1. The difference spectra calculated by subtracting the T<>T spectrum from each trinucleotide (dimer) spectrum closely resemble the spectra of the mononucleotides AMP and GMP, indicating the absence of strong ground-state interactions between the purine and the adjacent T<>T. NMR chemical shifts of the methyl groups in the trinucleotide dimers are shifted upfield slightly from those of T<>T,[29] indicating the presence of ground-state conformations in which these methyl groups lie within the shielding region of the purine.[30] However, we expect the population of well-stacked purine-T ground-state conformations to be low, as is the case for the parent trinucleotides.[31,32]

Irradiation of the dimers at 240 nm results in cleavage and regeneration of the parent trinucleotides. Plots of conversion of dimers to trinucleotides shown in Figure 2 are linear to ca. 50% conversion with optimum conversions of ca. 80%. Small amounts of the minor (2 + 2) *trans*−*syn* and (6−4) photodimers can be observed at high conversions; however, the parent trinucleotides are the only products detected by HPLC analysis at conversions <30% (Supporting Information, Figure S1). The optimum conversions do not represent true photostationary states because of the formation of a minor amount of other (non reversible) products in the photodimerization process.

The initial slopes of Figure 2 provide relative quantum yields for T<>T dimer cleavage. Quantum yields for dimer cleavage, Φ, were calculated from the slopes using potassium ferrioxalate actinometry and after correction for incomplete absorption of the incident light. The resulting values for the dimer-containing trinucleotides are reported in Table 1 along with the value determined for T<>T. The reported values have an error of ±10% estimated from the cumulative small errors from actinometry and HPLC analysis.

3.2. Time-Resolved Photoreversal Experiments. Aqueous solutions of purified *cis*−*syn* thymine dimer were studied in femtosecond transient absorption experiments using a pump wavelength of 240 nm and a probe wavelength of 266 nm. The dimer solution absorbance was 0.50 at 240 nm in a 1 mm path length, corresponding to a dimer concentration of 2.7×10^{-3} M, assuming a value of 1840 M^{-1} cm^{-1} for the molar absorption coefficient of the dimer (Supporting Information, Table S1).

Table 1. Cleavage Quantum Yields, Φ, and Conditional Cleavage Quantum Yields, $\overline{\Phi}_X$ and $\overline{\Phi}_{T<>T}$, for Thymine Dimers in the Listed Substrates at Irradiation Wavelength, λ_{irrad}

sample	λ_{irrad}	Φ	$\overline{\Phi}_X$[a]	$\overline{\Phi}_{T<>T}$[b]
T<>T	240	0.41 ± 0.04	–	–
AT<>T	240	0.065 ± 0.004	0.085	0.27
	280	0.00806	0.0085	0.16
T<>TA	240	0.074 ± 0.007	0.097	0.31
	280	0.00796	0.0084	0.16
GT<>T	240	0.097 ± 0.01	0.12	0.62
	280	0.00418	0.0042	0.32
T<>TG	240	0.091 ± 0.009	0.11	0.58
	280	0.00393	0.0040	0.30

[a] $\overline{\Phi}_X = \Phi/P(X)$, where the probability of exciting the purine base X, $P(X)$, is estimated from the molar absorption coefficients in Table S1 in the Supporting Information. [b] $\overline{\Phi}_{T<>T} = \Phi/P(T<>T)$, where the probability of exciting the dimer T<>T, $P(T<>T)$, is estimated from the molar absorption coefficients in Table S1.

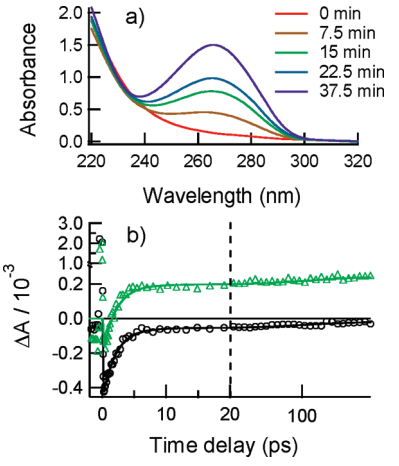

Figure 3. (a) UV/vis spectra of the *cis*−*syn* thymine dimer solution after the indicated irradiation times at 240 nm. (b) Average transient absorption signal (pump, 240 nm; probe, 266 nm) recorded between 0 and 38 min of 240 nm excitation (green triangles) and the transient absorption signal recorded between 50 and 65 min irradiation time (black circles). The solid curves are best-fit curves to the experimental points.

