Thymine dimer repair by electron transfer from photo-excited 2′,3′,5′-tri-O-acetyl-8-oxo-7,8-dihydroguanosine or 2′,3′,5′-tri-O-acetyl-ribosyluric acid- a theoretical study

Marzena Marchaj[1], Iwona Sieradzan[1], Iwona Anusiewicz[1], Piotr Skurski[1,2], Jack Simons[2,*]

[1] *Department of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland*

[2] *Chemistry Department and Henry Eyring Center for Theoretical Chemistry, University of Utah, Salt Lake City, Utah 84112, U.S.A.*

Abstract

Electronic structure calculations are combined with published experimental data from another laboratory to interpret trends in the rates of thymine dimer repair induced by photo-exciting the title molecules or their deprotonated derivatives. Opening of the thymine dimer's cyclobutane ring is believed to be initiated by electron transfer from the photo-excited molecule and to then pass over thermally accessible energy barriers. Therefore, the repair rates are determined by rates of accessing activation barriers connecting the photo-excited state to the electron-transferred state. These barriers are shown to depend on the electronic excitation energy and electron binding energy of the donor and the electron affinity of the thymine dimer acceptor. For neutral donors, the barriers also depend on the distance between the donor and the thymine dimer through a screened Coulomb interaction between the donor cation and acceptor anion. For the deprotonated (anionic) donors, this Coulomb-derived distance dependence is absent. For both neutral and anionic donors, the range for electron transfer is spatially limited by the strength of the electronic couplings. The model put forth here rationalizes why anionic donors can be expected to perform better than neutrals and offers a framework for designing electron transfer agents optimal for a given electron acceptor.

* Corresponding author: simons@chem.utah.edu

1. Introduction

We recently offered (1) a mechanistic rationalization for experimental data (2) related to the rate at which electrons injected from intra-DNA photo-excited 8-oxo-7,8-dihydroguanine (denoted OGH and shown in Figure 1) into a thymine dimer (denoted T=T and also shown in Figure 1) can induce opening of the cyclobutane ring in T=T and convert the dimer into two separated and intact thymine units (denoted T + T). As discussed in considerable detail in refs. 1 and 2, thymine dimers can be formed in DNA by exposure to ultraviolet light and pose danger as an initial stage in certain skin cancers. It is thus of much interest to identify and characterize species that can repair T=T damage by converting T=T into T + T.

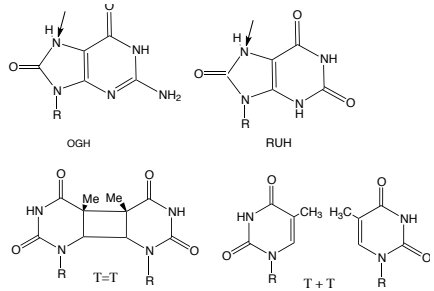

Figure 1. Structures of the OGH, T=T, and T + T units as well as of a species denoted RUH (see later in the text for details). The symbol R denotes where the OGH, T=T or T is bound to a sugar when this species is inserted into a DNA oligomer as in refs. 1 and 2. When nucleosides based on OGH or RUH are used in solution, as discussed in the present work, R = 2',3',5'-tri-O-acetylribofuranosyl, and R = H for the solution-phase T=T.

The cyclobutane ring-opening reaction is a [2+2] cyclo-opening and is known to be Woodward-Hoffmann forbidden on the electronic ground-state surface. However, as discussed in ref. 1, the symmetry-imposed barrier is removed when an electron is added to a π^* orbital of the T=T unit, as a result of which the anion-surface reaction

$$T = T^- \rightarrow T + T^- \qquad (1)$$

can occur with only a very small reaction barrier (in ref. 1, this barrier was estimated to be < 1.7 kcal mol^{-1}). Because the ring opening is so facile once the electron attaches to T=T, it is believed that the rate of electron transfer from the photo-excited donor to T=T is what governs the overall reaction yields.

In the experiments of ref. 2, an OGH unit was inserted into a DNA double strand oligomer containing one T=T damage site; an example is shown in Figure 2.

5′-CACAGCAT=TACAGTACAC-3′ **18mer**
3′-TCTGTGTCGOA ATGTCATGTGT-5′ **22mer**

Figure 2. An example of the T=T containing double-strand oligomers used in ref. 2 (taken from ref. 1). The symbol O is used here to label the OGH unit.

