# Triplet State Interactions between Nucleic Acid Bases in Solution at Room Temperature: Intermolecular Energy and Electron Transfer

**Paul D. Wood and Robert W. Redmond***

*Contribution from the Wellman Laboratories of Photomedicine, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114*

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**Abstract:** This study aims to provide information on the fate of triplet energy in DNA by considering the triplet-mediated reactions which occur between nucleic acid bases in solution, at room temperature. Following sensitization of base triplet states by acetone, the subsequent photophysics and photochemistry are highly dependent on the nature of the nucleotide pair under study. By establishing the direction of triplet energy transfer between pairs of mononucleotides, the relative triplet energy ordering was determined under physiologically relevant conditions, i.e. aqueous solution at room temperature. This order (C > U > G > A > T) is in agreement with that previously reported which was obtained at 77 K in rigid media. The absolute value for TMP triplet energy has been estimated as 310 kJ $mol^{-1}$, based on triplet energy transfer studies involving acetophenone and 3-methoxyacetophenone. Determination of the triplet energy gaps between all mononucleotides, to within 1 kJ $mol^{-1}$, has allowed an estimation of the absolute values of CMP (321 kJ $mol^{-1}$), UMP (320 kJ $mol^{-1}$), GMP (317 kJ $mol^{-1}$), and AMP (314 kJ $mol^{-1}$). The energy gaps between the triplet states are smaller than those reported at low temperature. This allows triplet energy transfer equilibria to be established in which a significant proportion of both triplets is present, unless thymine is present, in which case the dominant process is triplet energy transfer to the thymine. In any purine/pyrimidine (not thymine) pair, electron transfer from purine to pyrimidine is significant, producing the purine radical cation, which rapidly deprotonates to give the neutral radical. This process is most efficient for the guanosine−uracil combination and our evidence suggests that it is the purine triplet state which initiates the electron transfer. In the guanosine−adenosine system, there is no evidence of electron transfer and *net* triplet energy transfer is low due to a small triplet energy gap. This results in a relatively high proportion of both triplet states being observed in the equilibrium. These results show that DNA photochemistry should be highly sequence dependent and may have significance regarding the existence of "hot spots" in DNA photoproduct formation.

## Introduction

The photochemistry of DNA has been the subject of widespread experimental study but its complexity has resulted in a lack of a comprehensive description; many factors are known to be of importance in dictating the eventual consequences of DNA irradiation. In the photoexcitation of DNA, processes originating from the first excited singlet state dominate. In particular, a quantum yield for internal conversion of almost unity provides a high degree of self-protection.[1] Important processes, such as photoionization, have been shown to arise from the singlet excited state,[2−6] but the very short lifetimes of these species (picosecond range at room temperature[1]) complicates their experimental study. However, for the same reason, bimolecular reactions are less likely to involve the singlet state. Consequently, despite the fact that triplet state formation has a low quantum yield (<0.01 in water[2,7−9]), the longer-lived triplet state is of interest when considering excited state DNA chemistry. For example, it is known that the important cyclobutylpyrimidine dimer photoproduct can be formed *via* triplet state intermediacy.[10,11]

Literature concerning the photoexcitation of DNA at low temperatures suggests that facile intramolecular triplet energy transfer may occur between adjacent nucleic acid bases.[12,13] For chemical processes which involve the triplet state, intramolecular energy transfer will render the site of initial photoexcitation less important than the relative order of the base triplet energies and the sequence of nucleic acid bases; that is, energy transfer will continue along the strand until an energy minimum is reached.[7,12] Such interpretations are hampered by the limited information available on the triplet states of purines and pyrimidines and triplet interactions between neighboring bases on a DNA strand.[8,9,14−20] Despite their obvious importance, neither the absolute triplet energy values nor their relative order have been

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determined for physiologically relevant conditions. Literature values[21] are available only for blue-edge phosphorescence measurements at 77 K in rigid media.