Absorption spectra recorded after individual transient absorption scans are shown in Figure 3a. The initial absorption spectrum (red curve in Figure 3a) matches that of the thymine dimer. The absorbance near 270 nm increases dramatically with increasing irradiation time, and a broad band develops as expected when thymine dimers are cleaved to two separate thymine residues.

Figure 3b shows the transient absorption signal for a probe wavelength of 266 nm obtained by averaging five individual scans recorded between 0 and 37.5 min of irradiation time (green triangles). Because the probe transmission steadily decreases with irradiation time due to dimer cleavage, the probe signal level used to calculate ΔA was the average value measured before and after each scan with the pump pulse blocked.

After the positive spike due to simultaneous absorption of pump and probe photons near time zero, a negative ΔA signal is observed at earliest times, but this signal rapidly increases to yield a positive offset that then increases much more slowly out to the longest delay times studied. Signal averaging was necessary to

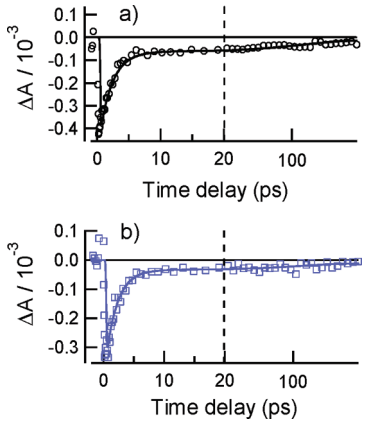

Figure 4. (a) Transient absorption signal from a *cis*−*syn* thymine dimer solution recorded at a probe wavelength of 266 nm and a pump wavelength of 240 nm after 50 min of 240 nm irradiation. (b) Transient absorption signal from a reference solution of TMP (pump, 240 nm; probe, 266 nm).

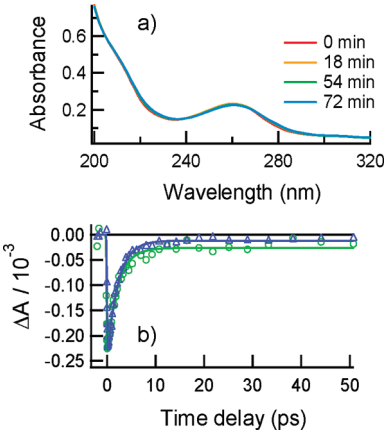

Figure 5. (a) UV/vis spectra of AT<>T after the indicated irradiation times during pump−probe experiments with 266 nm pump and 250 nm probe pulses. (b) Transient absorption signals (266 nm pump; 250 nm probe) from aqueous solutions of AMP (triangles) and AT<>T (circles).

obtain a reasonable signal-to-noise ratio, but lengthy averaging is not feasible in these experiments because continued photolysis by the pump pulses continuously changes the solution composition.

Despite the noise in individual scans, the positive signal amplitude seen at long delay times very clearly decreases from scan to scan. Averaging the signal between 10 and 500 ps produces average ΔA values for the first five scans of 0.38, 0.26, 0.21, 0.14, and 0.12 mOD. The positive signal decreases steadily with increasing irradiation time until after approximately 1 h of total irradiation time the signal is negative at all delay times (black circles, Figure 3b). As will be discussed below, the solution after 50 min of irradiation time consists of an approximately photostationary mixture of thymine dimers and cleaved dimers. The signal recorded from the approximately photostationary mixture is indistinguishable from the signal recorded from a solution of the mononucleotide TMP as shown in Figure 4.

