Model Studies of DNA Photorepair: Reduction Potentials of Thymine and Cytosine Cyclobutane Dimers Measured by Fluorescence Quenching

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Abstract: The interactions of various pyrimidines (1,3-dimethylthymine, DMT, 1,3-bis(N,N-dimethylcytosin-1-yl)propane, DMC) and their corresponding cis-syn cyclobutane dimers (DMTD and DMCD) with a series of excited-state electron donors were examined with the goal of understanding the energetics and mechanism of UV repair by DNA photolase. For each substrate there is a good correlation between the excited state oxidation potential (E_{ox}^*) and the quenching rate constant (k_q). The value for k_q increases as E_{ox}^* becomes more negative, asymptotically approaching a value that is at or below the solvent diffusion limit. These data all showed good fits to the Rehm–Weller equation. Reduction potentials for each of the substrates could be extracted from this analysis: −2.20 V (vs SCE) for DMTD; −2.14 V for DMT; −2.17 V for DMCD; and −2.16 for DMC. These values show that the initial electron transfer step in the photolyase mechanism is exergonic by ca. 10–15 kcal/mol. Thus these data support the reductive electron transfer mechanism for DNA photolyses proposed by Jorns et al. (J. Biol. Chem. 1987, 262, 486–491).

Introduction

Photoenzymes are a class of proteins that harness UV (ultraviolet) and/or visible light energy in order to effect specific chemical transformations. The best characterized example of these are the cis-syn DNA photolyases. These are monomeric proteins, found in a wide variety of organisms, that mediate the photoconversion of cis-syn pyrimidine cyclobutane dimers (eq 1). The dimers are formed as a consequence of UV light damage to the DNA molecule. The repair mechanism involves two distinct stages. The first is a light-independent binding to the damage site; the second is a light-dependent catalytic step in which the C5–C5’ and C6–C6’ carbon–carbon bonds are broken. There has been considerable interest in elucidating the detailed mechanism of the photoenzymatic repair process. Site-directed mutagenesis, substrate specificity studies, kinetic isotope competition experiments, time-resolved EPR, and laser flash photolysis have been employed. Catalytic antibodies that mimic the functions of the cis-syn pyrimidine dimer photolase have been characterized. Recently a crystal structure of the photolyase from E. coli, resolved to 2.3 Å, has been reported. While many details of the mechanism remain controversial, it is becoming increasingly clear that the splitting step (i.e. scission of the C5–C5’ and C6–C6’ bonds) is initiated by transfer of a single electron between the FADH− cofactor on the enzyme and the substrate.

Model studies indicate that the electron flow occurs from the FADH− to the dimer substrate (Scheme 1). Electron donors, such as indoles, have long been known to sensitize the splitting of thymine and uracil dimers. Recent studies have extended these observations to the cytosine dimers and cytosine–thymine heterodimers. Interestingly, pyrimidine dimer splitting reactions were discovered to occur with higher efficiency in nonpolar media. FADH2 is most effective.sets when it is in its conjugate base form (i.e. FADH−). Laser flash photolysis studies from this laboratory have been performed.

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Equation 2 shows a general scheme$^{24,25}$ for photochemical electron transfer where a sensitizer $S$ absorbs a photon and then transfers an electron to a quencher $Q$. A key consideration in evaluating any proposed photochemical electron transfer mechanism is the free energy change in the charge transfer step ($\Delta G_{ct}$). Generally speaking, photochemical electron transfer reactions occur only when the charge transfer step is either exergonic or $<5$ kcal/mol endergonic.$^{26}$ In this case charge transfer is fast enough to compete with nonradiative deactivation of the excited state sensitizer molecule. The value of $\Delta G_{ct}$ (in kcal/mol) can be determined from the oxidation potential of the donor ($E_{ox}$, in V), the reduction potential of the acceptor, ($E_{red}$, in V), the excited state energy of the sensitzers ($E_{oo}$, in kcal/mol), along with a term that accounts for desolvation and Coulombic interactions in the ion pair $q^2/e^r$ as described in eq 3.

$$\Delta G_{ct} = 23.03 \left( E_{ox} - E_{red} - \left( \frac{q^2}{e^r} \right) \right) - E_{oo} \quad (3)$$

Very little is known about the reduction potentials ($E_{red}$) of the pyrimidine dimers, or indeed even of monomeric pyrimidines. The functional groups present in these species (imido groups, enamines, etc.) are not generally considered to be electrochemically reactive. This consideration, along with a lack of knowledge about the precise nature of the enzymic chromophore, caused early workers to exclude the reductive single electron pathway.

