by hydroperoxide on the iron center, followed by peroxide heterolysis. H abstraction from the alkane by the high-valent intermediate generates a caged radical species, which collapses to the observed product. Clearly this scheme resembles the well-known oxygen rebound mechanism proposed for (porphyrinato)metal-oxo oxidation of alkanes,[15] but with one critical difference. Unlike the planar porphyrin ligands which enforce a diaxial configuration for the oxo and X ligands, the tetradentate tripodal ligand provides a cis coordination geometry. Such a configuration allows the caged radical a choice of either OH or X transfer in the rebound step. Our present observations suggest that the X group is transferred *preferentially* over OH in this chemistry (perhaps because of the lower oxidation potential of the X group) and lend credence to the proposed C-X bond forming mechanisms involving high-valent iron intermediates in the biosynthesis of β-lactam antibiotics.[4]

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Model Studies of DNA Photorepair: Radical Anion Cleavage of Thymine Dimers Probed by Nanosecond Laser Spectroscopy

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The major form of ultraviolet radiation (UV) damage to DNA results from [2 + 2] cycloaddition reactions between adjacent pyrimidines (eq 1).[1] DNA photolyase is an enzyme which mediates a net reversal of this damage.[2] This enzyme is somewhat unique[3] because the catalytic step is photochemical—absorption of a UV or visible photon by the enzyme-substrate complex is necessary for dimer cleavage. *Escherichia coli* photolyase possesses a 1,5-dihydroflavin cofactor which acts as a chromophore in the photochemical/catalytic step.[4] It has been proposed, based on indirect evidence, that dimer cleavage is initiated by single-electron transfer (SET) from the excited chromophore to the substrate.[5] The model studies described below were designed to determine if the anion radicals of thymine dimers cleave at a kinetically significant rate. The results support an SET mechanism for enzymatic photorepair.

Cycloreversion reactions initiated by reductive SET are relatively unknown.[6] To determine if reductive SET could initiate

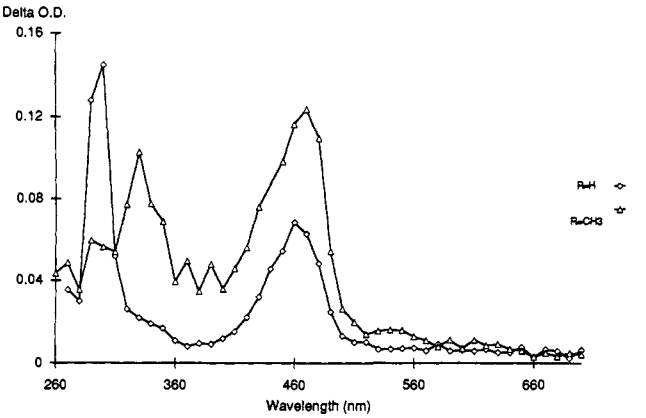
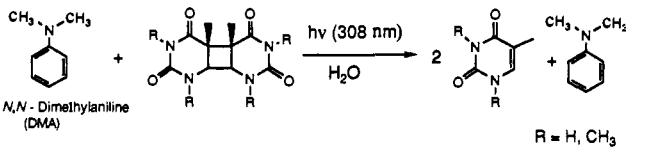


Figure 1. Transient spectra obtained after irradiating DMA with thymine dimer (diamonds) and dimethylthymine dimer (triangles) in pH 12 aqueous solution. Time is 3.0 ± 0.2 μs following the laser pulse.

dimer cleavage, we attempted to cleave simple pyrimidine dimers using *N,N*-dimethylaniline (DMA) as a sensitizer. This compound possesses an excited-state oxidation potential of −3.3 V.[7] The excited-state oxidation potential of the presumed enzymic sensitizer is calculated to be −2.5 V.[8] If simple SET is sufficient to cleave the thymine dimers,[9] then DMA should be an equally competent photosensitizer.



(1)

DMA is a photosensitizer for dimer cleavage. This was established by product studies and fluorescence quenching experiments. Irradiation of aqueous solutions (pH 12 or pH 7 0.05 M phosphate buffer, 308 nm) of DMA in the presence of either dimethylthymine dimers[10] or thymine dimers[11] results in efficient cleavage of the dimers. Only the corresponding monomers were detected by HPLC analysis of the reaction mixtures (80 ± 5% chemical yield). Dimethylthymine dimers quench the fluorescence of DMA in aqueous solutions. A Stern-Volmer[12] analysis gives a value for $k_q\tau$ of 38.3 M^{-1} (pH 12), where k_q is the bimolecular rate constant for quenching and τ is the singlet-state lifetime for DMA in the absence of dimer. The quantum yield for dimer splitting, Φ, depends on the concentration of dimers. A double-reciprocal plot of Φ^{-1} vs [dimer]$^{-1}$ gives a line with a slope of

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(2) (a) Sancar, A.; Sancar, G. *Ann. Rev. Biochemistry* **1988**, *57*, 29. (b) Friedberg, E. C. *DNA Repair*; W. H. Freeman: New York, 1985. (c) Sancar, A.; Smith, F. W.; Sancar, G. B. *J. Biol. Chem.* **1984**, *259*, 6028. (d) Sutherland, B. M. *Nature* **1974**, *248*, 109.