Pump−probe experiments were carried out on the purine-containing trinucleotides using a pump wavelength of 266 nm to excite mainly the purine base. The molar absorbance coefficient data in the last two rows of Table S1 (Supporting Information)

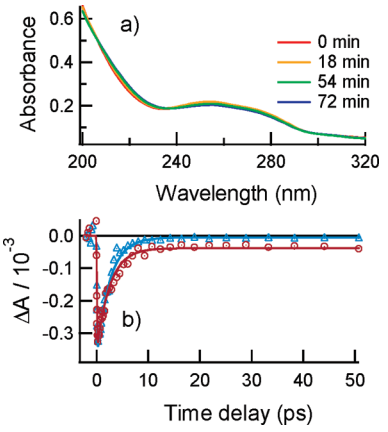

Figure 6. (a) UV/vis spectra of GT<>T after the indicated irradiation times during pump−probe experiments with 266 nm pump and 250 nm probe pulses. (b) Transient absorption signals (266 nm pump; 250 nm probe) from aqueous solutions of GMP (triangles) and GT<>T (circles).

suggests that 97−98% of all photons at this wavelength are absorbed by adenine or guanine. The trinucleotides are highly photostable and irradiation of AT<>T for over an hour caused virtually no change in the UV/vis absorption spectrum (Figure 5a). Transient absorption signals are independent of irradiation time within experimental uncertainty and are very similar to the signals observed using the same pump and probe wavelengths for a solution of AMP (Figure 5b). Similar results were observed for the trinucleotide GT<>T (Figure 6) and for T<>TA and T<>TG (data not shown).

4. DISCUSSION

Charge transfer from neighboring purine bases has been proposed as a mechanism for the repair of thymine dimers in DNA.[20,21] In this process, electron transfer from photoexcited A or G to T<>T is expected to be exergonic as the singlet energies of both purines are higher (ca. 4.5 eV) than the energy needed to form the corresponding radical ion pairs. Oxidation potentials for G and A (i.e., $E^0(G^{\cdot+}/G)$ and $E^0(A^{\cdot+}/A)$) in acetonitrile solution are 1.49 and 1.96 V vs NHE, respectively.[26] The reduction potential of T<>T is reported to be −2.2 V vs SCE,[33] corresponding to −1.95 V vs NHE. Therefore, reduction of T<>T is thermodynamically slightly more facile than reduction of thymine as $E^0(T/T^{\cdot-}) = -2.14$ V vs NHE in acetonitrile.[26] These facts make electron-transfer repair by photoexcited guanine a plausible hypothesis. Additionally, it has been proposed that singlet states in DNA decay to charge transfer states, especially when A is stacked with T.[27,34] In the following, the results of steady-state and time-resolved experiments investigating the relevance of charge-transfer processes in single-stranded trinucleotides are discussed in detail.

4.1. Steady-State Quantum Yields. The cleavage quantum yield, Φ, for T<>T at 240 nm (Table 1) is similar to that reported by Sztumpf and Shugar for 254 nm irradiation,[35] but lower than a previously reported value for 240 nm irradiation.[42] Because paper chromatography was used for product analysis and secondary actinometers were used to measure light intensity in the earlier study, our value should be more reliable. The value measured here of 0.41 ± 0.04 is consistent with cleavage

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occurring via a conical intersection which partitions approximately equally between product and starting material.[36]

Positioning a purine base next to the thymine dimer suppresses the quantum yield of cleavage in every case, but yields do not depend significantly on whether the purine base is on the 5′ or the 3′ side of the dimer (Table 1). The magnitude of suppression, however, does depend sensitively on wavelength. Cleavage yields are lower by a factor of between 4 and 6 at 240 nm. At 280 nm, yields are ∼50 and ∼100 times lower for the A- and, G-containing trinucleotides, respectively, compared to the T<>T cleavage quantum yield of 0.41 ± 0.04 measured at 240 nm. Table 1 indicates that the cleavage quantum yield, Φ, is about 30% larger for the G- vs A-containing trinucleotides at 240 nm. At 280 nm, however, yields are about twice as high for AT<>T and T<>TA as for the G-containing trinucleotides. These patterns are difficult to explain by the electron-donating ability of G, a property that should depend weakly, if at all, on excitation wavelength, but they are fully consistent with cleavage resulting from direct excitation of T<>T.

