Photoinduced Electron Transfer to Pyrimidines and 5,6-Dihydropyrimidine Derivatives: Reduction Potentials Determined by Fluorescence Quenching Kinetics

Michael P. Scannell, Gautam Prakash, and Daniel E. Falvey*

Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

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The dynamics of flourescence quenching of excited state electron donor sensitizers by various pyrimidine and 5,6-dihydropyrimidine substrates was examined. For all of the substrates studied the rate constant of fluorescence quenching (k_q) increases as the excited state oxidation potential (E_{ox}*) becomes more negative. The dependence of k_q on E_{ox}* in each case is well described by the Rehm−Weller relationship. Fits of the data to this relationship allow for the estimation of the reduction potentials of the substrates (E_{red}). The pyrimidines 1,3-dimethylthymine, 1,3-dimethyluracil, and 1,3,6-trimethyluracil give E_{red} values (in CH_3CN) ranging from −2.06 (vs SCE) to −2.14 V. Their dihydro derivatives, 1,3-dimethyl-5,6-dihydrothymine, 1,3-dimethyl-5,6-dihydrouracil, and 1,3,6-trimethyl-5,6-dihydrouracil gave E_{red} values ranging from −1.90 to −2.07 V. The higher E_{red} values for the dihydropyrimidines compared with their unsaturated derivatives is attributed to aromatic stabilization in the pyrimidines, which is not present in the dihydro derivatives. In addition, the E_{red} for both the *trans-syn* and *cis-syn* diastereomers of the dimethylthymine cyclobutane dimer was examined using the same method. The *trans-syn* dimer gives an E_{red} of −1.73 V and the *cis-syn* dimer gives an E_{red} of −2.20 V. This remarkable difference is attributed to a stereoelectronic effect. The *cis-syn* dimer anion radical suffers from an unfavorable charge−dipole interaction between the added electron and the O^4 carbonyl group in the remaining pyrimidine ring. In contrast, the *trans-syn* dimer anion radical shows mainly a stabilizing inductive electron-withdrawing effect of the remaining O^4 carbonyl group. Solvent effects on E_{red} were also examined. It is shown that the protic solvent, CH_3OH, significantly stabilizes the anion radicals, raising E_{red} by ca. 400 mV over the value in CH_3CN.

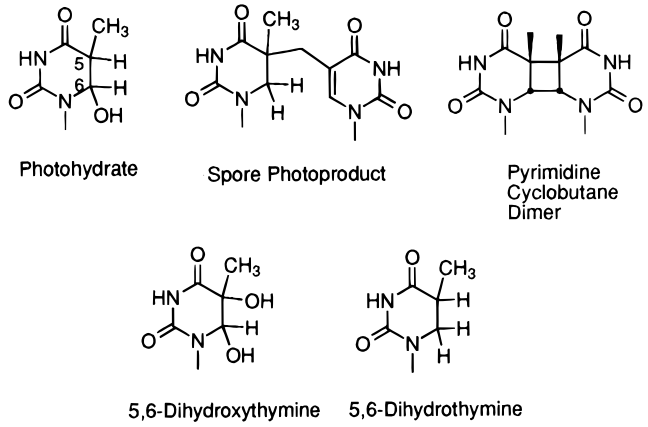
Introduction

Addition of an electron to DNA is a process of general interest. One of the mechanisms by which ionizing radiation damages DNA involves attachment of solvated electrons to the DNA bases.[1−3] It is generally understood that the attached electrons localize on the pyrimidines. The two pyrimidine bases in DNA, cytosine and thymine, have similar electron affinities,[4] and there has been some discussion[5−8] about which of these bases serves as the ultimate electron "sink". It is likely that this is determined by the local environment around the base in question. The initial electron attachment, or reduction of DNA, can lead to a variety of genotoxic lesions on the DNA molecule including strand scission and base modification.[1,2]

In addition to the question of radiation damage, there is also fundamental interest in the mechanism and rate by which single electrons migrate through DNA strands. Recent experiments using excited state metal complexes as electron donors and/or acceptors have led Barton and co-workers[9−11] to conclude that electron (and hole) migration through DNA is surprisingly fast. The charge carriers presumably migrate through the stacked bases, although the precise mechanism is still under investigation.[12,13]

Our interest in single-electron reduction of DNA comes from studies of photochemically-driven DNA repair enzymes known as DNA photolyases.[14−16] UV irradiation of DNA results in the formation of cyclobutane dimers between adjacent pyrimidine bases. The DNA photolyases bind to damaged bases and then, upon absorption of a photon, repair them to their normal forms. A number of studies[17−21] indicate that the initial photochemical step is transfer of a single electron from the protein to the damaged bases.

