Remote *cis*−*syn*-Thymine [2 + 2] Dimers Are Not Repaired by Radical Cations Migrating in Duplex DNA

Anthony K. Dotse, Edna K. Boone, and Gary B. Schuster*

Contribution from the School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

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Abstract: A series of DNA oligomers was prepared containing an anthraquinone derivative linked to a 5′-end, a *cis*−*syn*-[2 + 2]thymine dimer and strategically located GG steps. These compounds were designed to test the claim that one-electron oxidation of the DNA leads to repair of remote thymine dimers. Irradiation of the anthraquinone leads to remote damage at the 5′-G of GG steps that are positioned both before and after the thymine dimer, but there is no detectable (<3%) repair of the thymine dimer. These findings are in contrast to a previous series of reports in which repair of the thymine dimer is reported to occur 40 times more efficiently than reaction at GG steps when the remote one-electron oxidation is initiated by irradiation of a covalently linked Rh-containing metallointercalator.

Introduction

Photolesions are formed in DNA when it is exposed to ultraviolet light. The most prevalent lesion is the *cis*−*syn*-thymine dimer resulting from the [2 + 2] cycloaddition of adjacent thymines. These lesions are mutagenic,[1] and a gene having a single unrepaired dimer can be fatal to an organism.[2] In nature, these lesions are repaired by enzyme systems, which excise the dimer[3] or reverse the cycloaddition reaction.[4] Photolyase operates in the latter mode. It is an unusual enzyme because it is inactive until irradiated with visible light.[5] The light-activated enzyme transfers an electron to the thymine dimer forming its radical anion, which then reverts, eventually, to two thymines. The thymine dimer can also be repaired chemically. Deep-UV irradiation reverses the [2 + 2] cycloaddition,[6] and photoinitiated electron transfer from chemical sensitizers also repairs the dimer. Effective chemical sensitizers may either donate one electron to the dimer,[7−9] mimicking the reaction of photolyase, or remove one electron from the dimer to form its radical cation.[10−12] Anthraquinone derivatives, in particular, are among the light-activated sensitizers that have been reported to repair the *cis*−*syn*-thymine dimer by one-electron oxidation.[11,12]

Barton and co-workers are examining the photochemistry of Rh metallointercalators bound to DNA.[13−36] The central hypothesis of this extensive investigation has been that irradiation of the bound Rh compound with visible light (400 nm) causes long-distance reactions mediated by electron transport through the DNA helix. This ability of DNA to facilitate rapid, long-range electron transfer led them to characterize it as a "π-way" or "molecular wire".[14,16,22,28,32,35,37−39] Other investigators study-

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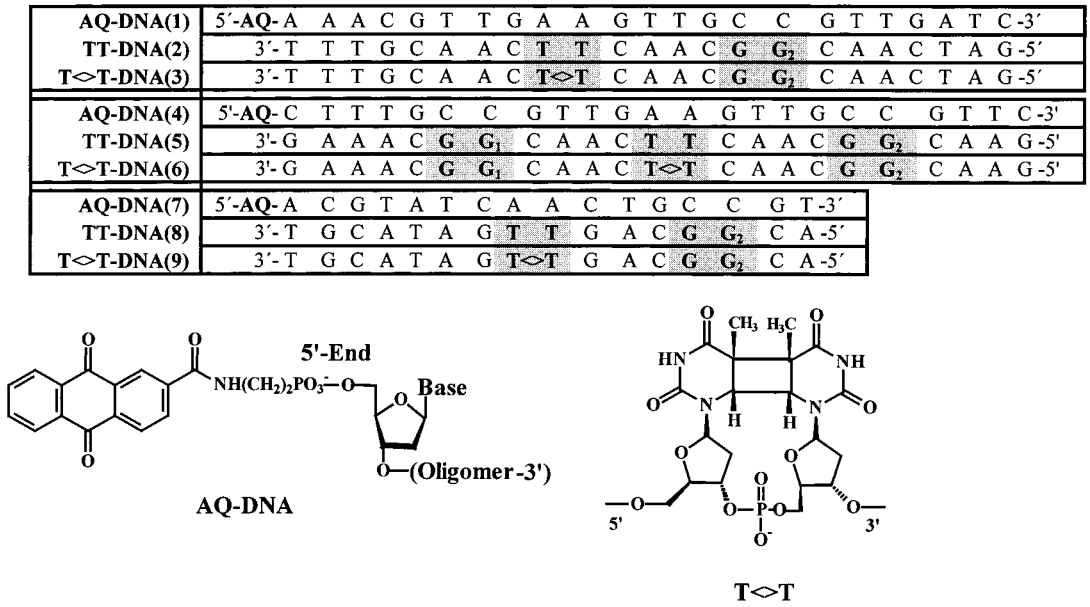

Figure 1. Structures of the DNA conjugates used in this work. Shown schematically are duplexes containing a tethered anthraquinone derivative serving as the oxidant and other duplexes containing the *cis*−*syn*-thymine dimer and 5′-GG-3′ doublets.

ing the ability of DNA to transport charge have not observed molecular wire-like behavior.[40−45] Our examination of long-range radical cation transport through DNA led to the formulation of the phonon-assisted polaron-hopping model.[46−48] In this view, introduction of a radical cation into the DNA helix causes a local structural distortion (the polaron) which hops through the duplex by thermally (phonon) activated processes. On the basis of models used for other synthetic polymers, Conwell and Rakhmanova recently concluded that a radical cation could form a polaron in a DNA stack.[49]

Barton and co-workers examined the effect of the thymine dimer on oxidative charge transfer in DNA assemblies containing tethered metallointercalators.[18,21,29,30] They report that irradiation of a tethered $Rh(phi)_2(bpy')^{+3}$ derivative (phi is 9,10-phenanthrenequinone dimine, bpy′ is a 4,4′-dimethyl-2,2′-bipyridine derivative) catalyzed the quantitative repair of a remote thymine dimer but that the quantum efficiency of this reaction is extremely low ($\Phi = 2 \times 10^{-6}$). Injection of a radical cation into duplex DNA leads to oxidative damage that is revealed as strand cleavage primarily at the 5′-G of GG steps.[35,50,51] Barton assessed the competition between remote thymine dimer repair and cleavage at G using the covalently linked $Rh(phi)_2(bpy')^{+3}$ system. They report that repair of the thymine dimer is 40 times more efficient than guanine damage and that incorporating a thymine dimer in the DNA sequence reduces damage at remote GG steps by a factor of 2.[30]

We have developed an anthraquinone derivative that is covalently linked to a 5′-end of duplex DNA through a four-atom tether.[46−48,52,53] The anthraquinone group is associated with the DNA by end-capping, and its irradiation efficiently injects a radical cation into the DNA duplex. We have employed this system to assess the remote repair of *cis*−*syn*-thymine dimers and to determine the effect of this dimer on oxidative damage at GG steps. Irradiation of an unbound analogue to the bound anthraquinone derivative induces conversion of the thymine dimer to thymine monomers, but there is no measurable repair of the thymine dimer when the remote, covalently bound anthraquinone is irradiated. We also find that the presence of a thymine dimer in the duplex DNA has only a very small effect on the efficiency of strand cleavage at remote GG steps. These findings highlight the care required in the interpretation of low quantum efficiency photochemical reactions.