In a series of experiments, an OGH was inserted at various locations within the 18mer or the 22mer in Figure 2. Each such sample was subjected to ultraviolet photons having energy < 4.1 eV (this is an energy range within which neither DNA's bases nor T=T absorb) for a time duration t after which the sample was subjected to HPLC analysis to determine what fraction of the sample remained T=T damaged and what fraction had been repaired. Analysis of the fraction of repair as a function of time displayed first-order kinetics behavior with rate constants in the range of $1x10^{-2}$ min^{-1} at 22 °C. The rate of T=T repair was found to depend upon (i) whether the OGH is in the same strand as or in the strand opposite the T=T, (ii) whether the OGH is to the 3' side of the T=T or toward the 5' side of the T=T, and (iii) how many bases separate the T=T from the OGH. A sample of the T=T rate data is shown in Figure 3 for several locations of the OGH. Explaining how and why these repair rates depend on the location of the OGH relative to the T=T was the primary focus of ref. 1

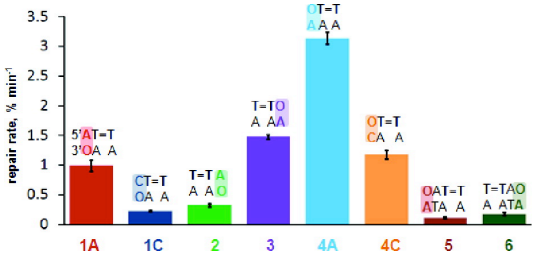

Figure 3. T=T ring opening rates (in % per min) for eight locations of the OGH unit (labeled O in this figure to avoid confusing OGH with the DNA base G) within the sequence shown in Figure 2 (taken from Figure 1 in ref. 1)

A primary conclusion of ref. 1 was that the Coulomb stabilization of the OGH^+ …$T=T^-$ ion pair formed when the photo-excited OGH (denoted OGH*) transfers an electron to the proximal T=T plays an important role in the energetics of the electron-transfer reaction. In particular, it was shown in ref. 1 that, in the absence of Coulomb stabilization, the reaction

$$OGH^*...T=T \rightarrow OGH^+...T=T^- \qquad (2)$$

although exothermic, has a Marcus activation barrier that is far too large to be consistent with ref. 2's observed rates of ring opening. However, after including the Coulomb stabilization energy, which depends on the distance R between the OGH and T=T units and hence on the position of the OGH within the oligomer of Figure 2, we were able to show in ref. 1 that the pattern of ring-opening rates illustrated in Figure 3 can be interpreted in terms of how the Marcus activation energies and the donor-acceptor coupling strengths vary with R. We showed that only for $R \leq ca.6\text{Å}$ is the Coulomb stabilization sufficiently strong to produce a thermally accessible Marcus activation barrier and that, for R-values in this range, the rates decay with R in a manner consistent with exp(-βR) as expected in electron-transfer processes (a value of β = 0.6 $Å^{-1}$ was inferred).

In the present work, we turn our attention to interpreting a subsequent related series of experiments (3) from the Burrows group. In those experiments a solution containing 0.2 mM T=T and 0.2 mM of an OGH or RUH based nucleoside or both (as explained in Figure 1, OGH and RUH are then 2′,3′,5′-tri-O-acetyl-8-oxo-7,8-dihydroguanosine 2′,3′,5′-tri-O-acetyl-ribosyluric acid, respectively) in an aqueous buffer solution whose pH could be controlled was used to generate the reactive T=T, OGH, and RUH species. These solutions were again subjected to ultraviolet photons having energy < 4.1 eV (where both OGH and RUH are known to have ππ* absorptions) at $22^{\circ}C$ for a length of time t, after which the yield of ring opening was determined by HPLC.

In Figure 4 we show the T=T repair yield for four samples as functions of the time over which the ultraviolet radiation is applied. One sample contains only the T=T species described above. The other three samples contained either the OGH or RUH nucleoside described above or a nucleoside containing the DNA base guanine (G) in place of the OGH or RUH. In all cases, the pH was held fixed at 7.0.

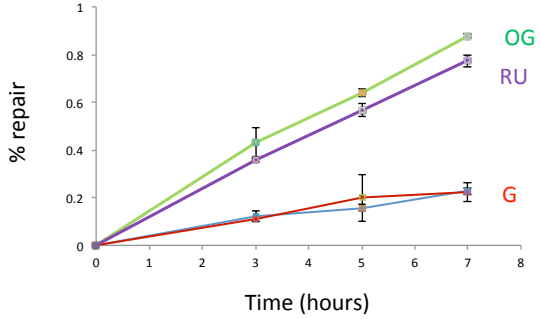

Figure 4. Yield of T=T repair as a function of ultraviolet light exposure time for four solutions containing T=T at pH = 7. The species labeled OG and RU in this figure are what we call OGH and RUH, respectively (provided by authors of refs. 2 and 3).