(3) Protochlorophyllide reductase is another example of a photochemical enzyme. (a) Begley, T.; Young, H. *J. Am. Chem. Soc.* **1989**, *111*, 3095. (b) Nielsen, O. F.; Kahn, A. *Biochim. Biophys. Acta* **1973**, 117.

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(5) (a) Hartman, D.; Van Camp, J.; Rose, S. *J. Org. Chem.* **1987**, *52*, 2685. (b) Jorns, M. S. *J. Am. Chem. Soc.* **1987**, *109*, 3133. (c) Sancar, G. B.; Jorns, M. S.; Payne, G.; Fluke, D.; Rupert, C. S.; Sancar, A. *J. Biol. Chem.* **1987**, *262*, 492. (d) Payne, G.; Sancar, A. *Biochemistry* **1990**, *29*, 7715.

(6) There are examples of pericyclic processes initiated by *oxidative* SET. See, for example: (a) Majima, T.; Pac, C.; Sakurai, H. *J. Am. Chem. Soc.* **1980**, *102*, 5265. (c) Lewis, F. D.; Kojima, M. *J. Am. Chem. Soc.* **1988**, *110*, 8664. (c) Bauld, N. L.; Bellville, D. J.; Harirchian, B.; Lorenz, K. T.; Pabon, R. A.; Reynolds, D. W.; Wirth, D. D.; Chiou, H.-S.; Marsh, B. K. *Acc. Chem. Res.* **1987**, *20*, 371. (d) Lamola, A. A. *Mol. Photochem.* **1972**, *4*, 107. Indole derivatives have been shown to sensitize the cleavage of thymine dimers by what is apparently a reductive SET mechanism: (e) Young, T.; Kim, S.-T.; van Camp, J. R.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1988**, *48*, 635. (f) Charlier, M.; Hélène, C. *Photochem. Photobiol.* **1975**, *21*, 31.

(7) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.

(8) This is based on the oxidation potential of dihydroflavin of −0.12 V (a) Anderson, R. F. *Biochim. Biophys. Acta* **1983**, *722*, 158 and a singlet-state energy of 56 kcal/mol from the fluorescence spectrum (b) Ghisla, S.; Massey, V.; Lhoste, J.-M.; Mayhew, S. G. *Biochemistry* **1974**, *13*, 589.

(9) In principle, it would be possible to determine the free energy change for reduction of the thymine dimer anion radical if its reduction potential were known. However, our attempts to measure the reduction potential for the thymine dimer (DMF solution with 1 M Bu_4NPF_6, Ag electrode) did not show a reduction wave >−2.1 V vs Ag/AgCl.

(10) Cis-syn [2 + 2] cycloadducts of 1,3-dimethylthymine: Kloepfer, R.; Morrison, H. *J. Am. Chem. Soc.* **1972**, *94*, 255.

(11) Wulff, D. L.; Fraenkel, G. *Biochim. Biophys. Acta* **1961**, *51*, 332.

(12) Turro, N. *J. Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978; Chapter 8.

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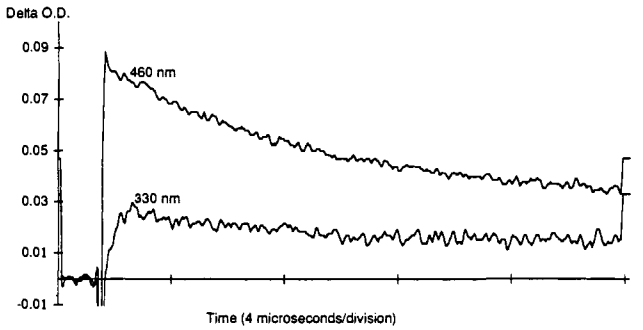
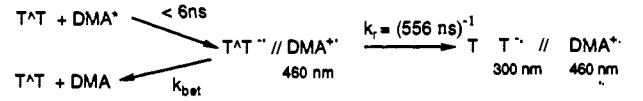


Figure 2. Kinetic traces from pulsed laser irradiation (308 nm, 6 ns, 50 mJ) of pH 12 thymine dimer/DMA solution. Absorbance change is monitored at 460 nm (DMA cation radical) and 330 nm (thymine anion radical).

$(k_q\tau\phi_r)^{-1}$.[11] The quantum efficiency of dimer anion radical cleavage, ϕ_r, is 0.4 at pH 12 and 0.1 at pH 7.