CHART 1: Some Base Modifications Resulting in Saturation of the 5,6-Double Bond in Thymine



While electron transfer in nucleic acids has been extensively studied in the context of the canonical bases, less is known about how relatively small changes in the structure of the bases affect electron transfer. Base modification occurs both enzymatically and through various types of enviromental insults. Pyrimidine cyclobutane dimers, spore products, and photohydrates (Chart 1) represent base modifications that result from UV irradiation of DNA.[22,23] Exposure of DNA to ionizing radiation generates (among other products) 5,6-dihydroxypyrimidines and 5,6-dihydropyrimidines.[1,2]

Since naturally occuring DNA is likely to have some fraction of modified bases, it would be useful to understand how these modifications affect electron transfer processes. If modified bases are easily reduced, they might serve as traps for electrons. On the other hand if modifications render the base more difficult to reduce, then such sites may constitute a barrier for electron

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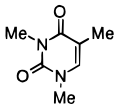
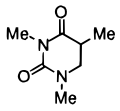
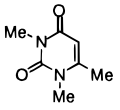
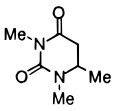
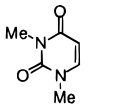
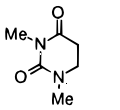
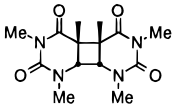
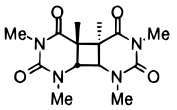
TABLE 1: Rehm−Weller Parameters for Some Pyrimidine Derivatives Determined in CH_3CN

Compound		E_{red} (V vs SCE)	λ (kcal/mol)	$k_{max}K_{diff}$ ($\times 10^{-9}$ $M^{-1}s^{-1}$)
	**DMT**	-2.14	22	640
	**DMTH$_2$**	-2.07	25	160
	**TMU**	-2.06	20	160
	**TMUH$_2$**	-1.91	22	40
	**DMU**	-2.11	18	160
	**DMUH$_2$**	-1.90	25	80
	**csDMTD**	-2.20	13	20
	**tsDMTD**	-1.73	33	640

migration along the DNA strand. The present study focuses on how removal, or saturation, of the 5,6-double bond affects the reduction potentials of various thymine derivatives. As indicated in Chart 1, this particular structural motif is found in a variety of DNA photolysis and radiolysis products.

We have examined the pyrimidine derivatives listed in Table 1 and determined their one-electron reduction potentials using a kinetic method. Analysis of fluorescence quenching kinetics shows that saturation of the 5,6-double bond makes a given base easier to reduce. Additionally, we have determined the reduction potential for the *trans-syn*-cyclobutane dimer of 1,3-dimethylthymine and show that is significantly easier to reduce than than the previously examined *cis-syn* cyclobutane dimer.

Experimental Section

1,3-Dimethyl-5,6-dihydrouracil (DMUH$_2$). The general procedure for the synthesis of the dimethyldihydropyrimidines is a modification of the procedure for the synthesis of hydrouracils by Zee-Cheng et al.[24] 1,3-Dimethylurea (88.11 g, 1 mol) was refluxed with acrylic acid (36 g, 0.5 mol) and hydroquinone (0.4 g, 3.6×10^{-3} mol) at 200 °C for 2 h. After 2 h, the solution was poured into H_2O (200 mL) and then extracted three times with aliqouts of $CHCl_3$ (200 mL). The $CHCl_3$ layers were combined, washed with two aliquots of 0.5 M K_2CO_3 (200 mL), dried over $MgSO_4$, filtered, and rotary evaporated to yield a pale-yellow viscous liquid. This liquid was added dropwise (10 mL at a time) into rapidly stirring pentane (200 mL quantities). Under these conditions, the product dissolves in the pentane. A colorless solution is decanted from an insoluble colored oil. Crystals of the product form from the colorless fraction upon storage at 4 °C overnight. 1,3-Dimethyl-5,6-dihydrouracil crystallizes as short white needles (5.3 g, 7.5%): mp = 47−48 °C. ^{1}H NMR ($CDCl_3$): δ 3.32 (m, 2H), 3.13 (s, 3H), 3.00 (s, 3H), 2.69 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 169.35, 154.07, 42.93, 35.83, 31.43, 27.52. Low resolution MS: *m/z* (relative intensity) 142 (M^+, 100), 112 (23), 84 (12), 57 (31).