The first thing to notice is that the presence of guanine has no effect above background (i.e., results for T=T alone). This is not surprising since G is known to not absorb photons in the energy range < 4.1 eV. In addition, we note that both the OGH and RUH nucleosides enhance the T=T ring opening yields and by similar amounts, but with OGH being slightly more efficient. The latter fact was said to be surprising in ref. 3 because RUH was claimed by those authors to be slightly easier to oxidize than OGH and, because the ring opening is believed to require an electron transfer from OGH or RUH to T=T, these energetic factors would favor RUH over OGH. However, as we show later, our results suggest that RUH is actually more difficult to oxidize than OGH.

Next, we note that there appear to be orders of magnitude differences between the T=T repair yields in Figure 4 and those obtained in ref. 2 where the OGH and T=T units are constrained by their presence within the DNA duplex to distances of 3-6 Å. The highest yield (0.8% in 7 h) in Figure 4 corresponds to ca. $2x10^{-3}$ % min^{-1}, while the higher yields reported in ref. 2 were ca. 3 % min^{-1}, 700 times higher. To determine whether these differences likely derive from the fact that the OGH and T=T units are spatially unconstrained in the solution phase experiments of ref. 3 while, as noted above, they lie within ca. 6 Å in the DNA oligomer experiments of ref. 2, we offer the following analysis.

A concentration of 0.2mM for OGH or RUH corresponds to a nucleoside density of ca. $1.2x10^{-7}$ nucleotides per $Å^3$. Based on the results of refs. 1 and 2, we assume that the photo-initiated ring-opening reaction can occur only if the T=T and nucleoside are within 6 Å. We can estimate the number of nucleosides within 6 Å of any given T=T in solution to be $\frac{4}{3}\pi 6_{Å}^{3} x1.2x10^{-7}\,Å^{-3} = 1x10^{-4}$ nucleosides. In contrast to this solution-phase donor-acceptor pair density ρ, in the DNA oligomer experiments of ref. 2, the pair density ρ is unity (i.e., there is one donor within 6 Å of each T=T). Based on these density differences alone, we would expect the yield of T=T repair in the DNA oligomers to be 10^4 times that observed in the solution-phase experiments or ca.10^4 x $2x10^{-3}$ % min^{-1} = 20 % min^{-1}. In fact, the measured T=T repair yields in DNA are ca. 3 % min^{-1}, the main point being that the observed yields in DNA are <u>not</u> higher than one would expect if the only difference between the oligomer and solution-phase experiments were the density of donor-acceptor pairs. In the DNA experiments, the location of OGH relative to T=T within the oligomer governs the repair yield, and the Coulomb stabilization energy limits the OGH-to-T=T distance to within ca. 6 Å for the electron transfer to have a thermally accessible Marcus activation energy. In the solution-phase experiments, the concentration of the OGH or RUH in the solution determines the fraction of T=T species that have an OGH or RUH within ca. 6 Å which then determines the repair yields shown in Figure 4.

So, we believe the data suggest that the photo-induced electron transfer mechanisms operative in the experiments of refs. 2 and 3 are identical; only the local densities of donor-acceptor pairs are different. This interpretation of the yields observed in ref. 3 could be tested by repeating the experiments with different concentrations of OGH or RUH; the mechanism just proposed would predict yields that vary linearly with this concentration.

After finding that photo-excited OGH and RUH appear to induce T=T ring opening, the workers in ref. 3 carried out another series of experiments in which OGH,

RUH, and T=T concentrations and photon energies and intensities identical to those detailed earlier were used. However, in these experiments, the pH of the buffer solution was varied from 5 to 9 to explore the possibility that deprotonation of the OGH or RUH nucleoside (probably at the positions labeled with arrows in Figure 1 to generate anionic species we denote by OG^- or RU^-) would affect the photo-initiated T=T ring opening rate. Because the pK_a values of OGH and RUH are 8.6 and 6, respectively (see ref. 3), one would expect to observe significant changes in the ring-opening rates at pH values in these ranges if the anionic reagents behave qualitatively different than the neutral OGH or RUH. In Figure 5 we show the T=T ring-opening rates obtained after 5 h of exposure to ultraviolet radiation at a range of pH values.

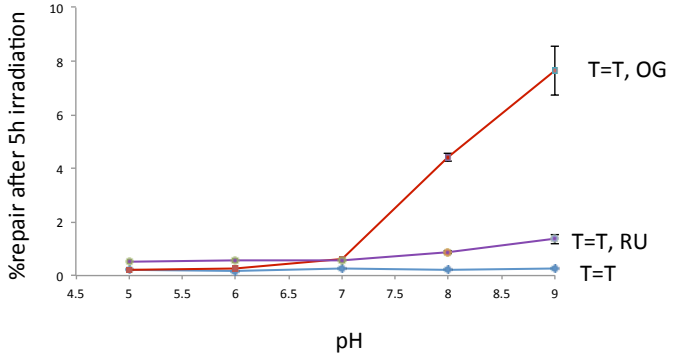

Figure 5. T=T repair rates for OGH or RUH containing samples as well as for a sample containing no OGH or RUH as functions of pH after exposure to ultraviolet light for 5 h (provided by authors of refs. 2 and 3).