We recently reported fluorescence quenching measurements with dimethylthymine dimer and a series of excited state electron donors having varying reduction potentials.$^{27}$ Based on this it was possible to estimate the $E_{red}$. The findings demonstrated that the proposed electron transfer step is thermodynamically feasible. This provided some quantitative support for the proposed mechanism. Here we provide a full account of the study in that preliminary communication which refines our original estimates, and extends our observations to cytosine-containing dimers.

### Results and Discussion

#### 1. Synthesis of Pyrimidine Dimers

Four substrates were employed in this study, the cis-syn cyclobutane dimer of dimethylthymine DMTD along with its monomer DMT, and a trimethylene linked cis-syn cyclobutane dimer of dimethylcytosine DMC along with its “monomeric” isomer DMC. Dimer DMTD was prepared from the irradiation of DMT frozen in ice according to the classical procedure.$^{28,29}$ Dimer DMD and monomer DMC were synthesized starting with 1,3-(1-uracilyl)-propane according our previously reported procedure.$^{19}$ Purity of samples was determined by $^1$H NMR and HPLC. The substrates used in this study are illustrated in Chart 1.

#### 2. Dimer Splitting Experiments

Previous work demonstrated that excited state electron donors photosensitize the splitting of pyrimidine dimers. Sensitzers employed include aromatic amines,$^{17,30}$ indoles,$^{31}$ tryptophan,$^{18}$ and dimethoxybenzene.$^{20,32}$ We have also demonstrated that N,N,N′-dimethylaniline sensitizes the splitting of DMTD.$^{22}$ Laser flash photolysis experiments confirmed the intermediacy of ion radical intermediates in this latter reaction.$^{22}$ Despite this earlier work it seemed worthwhile to re-examine this photochemistry using some of the sensitzers employed here to ensure that the fluorescence quenching events observed below resulted in the expected chemical reactions.

Each of the sensitizers listed in Table 1 was irradiated in the presence of 1–10 mM of either DMTD or DMD. Pyrene, naphthalene, and TMPD were chosen as representative excited state electron donors. All samples were purged with Ar, irradiated for the times indicated, and analyzed by HPLC to determine the amount of monomers formed and the amount of dimer remaining. Irradiations were carried out using cutoff filters to ensure that the light was absorbed by the sensitizer and not the dimers. The results are compiled in Table 1.
The efficiency and ability of the sensitizers to effect dimer splitting is qualitatively related to their excited-state oxidation potentials, \( E_{\text{ox}}^* \), which are listed in Table 2.\(^{1,3,33} \) \( E_{\text{ox}}^* \) is determined from literature values for the oxidation potential (\( E_{\text{ox}} \)) and the singlet state energy, \( E_{\text{oo}} \), using eq 4 where \( E_{\text{ox}} \) and \( E_{\text{ox}}^* \) are in volts (vs SCE) and \( E_{\text{oo}} \) is in kcal/mol.

\[
E_{\text{ox}}^* = E_{\text{ox}} - \frac{E_{\text{oo}}}{23.06} \tag{4}
\]

Aromatic amines, TMB, and TMPD have given the cleanest and most efficient splitting of both dimers. Naphthalene was also effective at splitting the thymine dimer DMDT; however, much longer photolysis times were employed and even then the conversion was low. Naphthalene could not be tested with DMCD because their UV absorption bands overlapped and sensitized photolysis would not be distinguished from direct photolysis. Chrysene also sensitizes dimer splitting, but as with naphthalene, much longer photolysis times are required. Cytosine dimer DMCD was split to completion in 14 h, whereas with DMDT less than 5% conversion was detected after 36 h.

It should be noted that the photolysis rates provide only a semiquantitative indication of the electron transfer efficiency of the sensitizers. These rates also reflect the spectral overlap of the sensitizers with the medium-pressure Hg lamp, the lifetime of the excited state sensitizer, the efficiency of the initial electron transfer, and the ability of the splitting reaction of the dimers to compete with back electron transfer. For example, below it is shown that pyrene is not quenched particularly efficiently by any of the substrates. That it does cause a splitting reaction can be attributed to its relatively long lifetime and broader absorption spectrum.