Results

(1) Design, synthesis, and characterization of AQ and T<>T-containing oligonucleotides. Figure 1 shows the duplex DNA structures that were prepared to examine remote repair of the *cis*−*syn*-thymine dimer and the dimer's effect on radical cation transport to remote GG steps. The preparation of the AQ-linked phosphoramidite required for the solid phase syntheses of AQ-DNA(1,4,7) has been previously described.[46] These DNA oligomers were purified by HPLC and characterized both by MALDI-TOF mass spectroscopy and by sequencing using the Maxam−Gilbert method.[54]

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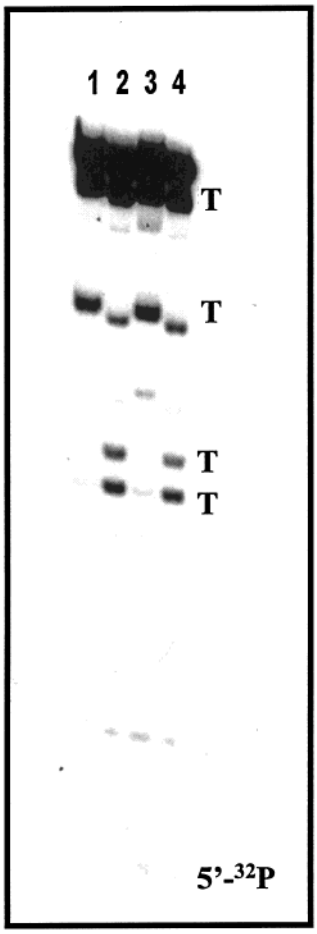

Figure 2. Autoradiogram demonstrating gel mobility and the repair by DNA photolyase of the *cis*−*syn*-thymine dimer in AQ-DNA(7)/T<>T-DNA(9). All experimental samples (8 μM in DNA) were treated first with $KMnO_4$ and then with piperidine: Lane 1, AQ-DNA(7)/T<>T-DNA(9) treated with photolyase but not irradiated (dark control); lane 4, AQ-DNA(7)/T<>T-DNA(9) treated with photolyase and irradiated for 10 min; lane 2, AQ-DNA(7)/TT-DNA(8) irradiated for 10 min; lane 3, AQ-DNA(7)/T<>T-DNA(9) untreated with photolyase and irradiated for 10 min.

The thymine dimer-containing oligonucleotides, T<>T-DNA(3,6,9), were prepared using solid-phase methods from authentic *cis*−*syn*-thymine dimer phosphoramidite, which was synthesized according to the procedure described by Taylor.[55] The thymine dimer-containing compounds were purified by HPLC and then characterized by mass spectrometry and sequenced by modification of the Maxam−Gilbert method.[56] The location of the dimer was confirmed in each oligonucleotide by its repair with *E. coli* photolyase.[57,58] Figure 2 shows an autoradiogram of the polyacrylamide (PAGE) gel for 5′-^{32}P-labeled TT-DNA(8) and T<>T-DNA(9). Lane 3 shows the results of treatment of DNA(9) with $KMnO_4$; as expected, there is no strand cleavage at the dimerized bases. Lane 4 reveals the effect of treating DNA(9) with photolyase: the thymine-dimer is repaired and $KMnO_4$ treatment yields strand cleavage with efficiency comparable to that seen (lane 2) for TT-DNA(8). This experiment unambiguously identifies the location and the stereochemistry of the dimer in DNA(9).[5,57] Related experiments with DNA(2,3) and DNA(5,6) give similar results.

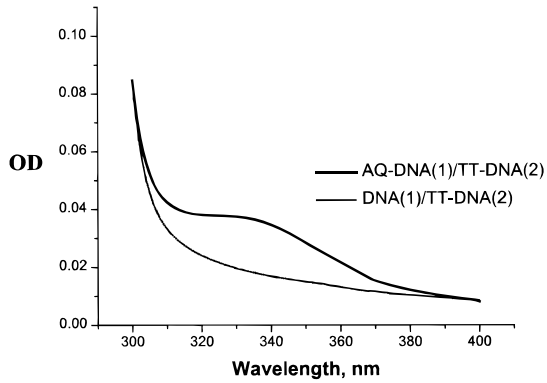

Figure 3. Absorption spectra of DNA(1)/TT-DNA(2) and AQ-DNA(1)/TT-DNA(2) duplexes (2.5 μM in DNA, phosphate buffer solution). The unique absorption of the linked anthraquinone in duplex AQ-DNA(1)/TT-DNA(2) is clearly visible.

The melting temperatures (T_m, the data are summarized in the Supporting Information) of the duplex DNA structures were determined to assess the effect of the thymine dimers and the anthraquinone groups on their thermal stabilities. Generally, conversion of two adjacent thymines to the dimer lowers T_m, and adding the anthraquinone group increases this value by ~3 °C. This behavior, which is typical of that observed for other covalently linked anthraquinone groups, is attributed to association of the AQ with the terminal base pairs of the DNA by end-capping.[46−48,53,59] The absorption spectra of DNA(1)/DNA(2) and AQ-DNA(1)/DNA(2) are shown in Figure 3. The difference in these spectra is the result of the covalently attached AQ, which absorbs at $\lambda > 310$.