The background T=T repair rate data of Figure 5 are consistent with those shown in Figure 4 as are the pH = 7 data shown for OGH and RUH. However, there are two features of the data in Figure 5 that are surprising and that constitute the focus of the present study:

1. The T=T repair rate displays very weak, if any, variation as the pH traverses the pK_a of RU. It appears that RUH (for pH values below 6) and RU^- (for pH values above 6) behave in similar ways as T=T repair agents.
2. The T=T repair rates for OGH increase considerably (by a factor of 10 or more) as one increases the pH from 7 through and beyond the pK_a = 8.6 of OGH. This suggests that the anionic OG^- is a better T=T repair agent than neutral (not deprotonated) OGH.

The reasons underlying these observations as well as comparisons between the T=T repair rates for anionic OG^- and RU^- and non-deprotonated OGH and RUH will be provided in this report. In Section 2, we detail the electronic structure methods used in the present study. In Sections 3 and 4, we present our results and summarize our findings.

2. Methods

The equilibrium structures of the anionic OG^- and RU^- and the non-deprotonated neutral OGH and RUH were determined at the Møller-Plesset (MP2) perturbation level with 6-31++G(d,p) (4,5) basis sets. Each anion's vertical electron binding energy (DE) was calculated by subtracting the anion's energy (in the presence of whatever solvation environment was being studied) from that of the corresponding radical neutral (in the presence of the same solvation environment but relaxed to accommodate the removal of the excess electron) at the equilibrium geometry of the anion. The neutral OGH and RUH vertical ionization potentials (IP) were calculated by subtracting the neutral's energy (in the presence of whatever solvation environment was being studied) from that of the corresponding radical cation (in the presence of the same solvation environment but relaxed to accommodate the removal of the electron) at the equilibrium geometry of the neutral. Adiabatic DEs and IPs were obtained in a similar manner but with the geometry of the neutral radical or radical cation optimized, respectively. The corresponding energy data for the neutral T=T and its anion $T=T^-$ were obtained using the same approach in ref. 1, and we make use of these electron affinity (EA) values here.

The electronic excitation energies for singlet states were obtained from the TD-DFT (6,7,8) technique using Becke's Three Parameter Hybrid Method with the LYP (Lee-Yang-Parr) correlation functional (B3LYP) (9,10) and 6-31++G(d,p) basis sets and at the equilibrium geometry of the absorbing species.

To approximate the effect of surrounding solvent molecules on the electronic energies of the neutral, cationic, and anionic species, we employed the polarized continuum (PCM) solvation model (11,12,13) within a self-consistent reaction field treatment, as implemented in the Gaussian09 program. From these calculations, free energies for the neutral, cation, and anion species are obtained that contain enthalpic and entropic contributions from the solvent. Hence, the DE and IP data we report later reflect these solvent thermodynamic effects.

Studies with dielectric constants ε of 1.0 (gas phase), 2.02 (cyclohexane), and 78.39 (water) were included to gain appreciation for how strongly the most important aspects of the resulting data depend on the solvation strength and to provide the data needed to determine Marcus-theory (14) thermodynamic and solvent reorganization parameters appropriate to our systems:

i. The TD-DFT electronic excitation energies were all obtained using ε = 78 because the experiments of ref. 3 were performed in dilute aqueous solutions.

ii. The OG^- and RU^- anion adiabatic DEs were obtained using ε = 78 to obtain energies appropriate for computing the adiabatic (i.e., after full solvent relaxation) energy difference between the donor states prior to and after electron transfer.

iii. The T=T acceptor's EA was obtained using ε = 2 to obtain an energy appropriate (15) for vertical (i.e., allowing the solvent's electron density to repolarize but not allowing the solvent to reorient fully) attachment of an electron to T=T. The T=T EA was obtained using ε = 78 to compute the adiabatic (i.e., after full solvent relaxation) energy difference of the T=T acceptor prior to and after electron transfer.

iv. The non-deprotonated OGH and RUH adiabatic IPs were obtained using ε = 78 to compute the adiabatic (i.e., after full solvent relaxation) energy difference between the

donor states prior to and after electron transfer. Corresponding vertical IPs obtained using ε = 2 were used to describe the vertical (i.e., allowing the solvent's electron density to repolarize but not allowing the solvent to reorient fully) removal of an electron from the donor. We need to determine both adiabatic and vertical energy differences because the Marcus theory formalism requires as input knowledge of the energies of the state after electron transfer has taken place both prior to and after full solvent relaxation.