3. Fluorescence Quenching Experiments. To better understand the photosensitized splitting reaction mechanisms, fluorescence quenching experiments were carried out. A series of sensitizers with varying redox properties and singlet energies were examined using dimers DMDT and DMCD and monomers DMTD and DMC as quenchers. It was reasoned that if the splitting occurred via the proposed ion radical intermediates (Scheme 1), then a correlation between the excited state oxidation potential (\( E_{\text{ox}}^* \)) and the quenching efficiency would be observed. In any case, we anticipated that comparing the quenching efficiencies with sensitizer properties would help identify the minimal requirements for an enzymatic photorepair system. Our results, outlined below, support the electron transfer mechanism.

The quantum yield of fluorescence without quencher, \( \Phi_o \), relative to that with quencher added, \( \Phi \), is given by the rate constant for the reaction of the excited sensitizer with the quencher (\( k_q \)), the lifetime of the sensitizer’s excited state (\( \tau \)), and the concentration of the quencher, \([Q]\), according to the Stern–Volmer equation (eq 5).

\[
\frac{\Phi_o}{\Phi} = 1 + k_q \tau [Q] \tag{5}
\]

The pyrimidine dimers DMDT and DMCD quench the fluorescence of various sensitizers. Figure 1 shows typical examples where CH3CN solutions of the electron donor sensitizer, \( N,N',N' \)-tetramethylbenzidine (TMB), were irradiated in the presence of various concentrations of cytosine dimer DMCD. In this case the fluorescence decreases and no new emission bands are observed. Similar behavior was observed with the other sensitizers.


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**Figure 1.** Fluorescence spectrum of \( N,N',N' \)-tetramethylbenzidine in Ar-purged CH3CN. The fluorescence intensity decreases as increasing amounts (0–13 mM) of DMC are added.

**Table 2.** Properties of Various Sensitizers Used in This Study

<table>
<thead>
<tr>
<th>Sensitizers</th>
<th>( \tau ) (ns)</th>
<th>( E_{\text{ox}}^* ) (V vs SCE)</th>
<th>( E_{\text{ox}} ) (eV)</th>
<th>( E_{\text{ox}} ) (V vs SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetramethyl-1,4 phenylene-diamine</td>
<td>7.1</td>
<td>3.15</td>
<td>3.45</td>
<td>0.20</td>
</tr>
<tr>
<td>tetramethyl-benzidine</td>
<td>10.0</td>
<td>3.17</td>
<td>3.60</td>
<td>0.43</td>
</tr>
<tr>
<td>dimethylaniline</td>
<td>2.78</td>
<td>0.04</td>
<td>3.87</td>
<td>0.83</td>
</tr>
<tr>
<td>aniline</td>
<td>3.10</td>
<td>0.04</td>
<td>3.97</td>
<td>0.95</td>
</tr>
<tr>
<td>acenaphthene</td>
<td>46.00</td>
<td>0.04</td>
<td>3.91</td>
<td>1.41</td>
</tr>
<tr>
<td>1-methoxy naphthalene</td>
<td>17.2</td>
<td>0.04</td>
<td>3.85</td>
<td>1.36</td>
</tr>
<tr>
<td>naphthalene</td>
<td>96.00</td>
<td>0.04</td>
<td>4.02</td>
<td>1.54</td>
</tr>
<tr>
<td>9-methylanthracene</td>
<td>5.80</td>
<td>0.04</td>
<td>3.42</td>
<td>0.96</td>
</tr>
<tr>
<td>2-methoxynaphthalene</td>
<td>15.00</td>
<td>0.04</td>
<td>3.70</td>
<td>1.42</td>
</tr>
<tr>
<td>1-acetamidopyrene</td>
<td>12.0</td>
<td>0.04</td>
<td>3.56</td>
<td>1.33</td>
</tr>
<tr>
<td>anthracene</td>
<td>5.30</td>
<td>0.04</td>
<td>3.11</td>
<td>1.04</td>
</tr>
<tr>
<td>pyrene</td>
<td>32.2</td>
<td>0.04</td>
<td>3.34</td>
<td>1.16</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>61.0</td>
<td>0.04</td>
<td>3.59</td>
<td>1.50</td>
</tr>
<tr>
<td>chrysene</td>
<td>43.0</td>
<td>0.04</td>
<td>3.43</td>
<td>1.35</td>
</tr>
</tbody>
</table>


The fluorescence intensities at various concentrations were fit to the Stern–Volmer relationship (eq 5). The rate constants for fluorescence quenching, \( k_q \), were determined from our measured \( k_q \tau \) values and literature data for the various sensitizer \( \tau \) values. Studies of DMCD and DMC with aniline and \( N,N \)-dimethylaniline could not be carried because the long-wavelength absorption band of the quenchers overlapped the absorption bands of these sensitizers making it difficult to distinguish quenching from inner filter effects.