Our experimental design is revealed by the structures of the duplex DNA sequences. DNA(1)/DNA(2) contains an AQ group tethered to the 5′-end of the DNA(1) strand. The DNA(2) strand contains a single GG step, which follows the TT sequence. We have shown previously in related experiments that irradiation of tethered AQ groups leads to selective reaction at the 5′-G of GG steps, which is revealed as strand cleavage following treatment with piperidine.[46−48,52,53] These reactions are attributed to injection of a radical cation into the DNA by the excited sensitizer. The radical cation migrates through the DNA and is trapped at remote GG steps.[35,60−62] Surprisingly, the distance dependence for radical cation migration ($\gamma = 0.02$ Å^{-1}) is relatively insensitive to the base sequence.[35,47,48]

A primary goal of our investigation is to assess the ability of a radical cation injected at one end of duplex DNA to repair a remote *cis*−*syn*-thymine dimer. Reactions observed at the GG step of AQ-DNA(1)/DNA(2,3) will confirm injection of the radical cation and measure its ability for migration past the thymine dimer. Duplexes AQ-DNA(4)/DNA(5,6) also contain the TT pair and its dimer, but they each have two GG steps (proximal, GG_1, and distal, GG_2) surrounding a symmetrical sequence containing the TT pair and its dimer. These compounds were examined to assess the effect of the thymine dimer on the relative efficiency of damage to GG steps that precede or follow it. AQ-DNA(7)/DNA(8,9) are precise sequences examined by Barton in the $Rh(phi)_2(bpy')^{+3}$-sensitized remote repair of the

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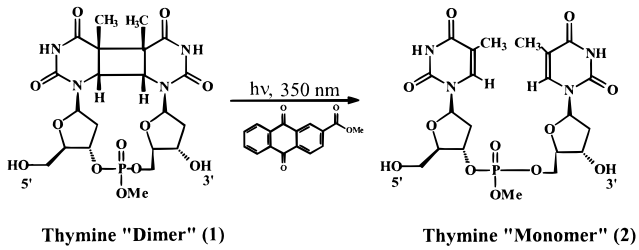
Scheme 1. Anthraquinone-Sensitized Oxidation of Thymine Dimer



thymine dimer.[29,30] We investigated them to assess the possibility that remote dimer repair is somehow very strongly sequence dependent.

(2) Photochemistry of AQ and the Thymine Dimer Radical Cation. There have been numerous investigations of the one-electron oxidation of thymine dimers.[11,12,63−70] One of the first studies in this field reported that the *cis*−*syn*-thymine dimer is efficiently repaired by photosensitized oxidation with 2-anthraquinone sulfonate.[12] However, a subsequent examination of photosensitization by anthraquinone sulfonate indicates efficient repair of the 3,3′-*N*,*N*′-dimethylated thymine dimer but not for the unmethylated "parent".[11] This failure of the parent to form monomeric thymines was attributed to the absence of steric repulsion between juxtaposed methyl groups, which is proposed to drive the reaction in the *N*-methyl substituted compound. Most of the other studies of oxidation of thymine dimers have been performed on N-alkylated analogues, where conversion of the dimer to monomers is regularly observed. Given these contradictory claims, we carried out a careful reinvestigation of the anthraquinone and radical cation sensitized reactions of the nonmethylated thymine dimer.

The reaction we examined is outlined in Scheme 1. The thymine dimer (**1**) was prepared from authentic *cis*−*syn* dimer (see above). The structures of the dimer and the thymine "monomer" (**2**) were verified by ^{1}H NMR spectroscopy. The reaction was carried out in acetonitrile solution to mimic the environment of the core of duplex DNA. This necessitated using methyl 2-anthraquinone carboxylate to sensitize the reaction, because anthraquinone carboxylate salts are sparingly soluble. The results are shown in Figure 4 in the form of HPLC traces. The bottom trace shows, in order of elution, the sensitizer, the thymine monomer, the thymine dimer, and deoxycytidine, which is used as the internal standard for quantification of the results. The middle trace shows a chromatogram of the reaction mixture before irradiation at 350 nm in a N_2-purged solution; significantly, there is no detectable thymine monomer. The upper trace in Figure 4 shows the chromatogram recorded after 5 min of irradiation. At this time, ∼6% of the thymine dimer has been consumed and the yield of thymine monomer is ∼25%. The identity of the monomer in this trace was confirmed by co-injection with authentic material, and by UV and mass spectrometry. The yield of the thymine monomer decreases as the reaction proceeds. After 30 min of irradiation (35% consumption of the thymine dimer), the yield of the thymine monomer is only ∼1%. The high reactivity of the thymine monomer under these conditions may explain why its formation has been difficult to confirm. However, photosensitization by anthraquinone clearly does cause the conversion of thymine dimer to thymine monomers in modest yield. A modest yield is not unexpected. The triplet state of anthraquinone is known to abstract hydrogen atoms rapidly from suitable donors,[71,72] and the deoxyribose units on the thymine dimer and monomer possess suitable hydrogen atoms. Furthermore, the radical cation of the thymine and its dimer are expected to undergo rapid proton tautomerization,[73] which may lead to side reactions. Finally, we verified that conversion of thymine dimer to monomers proceeds through the radical cation by carrying out a co-sensitization reaction modeled after that of Pac and co-workers.[66] The co-sensitization reaction more closely models the remote repair of the thymine dimer where an adjacent base is the oxidant. Irradiation (313 nm) of an air-saturated acetonitrile solution of 1,4-dicyanobenzene and naphthalene results in the conversion of the dimer to thymine monomer in ∼60% yield at 9% conversion. This reaction must involve oxidation of the dimer to its radical cation and cannot proceed through some other pathway such as triplet energy transfer or hydrogen abstraction.

(3) Photochemistry of AQ Linked to DNA Containing Thymine Dimer. Irradiation of AQ-DNA(1)/DNA(2) or AQ-DNA(1)/DNA(3) in air-saturated sodium phosphate buffer solutions (pH = 7.0) at 350 nm, where only the anthraquinone chromophore absorbs light, leads to piperidine-requiring strand cleavage selectively at the 5′-G of the GG step in TT-DNA(2) and in T<>T-DNA(3), but there is no detectable repair of the thymine dimer. Figure 5 shows the autoradiogram from this experiment when DNA(2) or DNA(3) is 5′-labeled with ^{32}P. Lanes 1−4 are controls; lanes 1 and 2 show that there is no strand cleavage for unirradiated DNA(2) or DNA(3), respectively; and lanes 3 and 4 show that irradiation of DNA(1)/DNA(2,3), which does not have an AQ group, does not cause strand cleavage. Lanes 5 and 6 show the results of 30 min of irradiation of AQ-DNA(1)/DNA(2,3) followed by piperidine treatment. Strand cleavage selectively at the 5′-G of GG_2 is readily apparent in both DNA(2) and DNA(3) samples. Lanes 7 and 8 show the results of treatment of these samples with $KMnO_4$ before their reaction with piperidine. This process reveals thymines, but the *cis*−*syn*-thymine dimer is unreactive.[56] Lane 7 clearly shows strand cleavage at the TT pair of DNA(2), but cleavage at this site is absent, lane 8, in the irradiated sample of AQ-DNA(1)/T<>T-DNA(3). Within the limit of detectability for this experiment, there is no repair of the thymine dimer.