1,3-Dimethyl-5,6-dihydrothymine (DMTH$_2$). 1,3-Dimethylurea (88.11 g, 1 mol) was refluxed with methacrylic acid (43 g, 0.5 mol) and hydroquinone (0.4 g, 3.6×10^{-3} mol) at 200 °C for 2 h. The product was separated as described for 1,3-dimethyl-5,6-dihydrouracil. 1,3-Dimethyl-5,6-dihydrothymine cystallizes as long white needles (15 g, 10%), mp 37−38 °C. ^{1}H NMR ($CDCl_3$): δ 3.28 (dd, J = 6.2, 6.1 Hz, 1H), 3.13 (s, 3H), 3.09 (m, 1H), 3.03 (s, 3H), 2.71 (m, 1H), 1.21 (d, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 172.43, 154.09, 49.83, 35.80, 35.59, 27.79, 13.28. Low resolution MS: *m/z* (relative intensity) 156 (M^+, 100), 112 (35), 72 (27), 58 (35), 55 (46).

1,3,6-Trimethyl-5,6-dihydrouracil (TMUH$_2$). 1,3-Dimethylurea (88.11 g, 1 mol) was refluxed with crotonic acid (43 g, 0.5 mol) at 200 °C for 2 h. The product was separated as described for 1,3-dimethyl-5,6-dihydrouracil, with the exception that the $CHCl_3$ layers were not washed with K_2CO_3. 1,3,6-Trimethyl-5,6-dihydrouracil crystallizes as white cubes (34 g, 44%): mp 37−38 °C;. ^{1}H NMR ($CDCl_3$): δ 3.49 (m, 1H), 3.17 (s, 3H), 3.01 (s, 3H), 2.84 (dd, J = 16.4, 6.4 Hz, 1H), 2.53 (dd, J = 16.4, 2.7 Hz, 1H), 1.20 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 168.83, 153.10, 49.33, 38.09, 34.09, 27.47, 17.64. Low resolution MS: *m/z* (relative intensity) 156 (M^+, 62), 141 (84), 84 (100), 55 (40).

1,3-Dimethyluracil (DMU). Uracil (10 g, 0.9 mol) was added to H_2O (50 mL) containing KOH (9 g, 0.16 mol). Under rapid stirring, the solution turned clear and was placed in an ice bath. To it, dimethyl sulfate (20 mL, 0.2 mol) was added dropwise with stirring. After the addition was complete, the solution was removed from the ice bath and heated until it boiled. Upon cooling, the solution was extracted with three aliquots of $CHCl_3$ (50 mL). The $CHCl_3$ layers were combined, dried over $MgSO_4$, filtered, and rotary evaporated to remove the solvent. The solid was recrystallized twice from C_2H_5OH yielding 1,3-dimethyluracil (9.4 g, 75%): mp 121−123 °C (lit.[25] mp 120−121 °C). ^{1}H NMR ($CDCl_3$): δ 7.09 (d, J = 7.8 Hz, 1H), 5.63 (d, J = 7.8 Hz, 1H), 3.31 (s, 3H), 3.23 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 163.19, 151.76, 142.69, 101.11, 36.80, 27.52. Low resolution MS: *m/z* (relative intensity) 140 (M^+, 100), 83 (43), 54 (48).

1,3-Dimethylthymine (DMT). Thymine (10 g, 0.8 mol) was added to H_2O (50 mL) containing KOH (9 g, 0.16 mol). The procedure followed was similar to that for 1,3-dimethyluracil. 1,3-Dimethylthymine (10.4 g, 85%), is recovered as colorless crystals mp 152−153 °C (lit.[26] mp 153 °C); ^{1}H NMR ($CDCl_3$): δ 7.26 (s, 1H), 3.37 (s, 3H), 3.36 (s, 3H), 1.94 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 164.12, 152.00, 138.91, 109.64, 36.64, 27.96, 12.97. Low resolution MS: *m/z* (relative intensity) 154 (M^+, 100), 97 (26), 70 (59).