We have recently presented[22] comprehensive absorption spectra and an approximate energy level ordering of the triplet states of guanosine (GMP), adenosine (AMP), uridine (UMP), cytidine (CMP), and thymidine (TMP) monophosphates. The latter was obtained using sensitized triplet energy bracketing techniques. However, due to a lack of suitable high-energy triplet state sensitizers, the specific order of triplet energies for the mononucleotides could not be determined, other than that TMP was clearly the lowest, as expected. In experiments involving the purines GMP or AMP, reaction with acetone triplet led not only to triplet energy transfer but also to formation of the nucleotide radical cation which rapidly deprotonated at physiological pH to form the neutral radical. This confirmed that guanine is the most easily oxidized of the bases,[4] under comparable conditions, and highlighted the potential problems of using ketone sensitization of DNA to infer triplet excited state chemistry.[23,24]

Having observed both energy and electron transfer processes in acetone sensitization of mononucleotides in our previous work characterizing base triplet states, we have now turned our attention to triplet state processes which occur *between* different bases. A full rationalization of the interactions between any possible combination of two nucleic acid bases, following triplet state formation, should make it possible to infer the consequences of photoexcitation in a longer DNA strand.

Studies are described here in which the fate of triplet excitation energy has been studied in systems containing pairs of mononucleotides in neutral, aqueous solution at room temperature. The fate of the initial triplet excitation energy has been determined by monitoring time-resolved changes in transient absorption spectra and comparing the observed changes to those predicted in computer simulation programs. This is made possible by accurate knowledge of the triplet state[22] and radical[4,25,26] absorption spectra of the individual mononucleotides. The interaction between base triplets is shown here to be completely dependent on the specific base pair involved, their respective triplet energies, and oxidation potentials, and it leads to general conclusions concerning the dependence of DNA photochemistry on the base sequence and the generation of specific photoproducts at particularly favorable sequences.

## Experimental Section

**Chemicals.** Uridine 5′-monophosphate (UMP), thymidine 5′-monophosphate (TMP), cytidine 5′-monophosphate (CMP), adenosine 5′-monophosphate (AMP), and guanosine 5′-monophosphate (GMP) were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Acetone (Ac), acetophenone (AP), and 3-methoxyacetophenone (3MAP) were of the highest purity available from Aldrich (Milwaukee, WI) and were used without further purification.

**Laser Flash Photolysis.** The laser flash photolysis apparatus was essentially as previously described.[27,28] The excitation source for the laser flash photolysis was a Lambda Physik EMG 103 MSC XeCl excimer laser emitting 8 ns duration pulses at 308 nm. As acetone was shown previously to be of sufficiently high triplet energy to sensitize the triplet states of all the mononucleotides with rate constants $k_{Ac} = 1.9 \pm 0.7 \times 10^9\ M^{-1}\ s^{-1}$, it was used for the same purpose in these experiments. Acetone absorption was $0.68 \pm 0.02$ (i.e. ~1.0 M) at the laser excitation wavelength (308 nm), where direct absorption by mononucleotides is negligible. The laser energies were attenuated to $\leq 18$ mJ $cm^{-2}$ $pulse^{-1}$ through the use of neutral density filters and the variation in laser energy from flash to flash was within ±5%. Data acquisition was controlled by a Macintosh Quadra 630 computer using programs written using the LabView 3 software package in conjunction with NB-GPIB and LAB-NB boards (National Instruments, TX). Unless otherwise stated, experiments involved nitrogen saturated, aqueous solutions of 4.0 mM total mononucleotide concentration. A flow-through system was used to ensure irradiation of a fresh sample of solution with each laser pulse. Based on measured kinetic parameters, it was calculated that 4.0 mM nucleotide quenches 98.2% of the acetone triplet states. Experiments were carried out in both unbuffered and buffered (50 mM potassium phosphate/HCl; pH = 7.5) aqueous solution; no significant difference was found between the two systems.