The radiolabeling procedure has remarkable sensitivity for revealing strand cleavage. We determined the limit of detection of TT-DNA(2) in samples of T<>T-DNA(3) by preparing authentic mixtures and treating them with $KMnO_4$ and piperidine. The amount of cleavage at the TT pairs in these samples was determined by quantitative measurement of the radioactivity in these bands. The results, the gel is shown in Figure 6 (quantitative findings are presented in the Supporting Information) reveal that 3% repair of the thymine dimer is readily detected. On this basis, the irradiation of AQ-DNA(1)/T<>T-DNA(3) for 30 min results in readily measurable reaction at its

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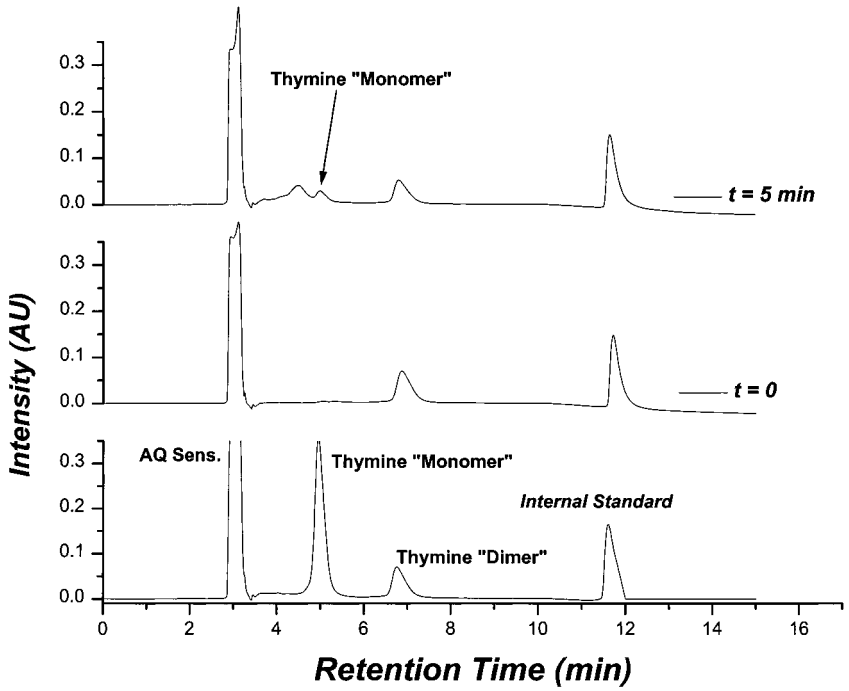

Figure 4. HPLC trace for the anthraquinone sensitized oxidative repair of *cis*−*syn* thymine dimer (**1**). Bottom trace (retention times are in parentheses): sensitizer (3.1 min), thymine monomer (**2**, 5.0 min), thymine dimer (**1**, 6.9 min) and internal standard, 2-deoxycitidine (11.7 min). Middle trace: reaction mixture before irradiation. Upper trace: after 5 min of irradiation at 350 nm.

GG_2 step, but there is < 3% repair of the thymine dimer. This result is expanded using quantitative HPLC in experiments with AQ-DNA(7)/DNA(8,9), see below, which is the precise sequence used in previous experiments reported to show remote thymine dimer repair.[30]

We assessed the effect of a *cis*−*syn*-thymine dimer on the ratio of radical cation-induced strand cleavage at preceding and following remote GG steps by examination of the photochemistry of AQ-DNA(4)/DNA(5) and AQ-DNA(4)/DNA(6). Figure 7 shows the results of 1 h of irradiation followed by treatment with $KMnO_4$ and then piperidine. Repetition of this experiment and quantification of the amount of cleavage by measurement of the radioactivity in the bands for 5′-G cleavage shows that replacement of the TT pair with the *cis*−*syn*-thymine dimer results in no more than ∼5% reduction in cleavage at the GG_2 step compared with the amount of cleavage at GG_1. This is in contrast to previous reports of a factor of 2 reduction in distal GG cleavage by conversion of an intervening TT to its dimer.[30]

Figure 8 shows the results of irradiation of DNA(7)/DNA(8) and AQ-DNA(7)/DNA(9) for 3 h. As shown by HPLC analysis, see below, 3 h of irradiation causes essentially complete reaction at the GG_2 step of this sequence. In contrast, inspection of lanes 3 and 4 of Figure 8 reveals essentially no repair of the thymine dimer. Analysis of authentic mixtures of DNA(8) and DNA(9) by the procedure described above shows that 3% repair of the thymine dimer can be detected in these compounds by this method.

The results of irradiation of AQ-DNA(7)/DNA(8,9) were also analyzed by HPLC on a Microsorb-MV C18 reverse-phase column at 65 °C. At this temperature the DNA is denatured, and under these conditions T<>T-DNA(9) elutes first and is followed by TT-DNA(8). The complementary anthraquinone containing strand, AQ-DNA(7), elutes much later and does not interfere with this assay for dimer repair. Analysis of irradiated samples of AQ-DNA(7)/DNA(9) by HPLC confirms the results of the PAGE experiment. After 2 h of irradiation, there is no detectable repair of the TT-dimer. In addition, the extent of reaction at the GG step of TT-DNA(8) can be assessed by HPLC. Figure 9 shows chromatograms recorded for AQ-DNA-(7)/DNA(8) after piperidine treatment following irradiation for 0, 0.5, 1 and 2 h. Nearly all of the DNA(8) is consumed after 2 h of irradiation. These experiments clearly show that remote oxidative reaction at GG steps is far more efficient than remote repair of the thymine dimer, which is essentially nonexistent. This result contrasts previous findings that oxidative dimer repair initiated by a Rh metallointercalator is 40 times more efficient than oxidative damage at GG steps.[30]

We determined the quantum efficiency of DNA disappearance (strand cleavage) for AQ-DNA(7)/TT-DNA(9) by HPLC using anthraquinone-2,6-disulfonate as an actinometer.[74] Irradiation of optically matched samples in identical cells gives $\Phi_{-DNA} = 1\%$. This is a minimum quantum yield for reaction of the DNA, since not all products of oxidative damage at guanine lead to strand cleavage when treated with piperidine.[75,76] Nevertheless, the efficiency of reaction for the AQ-linked compound is about 200 000 times greater than that reported for the Rh metallointercalator-linked compounds.[29,30]

Discussion

The central issue exposed in this investigation is a discrepancy between the report that irradiation of a remote Rh metallointercalator quantitatively repairs thymine dimers and our observation that there is no measurable repair when an excited anthraquinone derivative is the remote one-electron oxidant. In fact, absence of dimer repair was observed by Barton when a Ru metallointercalator was substituted for the Rh compound.[30] This behavior was attributed to the difference in oxidizing power between the Ru and Rh metallointercalators. Oxidation of the thymine dimer was calculated to be exothermic for the Rh compound and endothermic for the Ru compound. This explanation