1,3,6-Trimethyluracil (TMU). 6-Methyluracil (5 g, 0.4 mol) was added to H_2O (100 mL) containing NaOH (12 g, 0.3 mol). The solution turned clear and was placed in an ice bath. Dimethyl sulfate (50 mL, 0.5 mol) was added dropwise with stirring. After the addition, the solution was heated until it boiled, cooled, and extracted with three aliquots of $CHCl_3$ (100 mL). The $CHCl_3$ layers were combined, dried over $MgSO_4$,

and filtered, and the the solvent was removed by rotary evaporation. The solid was recrystallized twice from C_2H_5OH (5.6 g, 92%): mp 111−112 °C (lit.[27] mp 113 °C); ^{1}H NMR ($CDCl_3$): δ 5.57 (s, 1H), 3.35 (s, 3H), 3.28 (s, 3H), 2.19 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 162.29, 152.52, 151.32, 101.12, 31.57, 27.80, 20.07. Low resolution MS: *m*/*z* (relative intensity) 154 (M^+, 100), 97 (21), 82 (62), 55 (66).

Cyclobutyldimethylthymine Dimers (csDMTD, tsDMTD). 1,3-Dimethylthymine (8.5 L, 1 mM aqueous solution) was frozen in batches into sheets (ca. 4 mm thick) and irradiated for 2 h using a 450 W medium-pressure Hg-vapor lamp fitted with a Vycor filter. During irradiation the solution was kept frozen on a bed of dry ice. After 2 h, the solution was thawed, and the solvent was removed by rotary evaporation. The residue, a yellow oil, was dissolved in CH_3OH and spotted onto a preparatory TLC plate (2000 μm). The plate was developed first in 60:40 EtOAc:hexane and then in 85:15 EtOAc:hexane. The silica gel containing the *cis-syn* dimer, the lower band (R_f = 0.06), and the *trans-syn* dimer, the middle band (R_f = 0.15), were isolated and individually washed with CH_3OH (ca. 15 mL). The silica gel was removed by vacuum filteration, and the solvent was subsequently removed by rotary evaporation. The resulting solids were individually recrystallized twice from CH_3-OH.

***cis-syn*-Cyclobutyldimethylthymine Dimer (csDMTD)** (0.108 g, 4%): mp 249−253 °C (lit.[28] mp 251 °C,); ^{1}H NMR ($CDCl_3$): δ 3.69 (s, 2H), 3.11 (s, 6H), 2.97 (s, 6H), 1.47 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 169.39, 152.37, 60.51, 47.49, 35.69, 28.07, 19.24. Low resolution MS: *m*/*z* (relative intensity) 154 (100), 97 (38), 70 (74), 69 (47) (the M^+ peak is not visible because the compound splits into its constituent halves under the MS conditions).

***trans-syn*-Cyclobutyldimethylthymine Dimer (tsDMTD)** (0.22 g, 9%): mp 258−262 °C (lit.[29] mp 255 °C). ^{1}H NMR ($CDCl_3$): δ 3.32 (s, 2H), 3.14 (s, 6H), 3.06 (s, 6H), 1.56 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 169.54, 151.75, 64.35, 36.14, 27.76, 24.92, 12.02. Low resolution MS: *m*/*z* (relative intensity) 154 (100), 140 (24), 97 (15), 70 (79), 69 (78) (the M^+ peak is not visible because the compound splits into its constituent halves under the MS conditions).

Fluorescence Quenching Experiments. A stock solution of the fluorescent sensitizer is prepared by sonicating the desired compound (1−3 mg) in spectroscopic grade CH_3CN (100 mL) for 30 min. This process results in a sensitizer concentration of about 10^{-2} M. This solution (2.5 mL) is placed in a quartz cuvette, sealed with a septum, lined with Teflon tape to prevent contamination, and then purged with Ar for 15 min. A stock solution of the quencher (200 mM) is prepared by dissolving the appropriate amount in spectroscopic grade CH_3CN and sonicating the resulting solution for 30 min. Aliquots (12.5 μL) of the quencher solution are injected into the sealed cuvette containing the sensitizer. This results in the increase of the quencher concentration in the cuvette by 1 mM steps. The fluorescence scan of the sensitizer is recorded at each step (0−6 mM). The excitation wavelength of the sensitizer (330 nm) is chosen in order to ensure that none of the light is absorbed by the quencher. The fluorescence quenching rate constants k_q were determined from a Stern−Volmer analysis.[30]