(2)

DMA cleaves thymine dimers via a reductive SET mechanism. This was determined by time-resolved laser spectroscopy. Pulsed laser photolysis[13] of DMA with thymine dimers at pH 12 gives the transient absorption spectra shown in Figure 1. Two bands appear: one at 460 nm, due to the cation radical of DMA,[14] and another at 300 nm, due to thymine monomer anion radical. The assignment of the latter is based on three considerations. First, this is very similar to absorption maxima for thymine monomer anion radicals reported by earlier works.[15] Second, when the substrate is changed to dimethylthymine dimers the low wavelength absorption band shifts to 330 nm. This demonstrates that low wavelength band is associated with the substrate rather than the sensitizer. Finally, we have independently generated the dimethylthymine monomer anion radical on our apparatus. Pulsed laser excitation of DMA in the presence of dimethylthymine gives a spectrum almost identical to the corresponding spectrum in Figure 1.

DMA cation radical appears within the 6 ns duration of the laser pulse, indicating that SET occurs on a time scale fast relative to the measurement. The band at 300 nm does not appear promptly after laser excitation, rather it grows in exponentially with a rate constant (fitted to first-order) of 4.6×10^6 s^{-1} (k_{obs}). The time profiles of both absorbance bands are shown in Figure 2. The observed rate constant for monomer anion growth, k_{obs}, is the sum of all rate constants which deplete the dimer anion radical ($k_{obs} = k_{bel} + k_r$).[16] The rate constant for the splitting step, k_r, is given as $k_r = k_{obs}\phi_r = 1.8 \times 10^6$ s^{-1}.[17]

In the absence of dimer, laser irradiation produces DMA cation radical and solvated electron (detected by its broad absorbance >600 nm). We considered that the 300 ns rise for the monomer anion radical might simply reflect the rate of attachment of the solvated electron to the thymine dimer. In this case, the solvated electron absorbance should have the same initial absorbance, but its decay rate should increase with added dimer. With added dimer, the initial absorbance at 600 nm is reduced to ca. 1/13 of its original intensity, indicating that dimer is interacting directly with DMA excited state. The fluorescence quenching experiment also demonstrates that the dimers are interacting directly with excited-state DMA and that solvated electron attachment is not a significant pathway.

For the reductive SET pathway to be operative, the pyrimidine dimer anion radicals must cleave rapidly enough to avoid nonproductive back electron transfer. The rate of back electron transfer in the enzymatic reaction is not known. However, the quantum yield for photorepair is ca. 0.7.[5d] This implies that the rate of back electron transfer is slower than cleavage. An upper limit for back electron transfer in the enzymatic reaction of $<10^6$ s^{-1} is predicted based on our data.[18] This is not unreasonable. Rates of SET are determined by properties of the external medium, the free energy change, distance between the donor and acceptor, and the relative orientation of the donor and acceptor.[19] The ordered environment of proteins can often hold the donor and acceptor at unfavorable distances and orientations.[20] Therefore, our results are entirely consistent with a reductive SET mechanism for DNA photorepair.[21]

Acknowledgment. We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work.

(13) Excitation: 308 nm, 50 mJ, 6 ns. Sample solutions were purged with nitrogen and sealed in a 40-mL flow cell with quartz windows. Typical concentrations were 1.4×10^{-6} M sensitizer, 10 mM dimer, and 0.1 M phosphate buffer. To avoid complications due to proton transfer either to the dimer or monomer anion radicals the experiments were done at pH 12. Under these conditions the monomer anion radical is not protonated; see ref 15b. The role of proton transfer in the cleavage mechanism is currently under investigation in our laboratory and will be discussed in the full paper.

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(17) Earlier workers have reported a 200-ns lifetime for thymine dimer anion radicals obtained by pulse radiolysis. In this case the spectroscopic behavior is more complex than that observed by our method. (a) Grossweiner, L. I.; Kepka, A. G.; Santus, R.; Vigil, J. A. *Int. J. Radiat. Biol.* **1974**, *25*, 521. (b) Santus, R.; Hélène, Ovadia, J.; Grossweiner, L. I. *Photochem. Photobiol.* **1972**, *16*, 65.

(18) This assumes that the enzyme does not actively promote the bond cleavage. It is also possible that the dimer cleavage occurs in a stepwise fashion whereby the 5-5 bond cleaves rapidly followed by rate-determining cleavage of the 6-6 bond. See: Witmer, M. R.; Altmann, E.; Young, H.; Begley, T. P.; Sancar, A. *J. Am. Chem. Soc.* **1989**, *111*, 9264.

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A Convergent Enone Synthesis. Three-Component Coupling of Alkyl Iodides, Carbon Monoxide, and Allylstannanes by Free-Radical Carbonylation

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Free-radical carbonylation is now emerging as a new tool for the introduction of carbon monoxide into organic molecules, and we recently reported tin hydride mediated carbonylation of organic halides.[1] The tin hydride mediated system usually required moderate CO pressures (70–90 atm) and high-dilution conditions so as to cause the trapping of an alkyl radical by CO to predominate over the competing direct abstraction of a hydrogen atom from tributyltin hydride by the alkyl radical. In principle, if a competing reaction is much slower than the trapping of the alkyl

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