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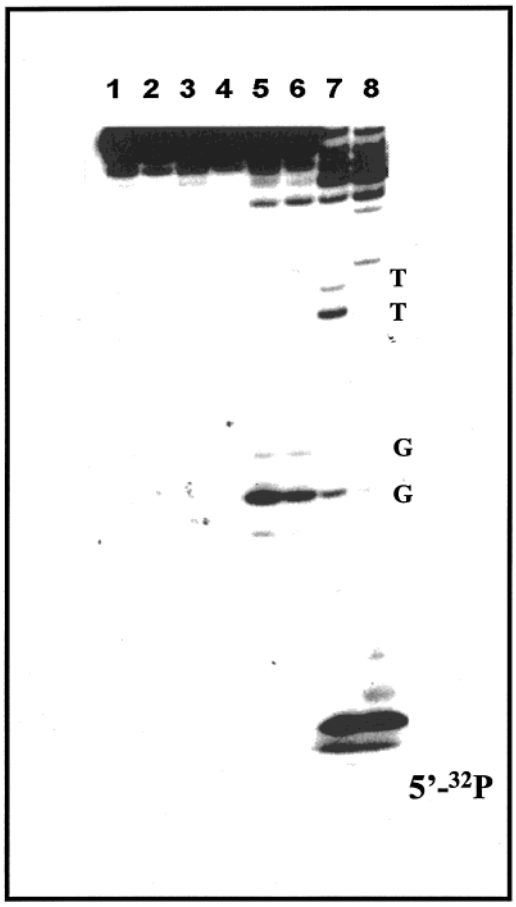

Figure 5. Autoradiogram from UV irradiation of AQ-DNA(1)/DNA-(2,3). Samples are 8 μM in duplex DNA in a phosphate buffer solution and were irradiated at 350 nm for 30 min (where indicated) at 30 °C. All samples were piperidine-treated, the samples in lanes 7 and 8 were thermally denatured at 90 °C prior to reaction with $KMnO_4$ and treatment with piperidine: Lane 1, AQ-DNA(1)/TT-DNA(2) dark control; lane 2, AQ-DNA(1)/T<>T-DNA(3) dark control; lane 3, DNA(1)/TT-DNA(2) light control; lane 4, DNA(1)/T<>T-DNA(3), light control; lane 5 is AQ-DNA(1)/TT-DNA(2) and lane 6 is AQ-DNA(1)/T<>T-DNA(3) irradiated for 30 min; lane 7 is the same as lane 5 but treated with $KMnO_4$; lane 8 is the same as lane 6 but treated with $KMnO_4$.

cannot accommodate the absence of repair for the linked anthraquinone derivative.

It has been established experimentally that a radical cation introduced at one location in DNA will migrate and cause oxidative reactions at remote guanines.[41,46−48,52,53,77] The DNA-linked anthraquinone derivatives we are examining are particularly well-suited for investigation of this process. Irradiation of the AQ group generates a singlet excited state that rapidly intersystem crosses to the triplet. The triplet state of the anthraquinone is a powerful one-electron oxidant. The anthraquinone group has a measured triplet energy (E_T) of 2.76 eV[78] and a ground-state reduction potential (E_{red}) of −0.58 V vs SCE,[79] which gives a calculated E_{red} for the triplet state AQ equal to 2.18 V vs SCE.[80] Application of the Rehm−Weller equation[81] indicates that the triplet anthraquinone has sufficient oxidizing power to convert any of the four DNA bases to their radical cation in an exothermic reaction.[82] The anthraquinone

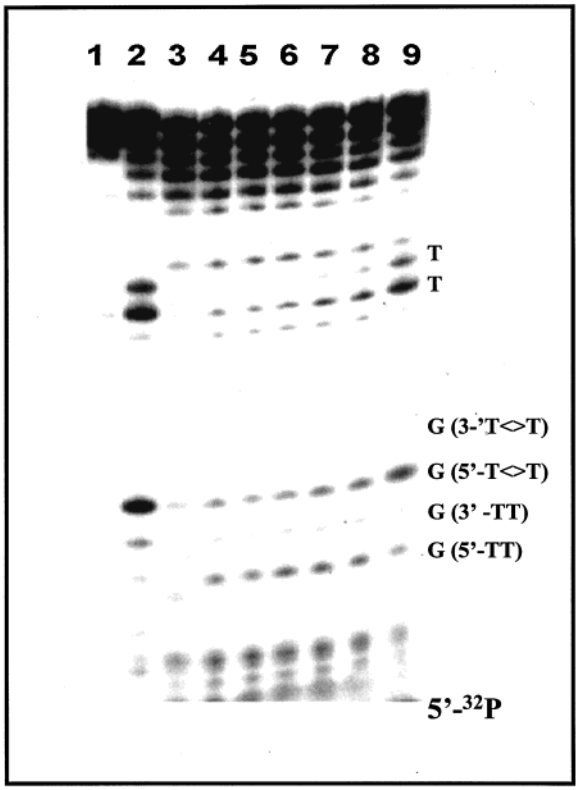

Figure 6. Autoradiogram revealing the detection limit of thymine dimer repair using authentic mixtures of AQ-DNA(1)/TT-DNA(2) and AQ-DNA(1)/T<>T-DNA(3). Samples were treated with $KMnO_4$ and piperidine. Lane 1, 100% DNA(1)/T<>T-DNA(3) (light control); lane 2: 100% AQ-DNA(1)/TT-DNA(2); lanes 3−9: are mixtures of AQ-DNA(1)/T<>T-DNA(3) and AQ-DNA(1)/TT-DNA(2) in the following proportions: lane 3, 100: 0; lane 4, 99:1, lane 5, 97:3, lane 6, 95:5, lane 7, 90:10, lane 8, 83:17, lane 9, 50:50.

radical anion formed in this reaction is converted back to the starting AQ group by reaction with O_2, which, in turn, is converted to superoxide ($O_2^{-\bullet}$).[78] This process leaves a radical cation in the DNA with no partner for charge annihilation. This radical cation migrates through the duplex DNA by the process we identified as phonon-assisted polaron hopping and causes oxidative damage at GG steps.[47,48]

Significantly, the triplet anthraquinone group is a strong enough oxidant to oxidize the *cis*−*syn*-thymine dimer. Empirical observations support this assertion. In particular, we showed that irradiation of a noncovalently linked anthraquinone initiates dimer repair through a radical cation intermediate. The oxidation potential of the *cis*−*syn*-thymine dimer in DNA is unknown. Pac and co-workers[10] report that cyclic voltammetry reveals a peak in the oxidation wave at 1.69 V vs SCE for the *cis*−*syn*-dimer of *N*,*N*′-dimethythymine in CH_3CN solution. This value is not the standard oxidation potential of the thymine dimer, as it is purported to be,[30] nor can it reliably be converted to that value. Considering the likely effect of the *N*-methyl substituents and the irreversibility of the observed oxidation wave, it can be concluded only that E_{ox} for the thymine dimer is >1.69 V vs SCE.