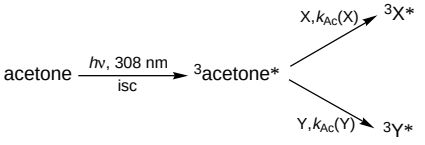
## Results

In the simplest possible triplet energy transfer experiments (eq 1), *selective* excitation is used to form only $^3X^*$, which then

$$X \xrightarrow{h\nu,\ \text{isc}} {}^3X^* \xrightarrow{Y} {}^3Y^* + X \qquad (1)$$

transfers energy to an acceptor Y. Such processes are routinely monitored by transient absorption spectroscopy. The similarity in ground state absorption spectra of nucleic acid bases[29−31] and their inherently low quantum yields of triplet state formation ($\Phi_T$) on direct excitation[2,7−9] make such an approach impractical for the study of two bases, X and Y. Acetone sensitization of nucleotide triplet states (Scheme 1) has therefore been used to

**Scheme 1**




facilitate the study of the transient absorption spectra and associated decay kinetics for mixed pairs of mononucleotides. In this case a step is added in which the triplet states of the bases ($^3X^*$ and $^3Y^*$) are sensitized by the higher energy sensitizer, acetone. Acetone has a $\Phi_T$ of unity[32] and a high (337 kJ $mol^{-1}$) triplet energy,[33] so that all of the individual mononucleotides react with the acetone triplet state with high rate constants ($k_{Ac} \approx 2.0 \times 10^9\ M^{-1}\ s^{-1}$).[22] This approach has

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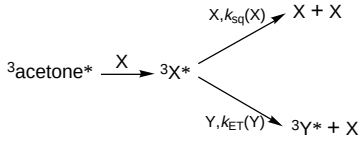
the advantage of generating nucleotide triplet states in sufficiently high concentrations (typically in the region of 50 $\mu$M in these studies) to allow their observation by transient absorption spectroscopy. However, the values of $k_{Ac}(X)$ and $k_{Ac}(Y)$ do not permit *selective* excitation of one base in the presence the other. This situation is analogous to pulse radiolysis studies in organic solvents where the initially formed triplet state of the solvent acts as the energy donor for the study of triplet interactions of multisolute systems.[34−36] In such situations, it is usually possible to effect selective excitation by using concentrations of X and Y which differ by a factor of 100. In the current study, this is not feasible due to the high self-quenching rate constants ($(0.4-4.0) \times 10^8$ $M^{-1}$ $s^{-1}$)[22] of the mononucleotide triplet states: i.e. if [Y] ≪ [X], triplet energy transfer from X to Y will not compete with self-quenching by ground state X (Scheme 2). Consequently, the best way of

**Scheme 2**




monitoring triplet energy transfer in these systems is by consideration of the time-dependent transient absorption spectra as described below.

Our previous work[22] confirmed that the mononucleotide triplet states are relatively close in energy at room temperature in aqueous solutions. Rate constants for energy transfer between two triplet states, $^3$X* and $^3$Y* (eq 2), and hence the equilibrium triplet state concentrations, depend only on the triplet energy gap, $\Delta E_T$, and are given by the Sandros equation (eq 3),[37] where $k_{max}$ is the optimum rate constant for the system.[38] The values of $k_f$ and $k_r$ differ because the energy transfer between X and Y is either "uphill" or "downhill" in terms of energy and so $\Delta E_T$ is either positive (uphill) or negative (downhill).

$$^3X^* + Y \underset{k_r}{\overset{k_f}{\rightleftharpoons}} X + {}^3Y^* \tag{2}$$

$$k_{ET}(\text{energy transfer}) = \frac{k_{max} e^{-\Delta E_T/RT}}{e^{-\Delta E_T/RT} + 1} \tag{3}$$

$$\Delta E_T = -RT \ln K \tag{4}$$

If $\Delta E_T \geq 12$ kJ $mol^{-1}$, the equilibrium constant, K, ≥127 (from eq 4) and back energy transfer is negligible. As the energy gap decreases, so the contribution from back transfer (and hence the equilibrium concentration of $^3$X*) increases. When the triplet states are isoenergetic, $k_r = k_f = k_{max}/2$. As the excited state concentrations are much smaller than the ground states ([X] = [Y] = 2 mM; [$^3$X*] and [$^3$Y*] < 50 $\mu$M), the equilibrium constant, *K* (eq 2), for our system is determined by the ground state concentrations and the relative triplet state energies (which in turn determine the rate constants for energy transfer in each direction, $k_f$ and $k_r$). In our experiments with equimolar X and Y, *K* will depend only on $\Delta E_T$.