The probability that an excitation is formed initially on T<>T is shown in parentheses in the final two rows of Table S1, assuming that molar absorption coefficients of the purine and T<>T are additive and neglecting energy transfer and the formation of delocalized excitations (excitons). These approximations are likely to hold for these dimer-containing trinucleotides which like T<>T-containing duplexes are expected to be poorly stacked.[37] In particular, hypochromism, which would cause the true molar absorption coefficients to be smaller than those estimated from summing coefficients for individual chromophores, should be small.

The steady-state quantum yield is the number of splitting events divided by the total number of absorbed photons. The observed strong variation of Φ with wavelength (Table 1) is one indication that cleavage depends acutely on which absorber (T<>T or the purine base) is photoexcited. Table 1 results show that the ability of an excited state on a neighboring purine to cleave a dimer cannot be substantial for irradiation at 280 nm. The cleavage quantum yields at 280 nm are less than 1% for all dimer-containing trinucleotides even though more than 95% of all excited states are localized on the purine base.

We seek to compare the likelihood of cleavage when T<>T is directly excited with the likelihood of cleavage when the flanking purine base is excited. The probability that an event takes place given that another event has occurred is described by a conditional probability.[38] We define the conditional quantum yield of cleavage, Φ_X, to be the cleavage probability given excitation of the purine base X, while the conditional quantum yield $\Phi_{T<>T}$ is our notation for the probability of cleavage given excitation of the dimer (see Supporting Information). Neither quantity can be determined from steady-state measurements, but an upper bound for each, written as $\overline{\Phi}_X$ and $\overline{\Phi}_{T<>T}$, can be calculated from the experimental data, as described in the Supporting Information. These values are listed in the last two columns of Table 1.

Notably, all $\overline{\Phi}_{T<>T}$ values in Table 1 are comparable in size to the splitting quantum yield of 0.41 measured for T<>T. This indicates that direct photoreversal is the dominant cleavage mechanism because efficient electron-transfer repair from the purine would otherwise cause $\overline{\Phi}_{T<>T}$ values to exceed unity. $\overline{\Phi}_{T<>T}$ is twice as large for the G-containing than for the A-containing trinucleotides at both 240 and 280 nm (Table 1). This could indicate that direct photoreversal is less efficient when A versus G is attached to the dimer. However, the molar absorption coefficients of the constituent subunits are uncertain, as is the procedure for properly partitioning excitation probabilities in multichromophoric molecules like DNA. Trends in the conditional quantum yields should therefore be interpreted cautiously.

According to Table 1, the conditional quantum yield for T<>T cleavage by electron transfer from a neighboring purine is less than 0.009 at 280 nm, and less than about 0.1 at 240 nm. Although $\overline{\Phi}_X$ increases 10−30 times from 280 to 240 nm, it is unlikely that the true value of Φ_X increases in this way. Because $\Phi_{T<>T}$ is large, the difference between $\overline{\Phi}_X$ and Φ_X increases with decreasing wavelength as the probability of exciting the purine decreases (see eq S5 in the Supporting Information). If the reasonable assumption is made that cleavage efficiency is independent of wavelength, whether cleavage occurs by direct excitation of T<>T or following excitation of the purine, then Φ_X should be no greater than the smaller of the two values, or no greater than about 0.008. We conclude that there is negligible repair of thymine dimer due to excitation of the purine base at both 280 and 240 nm.

4.2. Cleavage Dynamics. The steady-state experiments establish that excitation of the flanking purine does not contribute significantly to cleavage in these trinucleotides, but do not necessarily rule out photoinduced electron transfer from the purine to the thymine dimer. The absence of efficient cleavage could be due to back electron transfer that occurs more rapidly than the dimer radical anion can dissociate. For example, past studies have shown that the cleavage quantum yield is often much less than one due to efficient back electron transfer when a thymine dimer is covalently tethered to an electron-donating chromophore such as indole[14] or dimethoxybenzene.[17] Photolyase, on the other hand, repairs dimers with high efficiency and this is attributed to factors that suppress back electron transfer.[15,39] Given the ultralow fluorescence yields of guanine and adenine,[40] it is impractical to look for fluorescence quenching as evidence of ET as has been done with other sensitizer−dimer compounds.[14,15] Instead, pump−probe experiments were carried out to look for dynamical signatures of electron transfer. Experiments were first carried out on a sample of pure *cis*−*syn* thymine dimer in order to first establish the kinetics of direct cleavage.