It is revealing to compare reduction potentials of the covalently linked oxidants that have been used to examine

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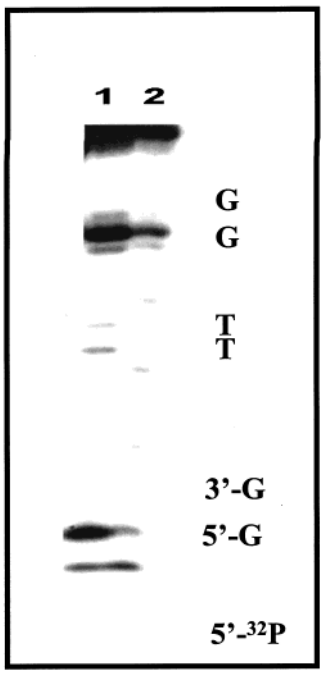

Figure 7. Autoradiogram from irradiation of AQ-DNA(4)/TT-DNA-(5) and AQ-DNA(4)/T<>T-DNA(6)at 350 nm (~30 °C) for 1 h. Samples contained duplex oligonucleotides (8.0 μM) in sodium phosphate buffer (pH 7.0, 16.0 mM). Samples in lanes 1 and 2 were thermally denatured at 90 °C before treatment with $KMnO_4$ and reaction with piperidine.

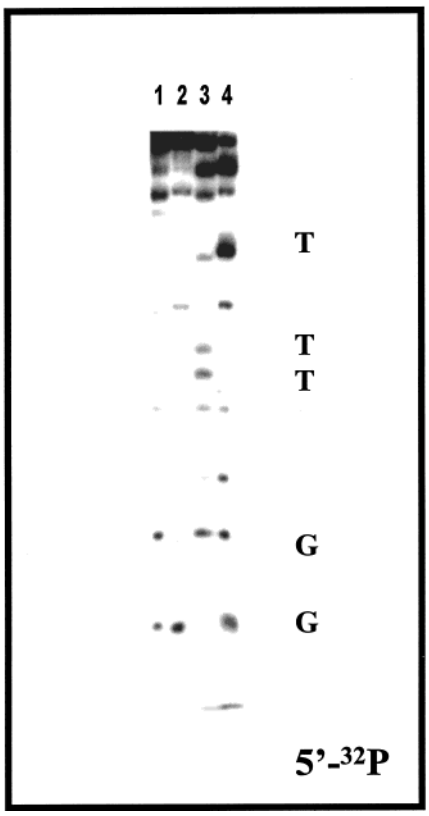

Figure 8. Autoradiogram from UV irradiation of AQ-DNA(7)/DNA-(8,9). Samples are 8 μM in duplex DNA in a phosphate buffer solution (16.0 mM, pH = 7.0) and were irradiated at 350 nm for 3 h at 30 °C. Sample lanes 3 and 4 were thermally denatured at 90 °C before treatment with $KMnO_4$. All lanes are piperidine-treated. Lane 1, AQ-DNA(7)/TT-DNA(8), no $KMnO_4$ treatment; lane 2, AQ-DNA(7)/T<>TDNA(9), no $KMnO_4$ treatment; lane 3, AQ-DNA(7)/TT-DNA-(8), $KMnO_4$-treated; lane 4, AQ-DNA(7)/T<>TDNA(9), $KMnO_4$-treated.

remote repair of the thymine dimer with E_{ox} of the dimer. It is reported that photoexcitation of $Rh(phi)_2(bpy)^{+3}$ generates an interligand charge-transfer state with an E_{red} estimated to be ~1.76 V vs SCE.[30,83] The E_{red} of the tethered Ru(phen)(bpy′)-$(dppz)^{3+}$ complex is reported to be ~1.4 V vs SCE.[30] Thus, the reduction potentials of the Rh and Ru metallointercalators could bracket the E_{ox} of the thymine dimer, which leads to the reasonable expectation that the Rh compound can initiate repair by one-electron oxidation and the Ru compound cannot.[30] The E_{red} of the excited tethered anthraquinone derivative is 2.18 V vs SCE, which is above that of excited $Rh(phi)_2(bpy')^{+3}$, and, therefore, the triplet AQ is certainly able to oxidize the thymine dimer if excited $Rh(phi)_2(bpy')^{+3}$ can. Thus, the discrepancy between our findings and those previously reported cannot be attributed to the inability of the linked anthraquinone to oxidize the thymine dimer.

It is informative to compare the E_{ox} of the thymine dimer with that of guanine. The oxidation potentials of the DNA bases in DNA are not known, and these values will surely be different from those that have been determined for free nucleosides in solution. Steenken and Jovanovic[82] determined E_{ox} of guanosine in H_2O at pH = 7 to be 1.34 V vs SCE. It is unreasonable to expect that incorporating guanosine in DNA will cause its E_{ox} to increase to a greater extent than does incorporating the thymine dimer into DNA. Consequently, we expect that the E_{ox} of guanine will be at least 0.35 V below that of the thymine dimer. Therefore, simply based on an expected Boltzman distribution, it is not likely that there will be a significant population of thymine dimer radical cations in a DNA sequence that contains guanines. This conclusion is consistent with the analysis of long range repair in DNA recently presented by Giese, Jortner and co-workers[84] and with our finding of immeasurably small remote dimer repair even when the reaction at guanine has reached completion.

The results reported above indicate that for the AQ-DNA-(4)/DNA(5,6) system, replacement of the TT pair with a *cis*−*syn*-thymine dimer causes only a very modest reduction in the efficiency of strand cleavage at the 5′-G of GG_2, which follows the dimer in the duplex. This finding should be compared with the previous report that this change results in a 2-fold reduction in the efficiency of cleavage at a following GG step.[29,30] The difference between these results may be attributed to sequence effects, since the DNA duplexes examined are different. But, since the quantum yields of the reactions induced by irradiation of the Rh metallointercalators are so small, the difference may be attributed to factors that cannot be specified precisely.

The modest reduction in relative reaction efficiency at GG_2 seen with the thymine dimer in the duplex is consistent with our previous work, which showed that introduction of an abasic site before a remote GG step does not cause a measurable reduction in cleavage at its 5′-G.[46] This may be a consequence of an extrahelical conformation for the thymine dimer and collapse of the DNA around the gap, as has been suggested.[30] Inter-strand hopping of radical cations migrating in DNA has also been observed,[41,48] and it is possible that the radical cation migrates through the AA sequence regardless of whether the complementary strand contains a TT or a thymine dimer.