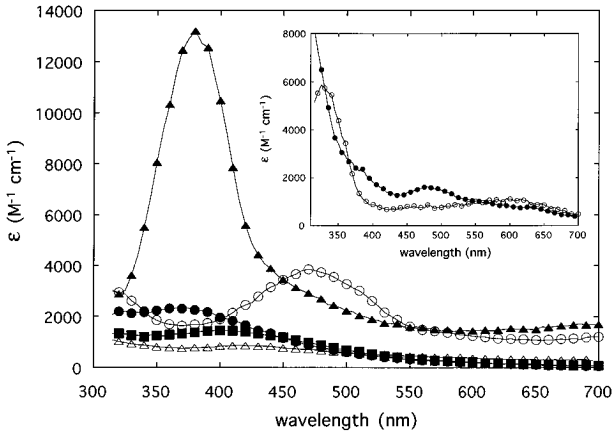



**Figure 1.** Corrected triplet−triplet absorption spectra of GMP (▲), AMP (○), CMP (△), TMP (●), and UMP (■). Insert: Transient absorption spectra of GMP (●) and AMP (○) neutral radicals.

Triplet energies can be accurately determined by monitoring the kinetics of either the approach to or the decay of triplet energy transfer equilibria involving standard sensitizers.[34−36] However, it proved impractical to use this kinetic approach to determine the triplet energy levels for the mononucleotides due to (a) spectral overlap and relative absorption coefficients of the respective triplet−triplet absorptions which prevent discrimination, (b) complications in analysis of the decay kinetics associated with significant self-quenching, and (c) the suspected involvement of electron transfer in the quenching process for some combinations (*vide infra*).

Rather than carry out kinetic analyses at a few wavelengths, an alternative approach is to analyze the complete time dependent transient absorption spectra of such systems in terms of contributions from the individual components. The transient absorption spectra of mononucleotide mixtures will comprise the absorption characteristics of the two individual mononucleotide triplet states and any other transient species which are formed. Its time evolution will be determined by kinetic parameters of the processes which occur in the system as a whole. For reasons outlined above, if only 1:1 mixtures are employed, then the proportion of each triplet state present at equilibrium will depend directly on $\Delta E_T$. In order to carry out this procedure, the triplet absorption spectra of the individual mononucleotide triplet states were used as the reference data. For comparison, these are reproduced in Figure 1, together with the residual purine neutral radical absorptions.[22]

**Transient Absorption Spectra.** Transient absorption spectra were obtained for all possible 1:1 combinations of the five mononucleotides in deaerated, aqueous solution. The spectra were obtained under identical experimental conditions[39] as those employed to obtain the individual mononucleotide spectra, thus making all spectra directly comparable. The time-dependent change in the transient absorption was the sum of two first-order processes: a growth due to reaction of both bases with acetone triplet, followed by a decay. The rate constant for the latter process was independent of monitoring wavelength. Electron transfer to acetone triplet was seen to occur in significant yields for the purines. In these cases, the triplet absorption decayed to reveal the relevant purine neutral radical,

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(38) Acetone is of sufficiently high triplet energy for energy transfer to the bases to occur at optimum rates and it was assumed that a value of 2.0 $\times 10^9$ $M^{-1}$ $s^{-1}$, typical of the rate constants determined under the present conditions, corresponded to $k_{max}$ for the present system.

(39) All solutions were optically matched at the excitation wavelength and laser energies were kept constant throughout. The optical path was identical for all of the experiments described. These practical considerations and knowledge of the energy transfer rates together make it possible to compare directly the 4 mM mononucleotide solutions used in this study.