Data Analysis. The procedures for fitting the k_q data to the Rehm−Weller relationship is described in some detail elsewhere.[31] Basically a simplex minimization algorithm was used to minimize the sum of the squares of the differences between the experimental data and a theoretical curve that was generated from the parameters E_{red}, λ, and $k_{max}K_{diff}$. In order to determine the uniqueness of the fits, only two of the parameters were adjusted in the algorithm while the remaining parameter was held fixed at a number (20−50) of preselected values. The best fit was selected from the case where the sum of the squares was minimized and through visual inspection of the 20−50 fits. This procedure was repeated three times for each reported fit, "fixing" each of the three parameters in turn and allowing the remaining two to relax. For all of the cases reported here, this procedure converged on the same best fit values (to within the stated uncertainties) regardless of which parameter was "fixed" and which were allowed to relax. We estimate the uncertainty in E_{red} to be ±0.08 V and in λ to be ±5 kcal/mol.

Results and Discussion

Three types of structural variations were the focus of the present study. First, it was of interest to determine to what degree methyl substitution influences the E_{red} of the pyrimidine derivatives. To this end we examined 1,3-dimethyluracil (DMU) and 1,3,6-trimethyluracil (TMU) and compared their behavior with that of 1,3-dimethylthymine (DMT), which has been previously examined. The syntheses of these substrates follow well-known procedures which are described in the Experimental Section. Second, it was of interest to determine the effects of saturating the 5,6-double bond in the pyrimidines. To this end 1,3-dimethyl-5,6-dihydrouracil ($DMTH_2$), 1,3-dimethyl-5,6-dihydrothymine ($DMTH_2$), and 1,3,6-trimethyl-5,6-dihydrouracil ($TMUH_2$) were synthesized and examined. Finally, the *trans-syn*-cyclobutane dimer of dimethylthymine (tsDMTD) was examined in order to explore the effect of stereochemistry on the ability of the bases to accept electrons. The *trans-syn* dimer has the same bonds and connectivity as the previously studied *cis-syn* dimer (csDMTD). However the two diastereomers differ in the relative spatial arrangement of the thymine rings. In the *cis-syn* both thymine rings are on the same face of the cyclobutane ring and in the *trans-syn* they are on the opposite face. Structures of all of the substrates are shown in Table 1.

Analysis of fluorescence quenching rate constants was used to determine the reduction potentials of the substrates. Similar methods have been previously employed by us[31,32] and others.[33−35] This technique holds several advantages over the more typical electrochemical methods. First, the measurements can be made in homogeneous solutions in the absence of added salts. Second, the potentials of very unstable organic radical ions can be measured with reasonable accuracy. Of course, fluorescence quenching is less direct than any equilibrium-based measurement. The accuracy of the values thus derived are dependent on the accuracy of the model which relates the kinetic information to the desired thermodynamic quantities. However, as the goal here is to identify how small structural alterations affect E_{red}, the absolute values of E_{red} are of less importance than how they change and in what direction.

Electron transfer from the excited state sensitizers (*S**) to the pyrimidine derivatives (*Q*) follows the kinetic scheme given in eq 1.[36] The quenching of *S** involves a diffusive encounter of

$$S + Q \xrightarrow{h\nu} S^* + Q \underset{k_{-diff}}{\overset{k_{diff}}{\rightleftharpoons}} (SQ)^* \underset{k_{-ct}}{\overset{k_{ct}}{\rightleftharpoons}} (S^{+\bullet}Q^{-\bullet})^* \xrightarrow{k_3} S^{+\bullet} + Q^{-\bullet} \quad (1)$$

*S** with *Q*. (k_{diff}) to form the so-called precursor complex, followed by a charge transfer step (k_{ct}) to form the successor complex.

Fluorescence quenching rate constants, k_q, for a series of sensitizers with each of the substrates were determined by Stern−Volmer analysis.[30] The sensitizers employed are the same as the series that was used in a previously published study.[31] The list of sensitizers and their photophysical properties,

along with the k_q values for each substrate, is available as Supporting Information. In the series the excited state oxidation potential, E_{ox}* varies from −2.1 to −3.3 V (vs SCE). For each of the substrates, k_q increases as E_{ox}* for the sensitizer becomes increasingly negative. As shown below in Figure 1, log(k_q) approaches an asymptotic value that is at or near the diffusion limit, k_{diff}, in the solvent (CH_3CN; taken as 1.91×10^{10} M^{-1} s^{-1}).[37]

The strong correlation of k_q with E_{ox}* supports an electron-transfer mechanism for quenching. Energy transfer is ruled out for two reasons. First the lowest energy absorption bands of the substrates are all well below 300 nm. Thus, their singlet energies can be safely assumed to be >100 kcal/mol. This value is more than 15 kcal/mol higher than that of the highest energy sensitizer employed in this study. Secondly, the a plot of log(k_q) vs the sensitizers singlet energy reveals no discernable correlation (data not shown).