4.3. Pump−Probe Signals from Aqueous *Cis*−*Syn* Thymine Dimer Solution. The competing cleavage and dimerization reactions lead to the establishment of an approximate equilibrium or photostationary state. The rapid approach to the photostationary state during the course of our measurements causes the transient absorption signals to change with irradiation time as shown in Figure 3b. This results from the high quantum efficiency of the cleavage reaction and the finite amount of purified *cis*−*syn* dimer available for these experiments.

Our quantity of purified dimer limited us to working with no more than 1.0 mL of solution at a time. Given a pump beam radius of 0.5 mm, a sample path length of 1 mm, and the pump pulse repetition rate after chopping of 1000 Hz/3, nearly 16 mL of solution would be needed every minute if every pump pulse were to excite fresh solution. Because 5−10 min of signal averaging is required to obtain a reasonable signal-to-noise ratio using our spectrometer for a transient absorption scan, it was impossible to avoid re-exciting previously irradiated solution with the 0.3 mL volume spinning cell used in these measurements. This results in continuous photolysis and the establishment of photostationarity in under an hour. In fact, a true photostationary state is not reached for TpT because of the formation of nonreversible products like the (6−4) photoproduct. However, because the quantum yields of formation of

irreversible products like the (6−4) photoproduct are about an order of magnitude lower than for formation of thymine dimer, the solution becomes approximately photostationary under our experimental conditions (irradiation times of less than 1 h).

The evolution from a fresh sample of pure T<>T to one that is approximately photostationary yields the transient absorption signals shown in Figure 3b. Nonlinear least-squares fits to the data (see Table S2, Supporting Information) confirm that the time-dependent signals decay identically within experimental uncertainty, but the signals recorded before the photostationary state have a substantial positive offset that steadily decreases with irradiation time.

A reversible photoreaction that has attained photostationary concentrations of reactant and product will always have a transient absorption signal of zero at delay times long enough that all excited states and other intermediates have decayed away. In the case of T<>T, photostationarity means that the number of dimers cleaved by each pump pulse exactly equals the number of new dimers formed. The transient absorption signal from the photostationary solution is shown by the black curve in Figure 3b. The signal does approach zero, as expected at photostationarity, but only several hundred picoseconds after excitation. At shorter times, the signal agrees fully with the signal recorded from a solution of the monomer TMP as shown in Figure 4b. The TMP signal has fast and slow kinetics with time constants of 2.1 and 130 ps (Table S2 in the Supporting Information). The fast kinetics are due to vibrational cooling following ultrafast conversion, while the slower kinetics reflect the lifetime of $^1n\pi^*$ excited states that relax more slowly.[41] It was shown earlier that UV/UV pump−probe signals for $(dT)_{18}$ differ insignificantly from those of the mononucleotide TMP,[34] and this is the reason for the excellent agreement in Figure 4.

The photostationary concentrations depend sensitively on the irradiation wavelength (Figure S2 in the Supporting Information) because reactant (TT) and product (T<>T) differ so greatly in their absorption spectra. With 254 nm irradiation from a germicidal lamp, only about 25% of TpT can be converted to dimer products, but irradiation at 280 nm converts 90% of TpT to dimer.[42] Despite this variation, the ratio of the number of excited states of TpT to the number of excited states of T<>T formed by each pump is the same at all pump wavelengths for the photostationary solution, when the reasonable assumption is made that the quantum yields of the forward and reverse photoreactions are independent of wavelength. Given that the quantum yield of cleavage of T<>T (0.41, see Table 1) is 40 times higher than the quantum yield of dimer formation in TpT (~0.01[42]), then 40/41 or 98% of excited states formed by the pump pulse are localized on TpT under photostationary conditions regardless of the pump wavelength. This is the reason why the kinetics seen from the photostationary solution are identical within uncertainty to kinetics seen from TMP.