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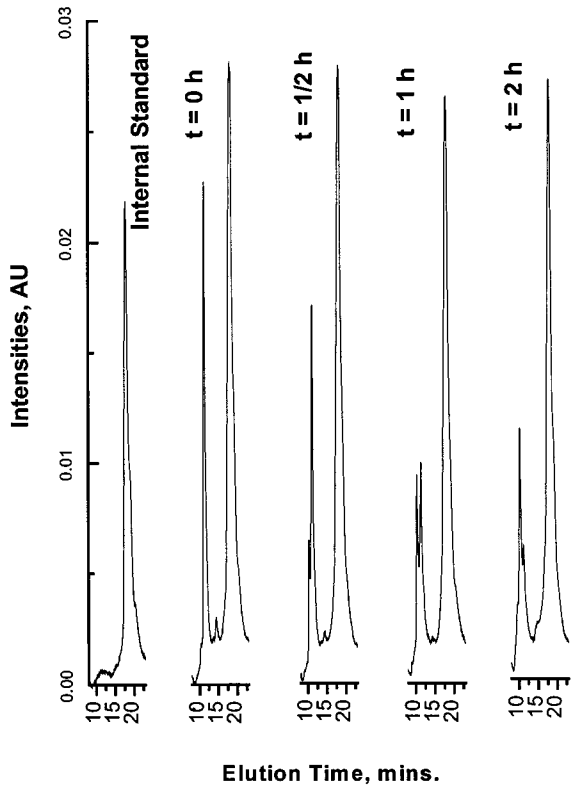

Figure 9. Reverse-phase HPLC traces of AQ-DNA(7)/TT-DNA(8) after irradiation at 350 nm for: 0, 0.5, 1, and 2 h. All samples were treated with piperidine, and then AQ-DNA(1) was added as an internal standard before analysis at 65 °C by HPLC. The sample under investigation, TT-DNA(8), elutes at 11.3 min, and the internal standard, at 18.1 min. The AQ-containing strand, AQ-DNA(7), elutes at 32.3 min and does not interfere with the analysis.

It is not possible to be definitive about the cause of the discrepancy between our finding of negligible thymine dimer repair compared with oxidative damage at guanine and the previous report that the former is 40 times more efficient than the latter. One clear difference between these systems is the nature of the initial oxidant. However, once a radical cation has been introduced into the DNA, its behavior should be independent of the initial oxidant. A second difference between the systems examined is in the measured quantum yields. The mechanistic interpretation of reactions having very low quantum yields is challenging because of the possible intervention of unanticipated minor pathways.

Conclusions

Injection of a radical cation into duplex DNA from a covalently linked AQ yields immeasurably little repair of *cis*−*syn*-thymine dimers compared with oxidative damage caused at the 5′-G of remote GG steps. This finding is in contrast to reports that irradiation of a linked Rh metallointercalator causes thymine dimer repair 40 times more efficiently than damage at remote guanines.

Experimental Section

Materials, Instrumentation, and Methods. Preparation and Purification of DNA Containing AQ-Modified and Thymine Photodimer Oligomers. [γ-^{32}P]ATP radioactive isotope was purchased from Amersham Bioscience. T4 polynucleotide kinase was purchased from Pharmacia Biotec and stored at −20 °C. Unmodified DNA oligomers (both gel filtration and HPLC grades) and AQ containing complementary oligomers (HPLC grade) were synthesized as described elsewhere on an Applied Biosystems DNA synthesizer[46] or purchased from Midland Certified Reagent Company. The *cis*−*syn*-thymine dimer was synthesized, purified, and characterized as previously described.[55] The thymine dimer was site-specifically incorporated into DNA oligomers by using standard solid-phase automated DNA synthesis procedures. The extinction coefficients of the oligomers were calculated using the Biopolymer Calculator Online, and the absorbance was measured at 260 nm. The concentrations of anthraquinone-modified oligomer solutions were determined the same way as that of the unmodified oligomers except that the anthraquinone was replaced with adenine in the extinction coefficient determination. A value of zero was substituted for the thymine dimer in calculating extinction coefficients for the thymine dimer-containing oligomers. Reverse-phase HPLC was performed on a Hitachi system using a Microsorb-MV C18 reversed-phase column (4.6 mm i.d. × 25 cm length, 300 Å) from Rainin with an oven temperature maintained at 65 °C. Normal-phase HPLC was performed on a Hypersil normal-phase silica gel column (4.6 mm i.d. × 25 cm length, 5.0 μm) from Phenomenex. HPLC/atmospheric-pressure electron impact ionization mass spectrometry (HPLC/APCI-MS, positive and negative mode) of the thymidines was performed at the School of Chemistry and Biochemistry of Georgia Tech, Atlanta. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry of the oligomer strands was performed at the Midland Certified Reagent Company. All oligonucleotides gave the expected mass spectrum. UV melting and cooling curves were recorded on a Cary 1E spectrophotometer equipped with a multicell block, temperature controller, and sample transport accessory.

Melting Temperature (T_m), Thermal Denaturation Measurements. Samples consisted of equimolar concentrations of DNA oligomers (2.5 μM) in sodium phosphate buffer (1.0 mL, 10.0 mM, pH 7.0). Samples were placed in cuvettes (1.5 mL capacity, 1.0 cm path length) and sealed with tape to prevent evaporation of water during heating/cooling cycles. The absorbance of the samples was measured at 260 nm as a function of temperature for four consecutive runs: heating from 20 to 90 °C at the rate of 0.5 °C/min and then cooling followed by reheating and cooling at 0.5 °C/min. Data obtained from cooling curves were found to be the same as those obtained from heating. T_m, the temperature at which half of the molecules are hybridized, was obtained as a first-order derivative using Microcal Origin software.

Cleavage Analysis by Radiolabeling and Polyacrylamide Gel Electrophoresis (PAGE). DNA oligonucleotides were radiolabeled at the 5′-end. 5′-OH labeling involved the use of [γ-^{32}P]ATP and bacterial T4 poynucleotide kinase. The labeling was performed according to standard procedures. Radiolabeled DNA was purified by 20% PAGE. Samples for irradiation were prepared by hybridizing a mixture of "cold" (5.0 or 8.0 μM) and radiolabeled (10 000 cpm) oligonucleotides with AQ- or non-AQ complementary strands (5.0 or 8.0 μM) in sodium phosphate buffer (pH = 7.0) and water (to a total volume of 20.0 μL each). Hybridization was achieved by heating the samples at 90 °C for 5 min, followed by slow cooling to room temperature overnight. Samples were irradiated in microcentrifuge tubes in a Rayonet photoreactor (Southern New England Ultraviolet Company, Barnsford, CT) equipped with 8 × 350 nm lamps at 30 °C. After irradiation, the samples were precipitated once with cold ethanol (100 μL) in the presence of glycogen (0.5 μL), washed with 80% ethanol (100 μL), dried (speedvac, low heat) and treated with piperidine (100 μL, 1 M solution) at 90 °C for 30 min. After evaporation of the piperidine (speedvac, medium heat), lypholization twice with water (20 μL), and suspension in denaturing loading buffer, the samples (3000 cpm) were electrophoresed on a 20% 19:1 acrylamide:bis-acrylamide gel containing urea (7 M) at 1500 V for 3 h. The gels were dried, and the cleavage sites were visualized by autoradiography. Quantitation of cleavage bands was performed on a phosphorimager.