In principle, some sort of bimolecular photochemical reaction could also give rise to quenching. To test for this, several combinations of sensitizers and substrates were subjected to prolonged irradiation and analyzed by HPLC. No decomposition of the substrates could be detected. The results from this control experiment are also consistent with the electron transfer mechanism.

The plots of log(k_q) vs E_{ox}* for each of the pyrimidine derivatives were fit to the Rehm−Weller relationship (eqs 2−4).[38,39] The adjustable parameters were E_{red}, the reduction

$$k_q = \frac{k_{diff}}{1 + \frac{k_{diff}}{k_{diff}k_{max}} \exp\left(\frac{\Delta G_{ct}^{\ddagger}}{RT}\right)} \quad (2)$$

$$\Delta G_{ct}^{\ddagger} = \left[\left(\frac{\Delta G_{ct}}{RT}\right)^2 + \left(\frac{\lambda}{4}\right)^2\right]^{1/2} + \frac{\Delta G_{ct}}{2} \quad (3)$$

$$\Delta G_{ct} = 23.03\left(E_{ox} - E_{red} - \frac{q^2}{r\epsilon}\right) - E_{oo} \quad (4)$$

potential of the pyrmidine derivative, λ, the reorganization energy, and $k_{max}K_{diff}$. The latter is the product of k_{max}, the pre-exponential term in the Eyring expression for the rate constant of the charge transfer step (k_{ct}), and K_{diff}, which is the equilibrium constant for formation of the reactive precursor complex. The curves calculated from the Rehm−Weller relationship show excellent agreement with the experimental data. Figure 1 shows representative fits for tsDMTD and DMU. The best fit parameters of these and the other substrates are presented in Table 1. Some data from the earlier study are also included for comparison purposes.

We also attempted to fit the data to the Marcus equation. However, in many cases, this model failed to give reasonable fits with physically meaningful parameters. It has been widely known that Marcus theory, while highly successful in predicting electron transfer rates in rigid systems[40,41] and ion pairs,[42] often fails to predict bimolecular excited state quenching behavior. This is because the inverted region predicted by Marcus theory is often absent[43,44] or obscured.[45]

To the extent that reasonable fits could be obtained, the same trends in E_{red} were observed with the Marcus relationship. The latter consistently predicts higher (i.e. less negative) values for E_{red} and much higher values for λ than the fits to the Rehm−Weller relationship. Soumillion[33] and Schuster[34,35] have both studied ion-neutral excited state quenching processes. In their systems agreement with Marcus theory could be obtained when $k_{max}K_{diff}$ was assumed to be larger (10^{12}−10^{14} M^{-1} s^{-1}) than

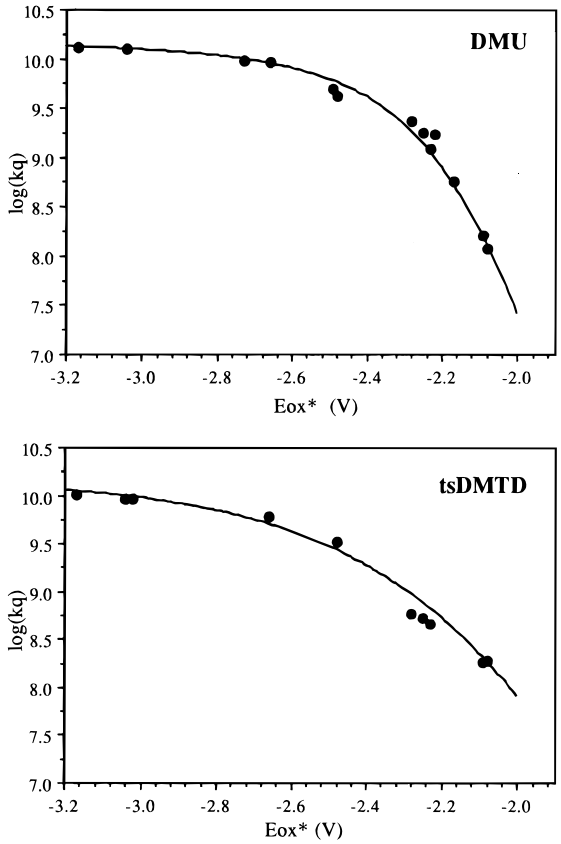
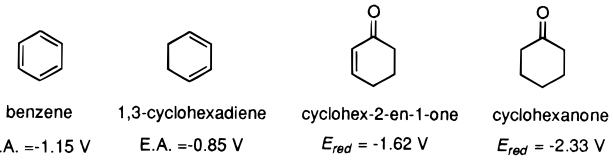