Finally, we note that all of the calculations were performed with the Gaussian09 program (16).

3. Results

In making use of the electronic structure data obtained in this study, we need to approximate the thermochemical and solvent-reorganization energy parameters entering into the conventional Marcus model for electron transfer. We will first analyze the cases in which an electron transfers from anionic OG^- or RU^- and then make comparisons with the cases in which the electron comes from the neutral OGH or RUH.

In Figure 6, we introduce the Marcus-theory parameters and show three parabolas that qualitatively represent the energies of the ground $OG^-\ldots T=T$, photo-excited $OG^-*\ldots T=T$, and charge-transferred $OG\ldots T=T^-$ states as functions of the phenomenological solvent reorganization coordinate. We will describe the process for the case of OG^-, but the same steps are involved for RU^-.

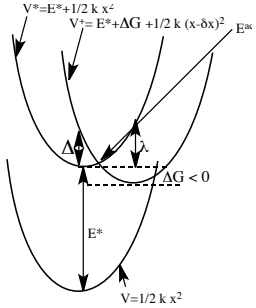

Figure 6. Qualitative depiction of the $OG^-\ldots T=T$ ground (lowest parabola), photo-excited $OG^-*\ldots T=T$ (upper left parabola), and charge-separated $OG\ldots T=T^-$ (upper right parabola) states for the photo-induced OG^- (or RU^-) to T=T electron transfer events.

The first parameter we need is the electronic excitation energy E* for which we can use either the experimental photon energy of 4.1 eV or our singlet-state TD-DFT data on the OG^- or RU^- system obtained using a solvent dielectric constant of 78, since the experiments of ref. 3 were carried out in dilute aqueous solutions. Using 4.1 eV for E* might offer a closer connection to the experimental situation. It should be stressed that

the ππ* absorption spectra of these species and their anions are quite broad (see Supplementary material of ref. 2), so photons throughout this range can access the same ππ* transitions to which our TD-DFT excitation energies relate.

To estimate the adiabatic energy of the charge-transferred state OG…T=T$^-$, we subtract the electron affinity (EA) of the T=T moiety from the adiabatic detachment energy (DE) of the OG$^-$ with both evaluated for a dielectric constant of ε = 78. In ref. 1, we evaluated the EA of T=T for ε = 78 to be 1.5 eV, and, as shown in Table 1, the ε = 78 adiabatic DE of OG$^-$ is 4.9 eV, with both the EA and DE determined at the MP2-level of theory as we do throughout this work. These data thus place the OG…T=T$^-$ state 4.9-1.5 = 3.4 eV above the ground state.

To compute ΔG, we subtract from the adiabatic energy of the charge-transferred state the electronic excitation energy, which we take to be the experimental value of 4.1 eV. This gives ΔG = 3.4 – 4.1 = - 0.7 eV as shown in Table 1 and is our estimate for the free energy change accompanying transfer of an electron from OG$^-$…T=T (in the presence of a dilute aqueous solution) to form OG…T=T$^-$ after allowing the donor, acceptor, and surrounding solvent to fully relax (i.e., to repolarize their electron densities and reorient to accommodate the electron transfer).

Table 1. Electron detachment energies[a] (DE) and singlet excitation energies E* at three solvent dielectric constants, and Marcus reorganization (λ), activation (E^{act}), and free energy change ΔG for OG$^-$ and RU$^-$ (all in eV). The experimental photon energy is hν=4.1 eV.

OG$^-$	DE	E*	Δ	ΔG	λ	E^{act}
ε =1	3.1-3.6	4.8				
ε =2	4.0-4.5	4.8				
ε =78	4.9-5.3	4.7				
		hν=4.1	0.7	-0.7	1.4	0.09
RU$^-$	DE	E*	Δ	ΔG	λ	E^{act}
ε =1	3.6-4.0	4.4				
ε =2	4.4-4.9	4.5				
ε =78	5.2-5.5	4.4				
		hν=4.1	0.9	-0.4	1.3	0.16

a. As explained earlier, these DE data reflect solvent entropic contributions as well. In each case, the first number is the adiabatic DE and the second is the vertical DE.