At times before photostationarity is established, transient absorption signals display a positive offset, but no new time constants are detected. A full kinetic analysis will be published elsewhere, but these results are consistent with prompt (i.e., subpicosecond) dissociation of excited T<>T, a reasonable result for a unimolecular photoreaction characterized by a high quantum yield.

4.4. Pump−Probe Signals from Dimer-Containing Trinucleotides. In the pump−probe experiments on purine-containing trinucleotides, the 266 nm pump wavelength is expected to excite the purine base 97−98% of the time. The presence of the purine base could alter the time needed for reaching photostationarity, but should not alter the ultimate levels of dimer versus nondimerized TT. The low steady-state quantum yields for dimer cleavage at 280 nm indicate that attainment of the photostationary state at the 266 nm pump wavelength will happen much more slowly than for the T<>T solution. This is indeed the case and transient signals shown in Figures 5 and 6 are invariant with irradiation time since the start of measurements. No positive offset could be seen at long delay times that would indicate the dimer cleavage that is responsible for the positive signals seen in Figure 3.

There is no evidence from the signals in Figures 5 and 6 of electron transfer from the purine to the thymine dimer. The decays are unchanged within experimental uncertainty compared to what is observed from AMP or GMP alone (Table S3 in the Supporting Information). The data therefore rules out the possibility of efficient electron transfer from the excited purine to T<>T followed by efficient back electron transfer before cleavage can occur.

Our results show that electron transfer from G or A to T<>T does not occur to a significant extent in single-stranded trinucleotides. Through-space or through-bond electron transfer in unstacked systems such as our trinucleotides may be too slow to compete with rapid nonradiative decay of the purine excited state. The $^1\pi\pi^*$ excited states of all native nucleobases can decay via ultrafast internal conversion with a time constant of less than 1 ps.[28] $^1n\pi^*$ states with lifetimes of several tens of picoseconds have been observed in pyrimidine, but not in purine bases.[41] It is interesting to note that the rate of forward electron transfer in photolyase takes place with biexponential kinetics and lifetimes of 60 and 335 ps,[43] or much more slowly than excited-state relaxation by native nucleobases. In addition, although definitive kinetic measurements are lacking, it has been suggested that electron transfer takes place on the nanosecond time scale in model compounds in which a sensitizer is tethered to a thymine dimer.[44,45]

It is possible that the purine effect on dimer cleavage requires π-stacking of the flanking purine with T<>T, a condition that is largely absent in our single-stranded substrates. Base stacking like that which occurs in duplex DNA can result in longer-lived singlet excited states[28] and a shorter separation between T<>T and an adjacent purine, although duplex base stacking is disrupted in the vicinity of T<>T.[37,46] Both effects could favor electron-transfer repair. In fact, Nguyen and Burrows[16] have recently reported that photorepair of T<>T by 8-oxoguanosine (OG) is more efficient when located adjacent to T<>T than when located in the complementary strand.

5. CONCLUSIONS

Thymine dimer photoreversal was studied in model trinucleotides in order to understand how this process affects the observed dimer yields in UV-irradiated DNA. Direct excitation of T<>T induces cleavage with high quantum efficiency, but is of limited biological significance because the high singlet energy of T<>T virtually precludes excitation by the lower-energy UV radiation incident on the Earth's surface. In the single-stranded trinucleotide substrates studied, the natural purines guanine and adenine, the best electron donors among the natural bases, do not repair T<>T by a photolyase-like mechanism within experimental uncertainty. Nonetheless, the steady-state quantum yields of reversal vary by about a factor of 2 depending on whether T<>T is flanked by adenine or guanine. This variation appears to originate in effects on direct photoreversal, suggesting that the

purine effect on dimer yields may have more to do with inhibiting cleavage than promoting cleavage via electron transfer.

■ ASSOCIATED CONTENT

Supporting Information. Discussion of conditional quantum yields; table of molar absorption coefficients used in this study; table of fitting parameters in the ultrafast transient absorption experiment and two additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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