Figure 1. Rehm−Weller plots for *trans-syn*-pyrimidine−cyclobutane dimer of dimethylthymine (tsDMTD, upper panel) and 1,3-dimethyluracil (DMU, lower panel). The k_q values were measured in CH_3CN solvent.

CHART 2: Aromaticity vs Increased Conjugation



originally assumed by Rehm and Weller. However with the compounds in our study, reasonable fits to the Marcus theory required $k_{max}K_{diff}$ to be much smaller than this (10^9−10^{11} M^{-1} s^{-1}).

One objective of this study was to determine how saturation of the 5,6-double bond in pyrimidines affects E_{red}. Examination of similar compounds in the literature reveals two limiting cases. First, reducing the extent of conjugation in a π-system usually makes one-electron reduction more difficult. For example, cyclohexanone has a much more negative E_{red} (−2.33 V) than its α-β unsaturated analog, cyclohex-2-en-1-one (−1.62 V)[46] (see Chart 2). This analogy would predict that the 5,6-dihydropyrimidines would have more negative E_{red}. On the other hand, uracil and thymine are, at least formally, aromatic systems. Generally aromatic molecules are more difficult to reduce than their homologs where one of the double bonds is reduced. For example, benzene has a lower gas phase electron affinity (−1.15 eV) than 1,3-cyclohexadiene (−0.85 eV).[47] This analogy would predict that the the 5,6-dihydropyrimidines would have more positive E_{red}.

The results from the current study show that the latter analogy is more accurate. That is, the effect of aromatic stabilization, which makes one-electron reduction more difficult, predominates over the effect of increasing conjugation, which should have made one-electron reduction easier. The 5,6-dihydropyrmin-

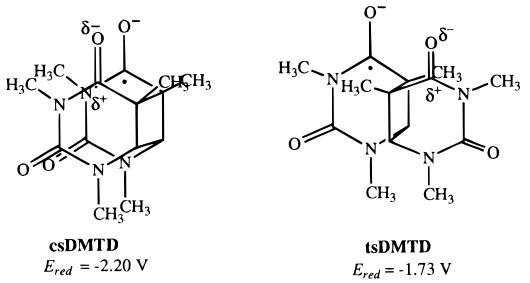


Figure 2. Stereoelectronic effects in cyclobutane dimer anion radicals csDMTD and tsDMTD.

idines are slightly more easily reduced having E_{red} values that range from −1.90 to −2.07 V. The corresponding pyrimidines have E_{red} values that range from −2.06 to −2.14 V. The differences are not dramatic, but they are consistent throughout the series when each pyrimidine is compared to its hydrogenated analog. We would therefore predict that, in the absence of any additional structural perturbations, modifications of pyrimidines that involve loss of the 5,6-double bond are likely to make the base more easily reduced.

The two cyclobutane dimers, csDMTD and tsDMTD, illustrate an interesting stereoelectronic effect on E_{red}. These two diastereomers are similar to the 5,6-dihydropyrimidines in that they have saturated 5,6-double bonds. However, unlike the 5,6-dihydro derivatives, the cyclobutyl dimers have inductively electron-withdrawing carbonyl groups attached directly to the C-5 position. One might predict that this additional carbonyl group would make the system easier to reduce, and thus E_{red} would be expected to become less negative. This does in fact appear to be the case for tsDMTD. The E_{red} for this compound (−1.73 V) is considerably less negative than its 5,6-dihydro analog, $DMTH_2$ (−2.07 V).

In contrast, the *cis-syn* diastereomer csDMTD (−2.14 V) is considerably *more* difficult to reduce than either $DMTH_2$ or tsDMTD. We attribute this to unfavorable charge−dipole interactions that are present in csDMTD but not in the other two species. This is illustrated in Figure 2.