To determine the Δ parameter, which gives the solvent reorganization parameter λ through

$$\lambda = \Delta - \Delta G \qquad (3)$$

we need to estimate the energy of the charge-transferred state after the system (solute and solvent) have had time to undergo polarization of their electron densities but before the solute or solvent has undergone geometry relaxation and reorientation to accommodate the electron transfer. We make this estimation by subtracting from the ε = 78 vertical DE of OG$^-$ (5.3 eV) the ε = 2 EA of T=T (in ref. 1 we determined this to be 0.5 eV), which

places the charge-transferred state at 5.3 – 0.5 = 4.8 eV relative to the ground state. Again, using 4.1 eV for E*, we obtain Δ = 4.8 – 4.1 = 0.7 eV as shown in Table 1. Once Δ and ΔG are in hand, Eq. (3) is used to obtain λ, and the Marcus activation energy is calculated as

$$E^{act} = \frac{\Delta^2}{4\lambda} \qquad (4)$$

When the above approach is applied to the OG$^-$…T=T case, an activation energy of 0.09 eV is obtained. Following the same procedure for RU$^-$…T=T, an activation energy of 0.16 eV is obtained. The main reason underlying the larger activation barrier for RU$^-$ is the higher (compared to OG$^-$).

To explore whether these activation barriers are consistent with what is seen experimentally, we recall that in ref. 1 the rate of electron transfer from photo-excited OG* to T=T when both occur within a DNA duplex was estimated (17) to lie in the range of 0.001 to 0.01 ps^{-1} or 10^9 to 10^{10} s^{-1}. The rate at which the barrier on the Marcus free energy surface is accessed must, therefore, be at least this high (i.e., once the barrier is reached, the electron transfer probability can reduce the rate but can not increase it). Assuming a pre-exponential factor of ca. 10^{12} s^{-1} to characterize the frequency at which the solvent attempts to surmount a 0.09 eV to 0.16 eV barrier, we estimate the rate for accessing the barrier to be

$$Rate = 10^{12} \exp(-\frac{0.09 - 0.16eV}{RT}) s^{-1} \qquad (5)$$

At a temperature of $22^{\circ}C$, RT = 0.58 kcal mol^{-1} = 0.025 eV, and thus

$$\exp(-\frac{0.09 - 0.16eV}{RT}) = 3x10^{-2} - 2x10^{-3} \qquad (6)$$

which could produce an electron transfer rate in the range of 3 x10^{10} s^{-1} to 2x10^9 s^{-1} if reaching the barrier were the rate-limiting step (i.e., if the electronic coupling were strong enough). As stated above, the electron-transfer rates deduced from the DNA experiments of ref. 2 are in the 10^9 -10^{10} s^{-1} range, and we concluded earlier that the rates in the solution-phase experiments should be similar. We therefore conclude that activation energies of ca. 0.16 eV or less are required to achieve rates consistent with the DNA and solution-phase experimental findings and that both OG$^-$ and RU$^-$ appear to have such Marcus activation energies, with OG$^-$ having a rate ca. 10 times that of RU$^-$ as shown in Figure 5 because it has a smaller activation barrier.

The above interpretation can be tested experimentally by increasing the maximum photon energy to, for example, 4.3 eV, which would lower the Δ values of OG$^-$ and RU$^-$ to 0.5 eV and 0.7 eV, respectively. This would cause the respective rates to change to 2x10^{11} s^{-1} and 2x10^{10} s^{-1} meaning that both RU$^-$ and OG$^-$ should display enhanced T=T repair yields comparable to those shown for OG$^-$ in Figure 5. Hopefully, this prediction can be tested experimentally in the near future by increasing the photon energy and using a pH value within which RUH and OGH should be deprotonated.

The analysis just presented offers one explanation for why OGH might be capable of efficient T=T repair in pH ranges where it is deprotonated and exists as OG^- and why RUH should be less efficient in pH ranges where it exists as RU^- when 4.1 eV photons are used. However, we still need to explain why both neutral OGH and RUH are less efficient than OG^- (see Figure 5). In Table 2, we present the IP and E* data pertinent to OGH and RUH that allow us to address this issue.

Table 2. Electron ionization energies[a] (IP) and singlet excitation energies E* at three solvent dielectric constants, and Marcus reorganization (λ), activation (E^{act}), and free energy change ΔG for OGH and RUH (all in eV). The photon energy is hν=4.1.

OGH	IP	E*	Δ	ΔG	λ	E^{act}
ε =1	7.5-7.9	4.2				
ε =2	6.6-7.0	4.3				
ε =78	5.6-6.0	4.4				
		hν=4.1	2.4	0.0	2.4	0.60
RUH	IP	E*	Δ	ΔG	λ	E^{act}
ε =1	8.0-8.4	4.5				
ε =2	7.0-7.3	4.5				
ε =78	5.9-6.2	4.5				
		hν=4.1	2.7	0.3	2.4	0.76

a. As explained earlier, these IP data reflect solvent entropic contributions as well. In each case, the first number is the adiabatic IP and the second is the vertical IP.