The monomeric bases DMTD and DMC quench fluorescence of the sensitizers. The \( k_q \) values for these are listed in Table 3. The quenching rate constants for both substrates increase as the \( E_{\text{ox}}^* \) of the sensitizer becomes increasingly negative. In both cases limiting \( k_q \) values of ca. 1.9 x 10^10 M^-1 s^-1 (the diffusion limit) are reached as \( E_{\text{ox}}^* \) becomes more negative than -2.4 V.

The dimeric substrates, DMTD and DMCD, show qualitatively similar behavior. For both dimers the limit is approached...
Table 3. Fluorescence Quenching Rate Constants, \( k_q \) (× 10^9 M\(^{-1}\) s\(^{-1}\)), for Excited State Electron Donors with Pyrimidines and Their Corresponding cis-syn Cyclobutane Dimers

<table>
<thead>
<tr>
<th>Substrates</th>
<th>DMDD</th>
<th>DMCD</th>
<th>DMC</th>
<th>DMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetramethyl-1,4-phenylenediamine</td>
<td>6.54</td>
<td>11.5</td>
<td>14.2</td>
<td>20.1</td>
</tr>
<tr>
<td>tetramethylenzidine</td>
<td>5.91</td>
<td>10.7</td>
<td>14.3</td>
<td>20.4</td>
</tr>
<tr>
<td>dimethylaniline</td>
<td>5.47</td>
<td>12.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aniline</td>
<td>5.06</td>
<td>12.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acenaphthene</td>
<td>3.31</td>
<td>9.04</td>
<td>8.08</td>
<td>9.80</td>
</tr>
<tr>
<td>1-methoxynaphthalene</td>
<td>3.34</td>
<td>7.01</td>
<td>7.51</td>
<td>7.23</td>
</tr>
<tr>
<td>naphthalene</td>
<td>2.48</td>
<td>6.32</td>
<td>2.05</td>
<td>6.87</td>
</tr>
<tr>
<td>2-methoxynaphthalene</td>
<td>0.821</td>
<td>6.17</td>
<td>1.61</td>
<td>6.652</td>
</tr>
<tr>
<td>9-methylanthracene</td>
<td>0.532</td>
<td>2.52</td>
<td>0.686</td>
<td>0.604</td>
</tr>
<tr>
<td>1-acetamidopyrene</td>
<td>0.623</td>
<td>2.34</td>
<td>0.528</td>
<td>0.521</td>
</tr>
<tr>
<td>anthracene</td>
<td>0.362</td>
<td>2.09</td>
<td>0.568</td>
<td>0.443</td>
</tr>
<tr>
<td>pyrene</td>
<td>0.152</td>
<td>1.20</td>
<td>0.228</td>
<td></td>
</tr>
<tr>
<td>phenantherene</td>
<td>0.103</td>
<td>0.201</td>
<td>0.0717</td>
<td>0.042</td>
</tr>
<tr>
<td>chrysene</td>
<td>0.287</td>
<td>0.178</td>
<td>0.0589</td>
<td>0.0366</td>
</tr>
</tbody>
</table>

Scheme 2. Kinetic Model for Fluorescence Quenching by Electron Transfer

\[
S + Q \xrightarrow{h_v} S^* + Q \xrightarrow{k_{an}} [S^* + Q] \xrightarrow{k_{ct}} [S + Q] \xrightarrow{k_{diff}} \text{products} \xrightarrow{k_{de}} \text{successor complex} \xrightarrow{k_{de}} \text{encounter complex} \xrightarrow{k_{diff}} \text{products}
\]