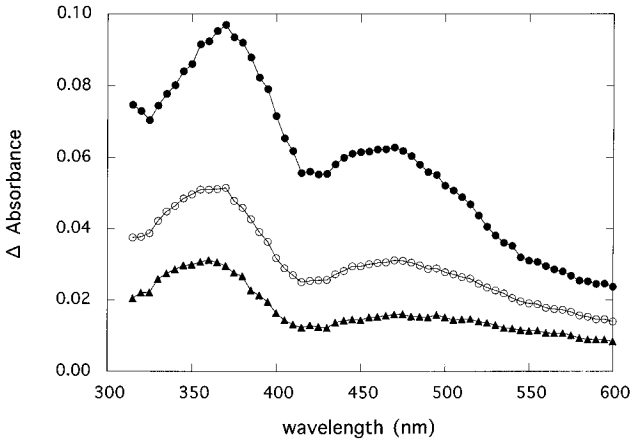



**Figure 2.** Transient absorption spectra (with residual radical absorption subtracted) obtained following 308-nm excitation of a deaerated aqueous solution of acetone (OD = 0.68) containing GMP and AMP (2 mM each) at delays of 1.4 (●), 2.8 (○), and 4.6 $\mu$s (▲) following the laser pulse.

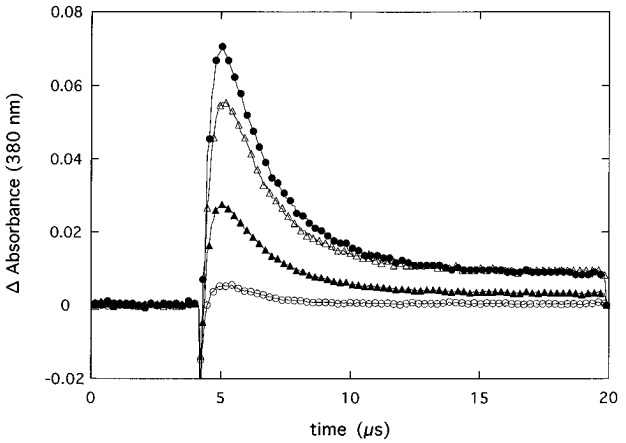



**Figure 3.** Transient decay profiles at 380 nm obtained following 308-nm excitation of a deaerated, aqueous solution of acetone (OD = 0.68) containing GMP alone (2 mM; ●), CMP alone (2 mM; ○), and GMP *and* CMP (2 mM each; △). A theoretical combination of the two individual mononucleotide absorptions in the ratio of the respective values of $k_{Ac}$[22] (i.e. the anticipated absorption of the 1:1 mixture in the absence of energy transfer between the bases) is shown for comparison (▲).

formed by rapid deprotonation of the radical cation.[40] The neutral radical absorptions decayed slowly *via* second-order kinetics over hundreds of microseconds. Decay of the radical was negligible on the time scale of triplet decay.

In the transient absorption spectra of the GMP/AMP system, maxima were observed which corresponded to the respective mononucleotide triplet states (Figure 2). The absorptions at both 380 and 450 nm decayed *via* exponential kinetics with the same rate constant ($k_{obs} = 1.0 \times 10^6$ s$^{-1}$) at all wavelengths. This is in contrast with the two isolated mononucleotides which have self-quenching rate constants which differ by a factor of 5 and hence significantly different decay rates at equal concentrations. The triplets were efficiently quenched by oxygen and decayed to leave a radical-like absorption, presumably comprising contributions from both purines. No evidence was found of additional electron transfer between these two nucleotides. Our experimental evidence is consistent with rapid establishment of a triplet energy transfer equilibrium for many of the 1:1 mixtures. The observed decay is thus that of the triplet equilibrium. Simple observation of the absorptions of both triplets is only possible with the GMP/AMP system since these two are the only combination for which triplet absorption maxima are well separated and the absorption coefficients are of comparable magnitude.