To illustrate for OGH, we take the ε = 78 adiabatic IP of OGH and subtract the ε = 78 EA of T=T to place the charge transferred state 5.6-1.5 = 4.1 eV above the ground state after full solvent relaxation. Using the photon energy for E* gives ΔG = 4.1 – 4.1 = 0.0 eV.

To obtain Δ, we take the ε = 2 vertical IP of OGH and subtract the ε =2 EA of T=T to place the charge-transferred state 7.0 – 0.5 = 6.5 eV above the ground state. Using E* = 4.1 eV gives Δ = 6.5 – 4.1 = 2.4 eV.

A similar path is used to obtain the Δ and ΔG values for RUH. Notice that, in contrast to what was stated earlier, the IP of RUH is actually higher than that of OGH, so there is no reason to expect RUH to be a better electron donor than OGH on this basis.

The activation energies of 0.60 eV for OGH and 0.76 eV for RUH would generate barrier surmounting frequencies many orders of magnitude too small to be consistent with the observed T=T rates of Figure 4 or Figure 5. As the analysis for the anionic systems offered above indicated, activation energies below 0.16 eV are needed to achieve rates that the experimental data suggest neutral OGH or RUH are capable of. Using Eq. (4) and a λ value of 2.4 eV for OGH and RUH, we conclude that Δ values of 1.2 eV or less would be needed to achieve activation energies of 0.16 eV. However, both OGH and RUH have much larger Δ values (2.4 and 2.7, respectively as shown in Table 2).

Building on the model put forth in ref. 1, we suggest that the screened Coulomb interaction between the OGH^+ or RUH^+ cation and the $T=T^-$ anion can lower the charge-transferred state's parabola thus decreasing both Δ and ΔG. A Coulomb stabilization of 2.4 – 1.2 = 1.2 eV would be needed to lower OGH's Δ value to 1.2 eV; for RUH, a

stabilization of 2.7 – 1.2 = 1.5 eV is needed. In ref. 1, we proposed that such a considerable Coulomb stabilization could result from the weakly screened (e.g., through a local dielectric constant in the ε = 2 range) interaction (14.4eVÅ/ εR(Å)) immediately after the electron transfers from OGH or RUH to T=T. A stabilization of ca. 1.2 eV could result from the OGH^+ cation and $T=T^-$ anion being within ca. 6 Å for ε = 2; for RUH, a Coulomb stabilization of 1.5 eV requires the RUH^+ and $T=T^-$ to be within ca. 5 Å. Both of these distances are in the range (3-6 Å) over which we expect electron transfer to be facile for reasons explained earlier.

It might seem unrealistic for the solution-phase OGH^+ and $T=T^-$ ions to experience a Coulomb interaction described by a dielectric constant of 2 rather than a value closer to 78. However, we need to keep in mind that it is only the fraction of OGH and T=T that are within ca. 6 Å of one another when photon absorption occurs that are capable of effecting electron transfer; OGH and T=T that are more distant don't have sufficient Coulomb stabilization to produce Marcus activation energies near 0.16 eV nor do they have large enough electronic couplings. Much like the OGH and T=T groups bound within the DNA duplexes studied in ref. 2, solution-phase OGH and $T=T^-$ species within ca. 6 Å of one another are far from being fully solvated. They are closer to contact ion pairs than to solvent-separated ion pairs.

There is considerable precedence for using such small dielectric constants to describe interactions between charged groups that are in close proximity even when the surrounding medium is aqueous. Newton and co-workers (18) treat such situations by using three distance ranges within which separate dielectric constants apply, depending on the distance R between the charged species. At long distances, the bulk static dielectric constant is used; at very close distances, ε = 1 is applied, and at intermediate distances, the high-frequency dielectric constant is used. For water, they use a high-frequency dielectric constant of ε_∞ = 1.8; for many solvents, ε_∞ is in the 1.8-2.1 range. Along similar lines, Karplus and co-workers (19) introduced a distance-dependent dielectric constant ε(R) that (see Figure 1 in ref. 19) remains small and quite constant for R-values up to ca. 6 Å and then increases strongly. A distance-dependent dielectric constant was used earlier by Ramstein et al (20) and, as shown in Figure 1 of ref. 19, has similar R-dependence to that of ref. 19. On the basis of these earlier studies, we believe our description of the interaction between proximal OGH^+ and $T=T^-$ in terms of a screened Coulomb interaction with $\varepsilon \approx 2$ is reasonable.