The free energy barrier for the charge transfer step, \( \Delta G_{ct}^{\mp} \), can be predicted from the driving force of the electron transfer reaction, \(-\Delta G_{ct}\), along with the reorganization energy, \( \lambda \). There are a number of treatments of this relationship,\(^{35,36}\) the most widely known being the Marcus theory (see eq 8, below). The latter is an extrathermodynamic treatment of reaction barriers which assumes a quadratic dependence of the barrier on the driving force. This predicts the so-called inverted region where the barrier begins to increase with increasing driving force. This treatment has been highly successful in predicting the rates of electron transfer in rigid systems\(^{39-41}\) and back electron transfer in photochemical systems (e.g. \( k_f \)).\(^{42-44}\) However, for photo-induced electron transfer reactions, inverted behavior has been observed only in a few special systems.\(^{45,46}\) More typical is behavior where the \( k_q \) increases with driving force and then saturates at the diffusion limit.\(^{47-49}\) Rehm and Weller\(^{36,37}\) demonstrated that the following monotonic relationship between \( \Delta G_{ct}^{\mp} \) and \( \Delta G_{ct} \) was successful at predicting rate constants for the latter types of reactions:

\[
\Delta G_{ct}^{\mp} = [\left( \frac{\Delta G_{ct}}{RT} \right) + \left( \frac{\lambda}{4} \right)]^{1/2} + \frac{\Delta G_{ct}}{2}
\]

The values of \( E_{ox}^* \) and the experimentally derived \( k_q \) in Tables 2 and 3 were analyzed using eqs 3, 6, and 7. The two adjustable parameters were \( \lambda \) and \( E_{ox} \). The desolvation term in eq 3 was estimated at 1.34 kcal/mol assuming a 700 pm separation distance for each of the sensitizer quencher pairs. The diffusion rate constant, \( k_{diff} \), was set at 19 × 10^10 M\(^{-1}\) s\(^{-1}\).

The appropriate value for the preexponential term, \( k_{max}K_{diff} \), has been the subject of some recent discussion. It was originally assumed to be 10^11 M\(^{-1}\) s\(^{-1}\).\(^{36,37}\) Subsequently it has been shown that certain fluorescence data can be made to conform to the Marcus theory by revising this factor upward.\(^{50-52}\)
**Figure 2.** Rehm–Weller analysis of the dependence of fluorescence quenching rate constants ($k_q$ in M$^{-1}$ s$^{-1}$) for DMTD (filled triangles) and DMT (open circles) on the excited state oxidation potentials ($E_{ox}*_{\text{red}}$ in V vs SCE) of various sensitizers in N$_2$-purged CH$_3$CN. Curves show fits calculated for DMTD (red line) and DMT (blue line). The value of $E_{ox}*_{\text{red}}$ varies from 2.30 V to 2.35 V.

**Figure 3.** Rehm–Weller analysis of the dependence of fluorescence quenching rate constants ($k_q$ in M$^{-1}$ s$^{-1}$) for DMCD (filled circles) and DMC (open triangles) on the excited state oxidation potentials ($E_{ox}*_{\text{red}}$ in V vs SCE) of various sensitizers in N$_2$-purged CH$_3$CN. Curves show fits calculated for DMCD (red line) and DMC (blue line). The value of $E_{ox}*_{\text{red}}$ varies from 2.17 V to 2.28 V.

**Table 4.** Parameters for Rehm–Weller Fits of Fluorescence Quenching Data

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$E_{ox}(V)$</th>
<th>$\lambda$ (kcal/mol)</th>
<th>$k_{max}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_{max}$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMTD</td>
<td>-2.20</td>
<td>13</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>DMT</td>
<td>-2.14</td>
<td>22</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>DMCD</td>
<td>-2.17</td>
<td>12</td>
<td>510</td>
<td>640</td>
</tr>
<tr>
<td>DMC</td>
<td>-2.16</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhCO$_2$Me</td>
<td>-2.28</td>
<td>28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$a \pm 0.08$ V, $b \pm 10$ kcal/mol.

Marcus$^{53}$ and Weaver$^{54}$ have analyzed the $k_{max}$ factor part of this and have argued that in CH$_3$CN, $k_{max}$ should take a value of $10^{12}$ to $10^{13}$ s$^{-1}$. The other factor in the preexponential term, $K_{diff}$, has apparently not been subjected to the same level of scrutiny. Of course, measurements of the sort reported here are sensitive only to the product of these two parameters and are incapable of resolving the individual contributions.