Analysis of *cis*−*syn*-Thymine Photodimer Repair by Photolyase. The thymine dimer oligonucleotides were labeled with ^{32}P at the 5′-end using standard techniques as described above. A "cold" (5.0 or 8.0 μM) and radiolabeled (10 000 cpm) oligonucleotide were mixed with AQ- or non-AQ complementary DNA (5.0 or 8.0 μM) in phosphate buffer (2.0 or 3.2 μL, 100 mM solution) containing enzyme assay buffer (50 mM Tris-HCl, 10 mM NaCl, 1.7 mM DTT, and 1 mM EDTA at

pH 7.4),[86,87] to give a total volume of 20 μL. Hybridization was carried out by heating the sample to 90 °C and then allowing it to cool to room temperature overnight. Photolyase (2.0 μL, 1.0 μM solution) was added to the sample, which was then incubated in the dark for 30 min to ensure complex formation and then irradiated for 10 min in a Rayonet photoreactor (8 × 350 nm lamps) light source at ∼15−20 °C. Denaturation was accomplished by adding cold oligomer (1.0 μL, 100 μM solution) and heating at 90 °C for 10 min followed by cooling on ice.

Sequencing by $KMnO_4$. The T sequencing by means of $KMnO_4$ was a modified version of the standard procedure.[56] Oligomer samples (5.0 or 8.0 μM) were added to calf thymus DNA (1.0 μL, 0.5 mM), phosphate buffer (2.0 or 3.2 μL, 100 mM solution), and water (to a 20 μL total volume), and were mixed by vortexing for 15 s and then centrifuged for 10 s at 12 000 rpm. A freshly prepared solution of $KMnO_4$ (0.5 μL, 0.5 M or 1.0 μL, 20 mM) was added to the samples. The reaction proceeded for 45 s and was then quenched by adding DNA precipitating buffer. The precipitated DNA was washed with 80% ethanol, dried, and subjected to piperidine treatment (100 μL of 1 M piperidine for 1 h at 90 °C). Loading buffer was added to the samples, and the samples were analyzed by 20% PAGE (19:1 acrylamide: bisacrylamide), followed by autoradiography.

Measurement of the Limit of Detection for TT-repair in T<>T-DNA(9). Samples of duplex DNA were prepared by combining unlabeled (5.0 or 8.0 μM) and radiolabeled (10 000 cpm) TT-DNA(8) or T<>T-DNA(9) with their complementary strand (5.0 or 8.0 μM) AQ-DNA(7) in phosphate buffer solution (2.0 or 3.2 μL, 100 mM), to a final volume of 20 μL. The samples were annealed by heating to 90 °C and then cooled to room temperature overnight. The duplex DNA samples were irradiated in a Rayonet photoreactor (8 × 350 nm lamps) for 30 min at ∼20 °C. Denaturation was accomplished by adding unlabeled oligomer (1.0 μL, 100 μM solution) and heating at 90 °C for 10 min followed by cooling on ice. A freshly prepared solution of $KMnO_4$ (1.0 μL, 20 mM) was immediately added to the irradiated, denatured samples. After 45 s, the reaction was quenched by addition of DNA precipitating buffer. The precipitated DNA was washed with 80% ethanol, dried, and treated with piperidine (100 μL of 1 M for 1 h at 90 °C). Loading buffer was added to the samples, and they were combined volumetrically to produce mixtures (3000 cpm each) of TT and thymine photodimer in amounts: 100% TT, 100% T<>T, 99% T<>T, 97% T<>T, 95% T<>T, 90% T<>T, 83.3% T<>T to 50% T<>T. The amount of TT-DNA(8) in each mixture was determined by 20% PAGE (19:1 acrylamide:bisacrylamide), and visualized by autoradiography and quantitated by counting the radioactivity with a phosphorimager.

Determination of Quantum Yield for Guanine Damage. The light flux of the Rayonet photoreactor was determined by using sodium 9,10-anthraquinone-2,6-disulfonate (AQDS(2,6)) actinometry at pH 13.5.[74] in triplicate before each experiment. The actinometer solutions had an optical density at 330 nm of ∼0.5 and were degassed by the freeze−pump−thaw technique at high vacuum. The extent of reaction of the actinometer was monitored by UV spectroscopy at various time intervals. Conversion was kept below 50%, where the extent of reaction was linear with irradiation time. The slope of a plot of extent reaction vs irradiation time yielded a light flux = $1.02 \pm 0.1 \times 10^{-9}$ Einstein/min. The actinometer system was shown to be linear for AQDS(2,6) concentrations from 9.2 to 92 μM.

AQ-DNA(7)/TT-DNA(8) samples (9.2 μM in air-saturated phosphate buffer solution) were irradiated in a 1.0-cm path length UV cell in the calibrated Rayonet photoreactor at ∼30 °C. Aliquots were withdrawn and treated with piperidine at various time intervals. After evaporation of the piperidine, the sample was lyophilized with water (2 × 20 μL) and then suspended in water. A unique DNA oligomer for use as an internal standard was added to the sample, and its total volume was adjusted to 25 μL. This mixture was analyzed by HPLC on a C-18 reverse-phase column under denaturing conditions (65 °C) using a gradient of 20 mM NH_4Ac/CH_3CN. The extent of reaction of the TT-DNA(8) was monitored and found to be linear with irradiation time. The quantum yield of disappearance of TT-DNA(8) was determined at 50% reaction.

Methyl Anthraquinone Carboxylate AQC(OMe) Sensitized Repair of *cis,syn*-Thymine Dimer A N_2-purged solution of *cis,syn*-thymine dimer (**1**, 4.04 mmol) and AQC(OMe) (5.0 μmol) in dry acetonitrile (1 mL) was irradiated at 350 nm (Rayonet reactor, 8 lamps). The extent of reaction was monitored by normal-phase HPLC with 2-deoxycytidine as internal standard (gradient: 13 to 30% MeOH in EtOAc, flow rate = 1.0 mL/min). The identity of the peak assigned to thymine monomer **2** was established by UV spectroscopy, co-injection, and by HPLC/APCI-MS mass spectrometry.

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Supporting Information Available: Tables showing T_m and detection limit of TT step (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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