Therefore, we suggest that Coulomb stabilization between the donor cation and acceptor anion lying within 5-6 Å lowers the Δ values for OGH and RUH to ca. 1.2 eV and generates activation energies in the ca. 0.16 eV range, thus producing T=T repair rates an order of magnitude lower than for anionic OG^- (whose activation energy is ca. 0.09 eV) but similar to anionic RU^- (whose activation energy is ca. 0.16 eV). This interpretation could be tested by increasing the photon energy from 4.1 eV to 4.4 eV, which would decrease the Δ values (including Coulomb stabilization) for OGH and RUH to ca. 0.9, thus lowering the activation barrier to ca. 0.08 eV and increasing the rate by an order of magnitude.

4. Summary

The electronic structure calculations performed in this study provide data that

supports the following description of the trends in the T=T repair rates and yields for the two donor species (OGH and RUH) that were studied.

i. The intrinsic rates of T=T repair as determined by the rates of electron transfer from photo-excited solution-phase OGH or RUH are quite similar to those observed when the OGH is spatially localized within a DNA duplex.

ii. The large difference in T=T repair yield over 5-7 h exposure to < 4.1 eV photons between the DNA-duplex and solution-phase experimental data derives from differences in local densities of OGH (or RUH) T=T pairs existing within ca. 6 Å of one another. In the DNA duplexes, the local densities of such pairs are much higher.

iii. Both in DNA duplexes and in solutions, screened Coulomb stabilization is needed to produce a thermally accessible Marcus activation barrier for the neutral OGH (or RUH) to T=T electron transfer. For the anionic donors, Coulomb stabilization is not operative, but accessible activation barriers can result if the detachment energy (DE) and electronic excitation energy E* provide a good energy match to the T=T acceptor's electron affinity (EA) (through $DE(donor;\varepsilon = 78) - EA(T = T;\varepsilon = 2) \approx E^*$).

iv. The rate of T=T repair for solution-phase deprotonated OGH (i.e., for OG^-) is considerably higher than for RU^- when < 4.1 eV photons are used because OG^- has a better $DE - EA \approx E^*$ match. It is predicted that using 4.3 eV photons could render RU^- as efficient as OG^- in repairing T=T damage because a smaller activation barrier would result.

v. The T=T repair rates for the anionic reagents (e.g., OG^-) can exceed those for the neutral reagents (e.g., OGH or RUH) because the former do not require (21) the donor and acceptor to be within ca. 6 Å since Coulomb stabilization is not operative for the anionic donors.

In addition to explaining the trends observed in the DNA-duplex and solution-phase data of refs. 2 and 3, the insights gained in this study provide a framework for designing new reagents to effect T=T repair. In particular, we suggest that
i. Anionic reagents are favored over neutral species because they do not require the substantial Coulomb stabilization that the neutrals do. As a result, the distances over which anionic reagents can be effective might be extended (of course, the decay of the electronic coupling strengths will also limit the anions' effective range).
ii. Anions whose detachment energy and electronic excitation energy allow for a good energy match ($DE - EA \approx E^*$) to the acceptor's electron affinity should be favorable.
iii. Although not addressed here, for repairing T=T damage within DNA, it would be beneficial to design anionic reagents having a molecular "shape" that would cause them to fit into and bind reversibly to the "kink" of DNA that occurs at the T=T site.

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17. These estimates were made as follows. The lifetime of the photo-excited OG* has not been measured but was estimated, based on comparison to lifetimes of several other bases and base pairs, to be between 1 ps and 10 ps. The fraction of photo-excited OG* species that transfer an electron to T=T was determined to be ca. 1% for the DNA oligomers with the highest T=T repair yields. From these facts, we concluded that the rate of electron transfer from OG* to T=T is between 0.001 ps^{-1} and 0.01 ps^{-1} or 10^{9} -10^{10} s^{-1}.

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21. This assumes that the electronic couplings strengths can extend the range over which electron transfer is facile a bit beyond 6 Å. In the DNA oligomers, the base spacing of ca. 3.5 Å per helical turn limits the OG-to-T=T distances to ca. 3.5, 7, 10.5, etc. Å. Using the exponential decay exp(-βR) fit inferred in ref. 1 with β =0.6 $Å^{-1}$, one predicts a falloff in electron transfer rates of $\frac{\exp(-0.6x10.5)}{\exp(-0.6x7)} = 0.12$ when the OG-to-T=T distance increases from 7 Å to 10.5 Å. This order of magnitude falloff is consistent with the decay of T=T repair yields seen in the data of ref. 2. On the other hand, for the solution-phase experiments, a decay governed by exp(-0.6R) could generate T=T repair for R-values between R = 6 Å and 8 Å at rates within the same order of magnitude as for R = 6 Å.