In view of the above considerations, the value for $k_{max}K_{diff}$ was not fixed. Instead, 10 to 20 fits were undertaken for each quencher as this parameter was systematically varied from $10^{10}$ to $10^{13}$ M$^{-1}$ s$^{-1}$. While the quality of the fits varied significantly over this range, the $E_{ox}$ were relatively insensitive to large changes in $k_{max}K_{diff}$. For example with DMC, $E_{ox}$ ranged only from $-2.25$ to $-2.14$ V, as $k_{max}K_{diff}$ was varied from $1 \times 10^{10}$ to $1 \times 10^{13}$ M$^{-1}$ s$^{-1}$.

Adjusting the $k_{max}K_{diff}$ term significantly improves the fits for the dimers DMTD and DMCD. Only through this consideration is it possible to capture the fact that the asymptotic $k_q$ values fall below the diffusion limit for these substrates. The best fit values $k_{max}K_{diff}$ were found to be $2 \times 10^{10}$ M$^{-1}$ s$^{-1}$ for DMTD and $4 \times 10^{10}$ for DMCD. We suggest that these low values are due to differences in the $K_{diff}$ term. On the basis of Fuoss’ model,$^{55}$ $K_{diff}$ is often taken to be 0.86 M$^{-1}$. However, this is based upon the assumption of spherical and isotropic reactants. In this case all relative orientations of the quencher and the excited state sensitizer would be presumed to be equally reactive. In cases such as the present, where the reactants are non-isotropic, $K_{diff}$ is the product of the diffusional equilibrium constant and any orientational equilibrium constants that lead to the reactive orientation. This lower value for $K_{diff}$ found in the dimer experiments causes us to assume that the precursor complexes involving the dimers and sensitizers must adopt rather specific relative orientations in order to achieve productive electron transfer. The geometries of these “productive” orientations are not clear at this time. Further experimental and/or computational investigations into this issue would be interesting as a knowledge of geometric constraints on electron transfer to pyrimidine dimers is of obvious relevance to the enzymatic system.

The reduction potentials ($E_{red}$) for all of the substrates were estimated from the $k_q$ values they each showed with the various sensitizers. The $k_q$ data sets from Tables 2 and 3 were then compared with theoretical curves determined using eqs 6 and 7. A simplex algorithm was used to minimize the sum of the squares of the residuals as the parameters $\lambda$ and $E_{red}$ were varied. The optimized plots along with the experimental data are presented in Figure 2 (DMTD and DMT) and Figure 3 (DMC and DMCD). Table 4 lists the best fit values.

It was of interest to determine the uniqueness of the fits and to estimate uncertainties in the best-fit parameters. To this end, the procedure described above for the $k_{max}$ and $K_{diff}$ parameter was applied to the remaining parameters, $\lambda$ and $E_{red}$. A series of fits were undertaken as $\lambda$ was held at 100 values between 1 and 50 kcal/mol while both $E_{red}$ and $\lambda$ were optimized. Likewise an additional series of fits was undertaken where $E_{red}$ was held at 100 different values between $-1.90$ and $-2.30$ V. Each of these three procedures converged on the same best fit parameters to within the stated uncertainties. We estimate the uncertainty in $\lambda$ as $\pm 10$ kcal/mol and the uncertainty in $E_{red}$ as $\pm 0.08$ V.

To further test the validity of this approach, the $E_{red}$ of methyl benzoate (PhCO$_2$Me) was determined in the same fashion. Figure 4 shows experimental data for this substrate as well as the optimized curve calculated from eqs 6 and 7. The value of $E_{red}$ for this compound ($-2.28$ V) compares favorably with a previously reported polarographic measurement of $-2.3$ V.$^{56}$ Fits to the classical Marcus theory were also undertaken. For these eq 7 was replaced with eq 8. In these cases the theoretical curves did not match the experimental data as well, but similar values for $E_{red}$ and $\lambda$ were extracted from the best fits.

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Rehm–Weller analysis of DMTD yields \( E_{\text{red}} = -2.20 \) V and \( \lambda = 13 \) kcal/mol. In the preliminary communication, we estimated a somewhat more negative value of \(-2.6 \) V. However in that experiment, the Rehm–Weller plot had only two sensitizers whose \( k_q \) values fell below the asymptotic limit. The \( E_{\text{red}} \) and \( \lambda \) thus extracted were highly dependent on the accuracy of these values. In this work we have repeated these determinations now using five sensitizers whose \( k_q \) values are below the asymptotic limit. This along with an improved fitting procedure permits a more accurate analysis.