Semiempricial calculations done on uracil−cyclobutane dimer anion radical by Rösch[48] show that the charge is localized on only one of the pyrimidine residues. Furthermore the negative charge is localized mostly on *O*^{4} (i.e. the carbonyl group α to the 5,6-bond). In csDMTD this charge is held next to the unreduced carbonyl group. The unreduced carbonyl has the negative part of its dipole directly across from the anion site. This results in an unfavorable charge−dipole interaction which destabilizes the anion radical and thus makes E_{red} more negative. In the tsDMTD the unreduced carbonyl group is held further away from the anion radical center, and the favorable inductive effects prevail.

Methyl substitution has a much less pronounced effect on the ability of the modified bases to accept an electron. Comparison of the three 5,6-unsaturated pyrimidines, DMU, DMT, and TMU, reveal no change in E_{red} beyond experimental uncertainty. With the dihydro systems $DMTH_2$ shows a more negative E_{red} than either $DMUH_2$ or $TMUH_2$. Methyl groups are weak, inductive electron-donating groups. It could be argued that the 5-methyl group in DMTH2 is located more closely to the site of higher charge density (i.e. the *O*^{4} in the anion radical) than it is in $TMUH_2$ (where it is in the 6-position) or $DMUH_2$ (which lacks the methyl group). A similar effect is not observed in the 5,6-unsaturated systems because the negative charge in the anion radical state is presumably much delocalized over the entire aromatic ring.

TABLE 2: Solvent Effects on the Reduction of $TMUH_2$

	THF	CH_3CN	CH_3OH
E_{red} (V)[a]	−1.97	−1.91	−1.51
λ (kcal/mol)[b]	21	22	36
$k_{max}K_{diff}$ ($\times 10^{-9}$ M^{-1} s^{-1})	80	40	160

[a] ±0.08 V. [b] ±10 kcal/mol.

Solvent effects were also examined. We were particularly interested in determining whether the hydrogen bonding would strongly alter E_{red}. Duplex DNA is a complex environment that is difficult to model accurately with any particular solvent. On one hand, the bases in duplex DNA are buried in a hydrophobic core and are thus not in a truly aqueous environment. On the other hand, the complementary base on the opposite strand provides specific and directional hydrogen bonds. Thus the duplex DNA environment also differs from an aprotic organic solvent. Both of these effects should be considered when comparing data from free solution to behavior in native DNA strands.

Previous studies on thymine and cytosine have revealed significant discrepencies in the E_{red} values determined in aqueous media compared with those determined in aprotic solvents. For example DMT gives an E_{red} of −2.14 V in CH_3CN,[31,32] but a value of −1.1 V was reported in H_2O.[4] In an earlier paper we attributed this large difference to specific H-bonding interactions between the solvent and the anion radical which were operative in protic solvents such as H_2O but not in aprotic solvents such as CH_3CN.[31] In addition to making E_{red} less negative such a solvent effect would also be expected to increase the λ value.

Fluorescence quenching experiments with Rehm−Weller analysis were carried out on $TMUH_2$ in three solvents: CH_3OH, tetrahydrofuran (THF), and CH_3CN. The best fit parameters from these experiments are presented in Table 2. The aprotic solvents, THF and CH_3CN, give almost indistinguishable values for E_{red} and λ. On the other hand, CH_3OH shows a large (ca. 400 mV) stabilization of the anion radical as seen in its less negative E_{red}. Moreover, as predicted, λ is increased by 14 kcal/mol from its value in the aprotic media. Thus, the data here are consistent with the earlier proposal of H-bonding to the anion radicals. We would therefore predict that the E_{red} values should be increased (i.e. made more favorable) by at least several hundred millivolts in aqueous media.

Conclusion

Three general trends in the reduction potentials are identified here. First, it is shown that 5,6-dihydropyrimidines undergo one-electron reduction more easily than their unsaturated analogs. This is attributed to aromatic stabilization which is present in the parent pyrimidines but not in the 5,6-dihydro derivatives. Second, the *cis-syn*-cyclobutane dimer of dimethylthymine is more difficult to reduce than its *trans-syn* diastereomer. In the *cis-syn* case, the corresponding anion radical apparently suffers from a destabilizing charge−dipole interaction that balances, and in fact exceeds, the stabilization afforded by the inductively withdrawing carbonyl groups. Finally it is shown that protic solvents significantly stabilize the anion radical of 5,6-dihydro-1,3,5-trimethyluracil, presumably through a specific hydrogen-bonding interaction.

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Supporting Information Available: List of sensitizers employed along with their photophysical parameters and their

k_q values for each of the quenchers (1 page). Ordering information is given on any current masthead page.

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