The \( E_{\text{red}} \) values derived from these experiments compare well with previous reduction potentials measured for similar systems in aprotic solvents. Aromatic amides have reduction potentials in ethanol that range from \(-2.0 \) to \(-2.4 \) V.\(^{(57)}\) Cyclic voltammetry of cytosine in DMSO gives a value of \( E_{\text{red}} = -2.36 \) V.\(^{(58)}\) This agrees reasonably with our fluorescence quenching value of \( E_{\text{red}} = -2.2 \) V for DMC in CH\(_3\)CN. It is interesting to note that earlier polarographic experiments on cytosine in aqueous solution showed an irreversible reduction wave near \(-1.4 \) V—a potential considerably less negative than that measured in DMSO.\(^{(59)}\) The electrochemical behavior of cytosine, and indeed all pyrimidines, in aqueous solution is complex. In the case of cytosine, the electron transfer is coupled with a fast and exothermic proton transfer. In aqueous solution, the equilibrium process, and thus electrochemically measured reduction potential, reflect a net H-atom transfer reaction (eq 9a). In contrast, the reduction potentials measured in the aprotic solvents, CH\(_3\)CN and DMSO, reflect only the electron transfer process.

![Diagram](image)

Figure 4. Rehm–Weller analysis of the dependence of fluorescence quenching rate constants (\( k_q \) in M\(^{-1}\) s\(^{-1}\)) for PhCO\(_2\)CH\(_2\) on the excited state oxidation potentials (\( E_{\text{ox}*} \) in V vs SCE) of various sensitizers in N\(_2\)-purged CH\(_3\)CN.

Another estimate for \( E_{\text{red}} \) of thymine dimers comes from the pulse radiolysis studies of Heelis’ et al.\(^{(62)}\) It was demonstrated that dimer splitting could be induced by CO\(_2^-\) although with only moderate efficiency. On this basis it was concluded that the \( E_{\text{red}} \) for thymine dimers in aqueous solution was near that of CO\(_2\), placing the value at ca. \(-1.9 \) V. We regard this as quite reasonable agreement with the values determined here considering the differences in solvent and method of determination.

The \( E_{\text{red}} \) values here are consistent with behavior from previous model studies. Rose et al.\(^{(20,21,63)}\) reported that pyrimidine dimers undergo dihydroflavin-sensitized decomposition through a radical anion chain mechanism. Such a mechanism requires that the pyrimidine anion radical generated in the splitting reaction be capable of transferring an electron to the dimer. This chain propagation step is plausible only if the electron transfer is exothermic or weakly endothermic. The values derived from this study predict that the propagation step should be slightly endothermic and are thus consistent with the proposed mechanism.

The values for \( \lambda \) extracted from the fitting procedures ranged from 12 to 28 kcal/mol. These fall into a range that is typical for organic sensitizer and quenchers in CH\(_3\)CN. For example, Rehm and Weller\(^{(37)}\) determined \( \lambda \) value of 9.6 kcal/mol for their series of aromatic compounds. On the other hand, Chen et al.\(^{(64)}\) report \( \lambda \) values of 23.5 kcal/mol for phenanthrene quenching by various amines. It is notable that the dimers give \( \lambda \) values that are roughly half that of the corresponding monomers. The \( \lambda \) is known to be inversely proportional to the radii of the reacting partners.\(^{(26)}\) The larger effective radii of the dimers could therefore account for at least part of this difference.

5. **Energetics of Enzymatic Photorepair.** The enzymatic chromophore responsible for electron transfer to the dimer is a reduced flavin (FADH\(^{-}\)). Anderson’s\(^{(65)}\) pulse radiolysis studies provide a redox potential of \(-0.124 \) V for the flavin radical to reduced flavin transition. The fluorescence spectrum of the FADH\(^{-}\)–chromophore in DNA photolyase\(^{(66)}\) shows an apparent 0–0 band at 450 nm. This corresponds to a singlet energy, \( E_0 = 63.6 \) kcal/mol. Application of eq 4, gives \( E_{\text{ox}*} = -2.8 \)

\[(\lambda + \Delta G_{\text{el}})^2 \]

\[
\Delta G^\circ = \frac{\lambda + \Delta G_{\text{el}}}{4\lambda}
\]

\[(8)\]


V for the proximate enzymic photosensitizer. This means it is a slightly more effective photosensitizer than 1,4-dimethoxybenzene. These values can be used to estimate the exergonicity of the charge transfer step in the enzymatic reaction. For both pyrimidine dimers, the initial charge transfer would be exergonic with \( \Delta G_{ct} \approx -10 \) to \(-15 \) kcal/mol. The uncertainty in this estimate is largely due to differing environmental effects in the model system and the photolyase active site.

The thermodynamic cycle illustrated in Figure 5 can be used to calculate the enthalpy change for the splitting of the dimethylthymine dimer anion radical (\( \Delta H_{anion} \)). The reduction potentials of the dimer DMTD (= DMT\( \times \)DMT in the figure) and monomer DMT are taken from this work (Table 4). The enthalpy for neutral splitting, \( \Delta H_{neutral} \), is taken from ref 67.

![Figure 5. Thermodynamic cycle used to estimate the enthalpy change for the splitting of the dimethylthymine dimer anion radical (\( \Delta H_{anion} \)). The reduction potentials of the dimer DMTD (DMT\( \times \)DMT in the figure) and monomer DMT are taken from this work (Table 4). The enthalpy for neutral splitting, \( \Delta H_{neutral} \), is taken from ref 67.](image)

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The thermodynamic cycle illustrated in Figure 5 can be used to calculate the enthalpy change of the splitting reaction. Using a photothermal technique, we have previously estimated the neutral-to-neutral enthalpy of dimer splitting \( \Delta H_{neutral} \) at \(-19 \) kcal/mol for DMTD.\(^\text{(67)}\) (Diogo et al.\(^\text{(68)}\) obtained a value of \(-26 \) kcal/mol using a derivative of DMTD where the two bases were linked at their respective N3 positions with a trimethylene bridge.) The reduction potentials of the DMT and DMTD determined here provide the energy difference between the neutral forms and the anion radicals. It is further assumed that the entropy change for the electron transfer to the dimer and monomer is equal. On the basis of these considerations, \( \Delta G = -20 \) kcal/mol is predicted for the splitting reaction.

**Experimental Section**

**Synthesis.** Dimethylthymine dimer 1 and monomer 3 were synthesized as previously reported.\(^\text{(29)}\) Dimethylcytosine dimer 2 and monomer 4 were also synthesized as previously reported.\(^\text{(19)}\)

**Product Studies.** A quartz test tube was charged with the monomer or dimer being studied in CH\(_3\)CN. An excess of sensitizer of interest was added (if used) and the tube was sealed with a septum. The solution was purged with Ar for 10 min to remove oxygen. The solution was irradiated with a 450-W medium-pressure Hg-vapor lamp. A filter for the experiment (Corex, flint, uranium, or none) was used so all the light was absorbed by the sensitizer and none by the dimer (monomer).

After irradiation the samples were analyzed by HPLC. The peak areas of the products were compared to the peak areas of authentic samples. For the cytosine system, an analytical amino-modified phase silica gel column (Microsorb-MV) was used with a MeOH:Et\(_2\)O mobile phase (2:3 until the monomer elutes, then 9:1). For the thymine system, an analytical C\(_18\) reversed phase column was used with a H\(_2\)O:CH\(_3\)CN (8.5:1.5) mobile phase. Products were detected by a UV detector set at 222 (1 and 3) and 246 (2 and 4).

**Fluorescence Quenching.** A stock solution of the fluorescent sensitizer was prepared by sonicating 1–3 mg of sensitizer in 100 mL of spectroscopic grade acetonitrile for 30 min. In general, the stock solutions had sensitizer concentrations of about 0.01 mM. Several quencher samples were prepared by sonicating various concentrations of the quencher being studied (monomers or dimers) in the stock sensitizer solution for 10 min. This resulted in several samples with quencher concentrations varying from 2 to 20 mM and constant sensitizer concentrations. The samples were each placed in a quartz cuvette and fitted with a septa lined with Teflon tape (to prevent leeching of any impurities present in the septa). Each sample was then purged for 15 min with argon and the fluorescence was measured and recorded.

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