While qualitative consideration of the spectral form revealed obvious trends in some systems (e.g. in GMP/TMP energy transfer was clearly in the direction of TMP), most combinations could not be easily interpreted in such a way. For example, the disparity in the values of the maximum absorption coefficients of $^3$GMP* (13 200 M$^{-1}$ cm$^{-1}$) and $^3$CMP* (850 M$^{-1}$ cm$^{-1}$) illustrates that although the shape of the absorption spectra (with residual absorption subtracted) of the 1:1 combination strongly resembled that of $^3$GMP*, the presence of a significant concentration of $^3$CMP* at equilibrium could not be discounted. This problem was accentuated by the fact that in the sensitization experiments carried out in this study, the rate of energy transfer, from the higher energy base to the lower, is expected to occur with similar rates as the initial sensitization step, i.e. [X] = [Y] and $k_{Ac}$(X) is comparable to $k_{ET}$(Y).

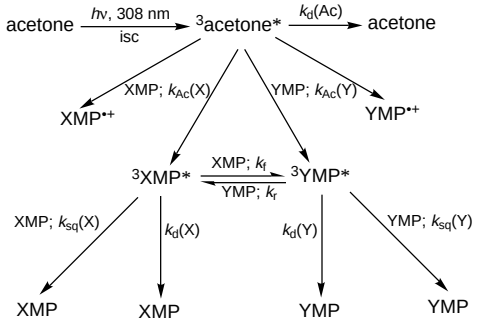
**Confirmation of Triplet Energy Transfer between Bases.** To confirm that triplet energy transfer was indeed taking place, the transient absorptions of each mononucleotide triplet (4 mM) were compared to those of the combinations (2 mM each) of two mononucleotides. In the absence of energy transfer, the amplitude of the transient absorption of the combination will depend only on the relative values of $k_{Ac}$. A deviation from this is good evidence for interbase energy transfer. Experiments confirmed the occurrence of energy transfer (Figure 3). The only combination which gave rise to essentially 100% of only one triplet state in these experiments was TMP/CMP. In this case, both the amplitude of absorption and the time-dependent decay profile were essentially identical to those of TMP alone. The conclusion is that for this pair, reverse energy transfer is negligible. This suggests an energy gap of ~12 kJ mol$^{-1}$ or more between CMP and TMP which is entirely consistent with the phosphorescence data.[21]

In theory, this type of experiment provides a way of determining the energy gaps between the triplet states since the absorption amplitude reflects the equilibrium concentrations of the two triplet states. Involvement of competing processes other than triplet energy transfer makes the analysis more complex. For example, at 380 nm the amplitude of the absorption of GMP/CMP shows that net energy transfer occurs in the direction of GMP (Figure 3). However, other experiments (*vide infra*) indicated that the reaction of $^3$GMP* with CMP (and also with UMP) also involves electron transfer. Clearly, a much more accurate consideration of the systems was necessary, one which comprised *all* relevant processes and which took into account not only the shape of the absorption spectra but also the amplitude and time dependence. Therefore a computer modeling program was written, based on our knowledge of the reactions involved. The program uses values determined for the relevant kinetic parameters to obtain accurate information about $\Delta E_T$.

**Modeling of Acetone Sensitization of Mixed Mononucleotide Systems.** Our study of acetone-sensitized mononucleotide triplet states has enabled us to describe fully the reactions occurring in the 1:1 mononucleotide systems (Scheme 3). In this scheme, $k_{Ac}$ is the rate constant for reaction with acetone triplet, $k_d$ is that for natural decay, and $k_{sq}$ corresponds to self-quenching. $^3$XMP* is higher in energy than $^3$YMP* and so triplet energy transfer between the two is accordingly "forward" ($k_f$) and "reverse" ($k_r$). A computer program was written to model this series of reactions using an appropriate series of differential equations (eqs 5−9).

(40) The p$K_a$ values for G$^{\bullet+}$ and A$^{\bullet+}$ are given by Steenken (refs 25 and 26) as 3.9 and ≤1.0, respectively, significantly below our typical pH conditions (7.0−8.0).

**Scheme 3**




$$\frac{d[^3\text{acetone*}]}{dt} = (-k_d(\text{Ac})[^3\text{acetone*}] - k_{Ac}(\text{X})[\text{XMP}][^3\text{acetone*}] - k_{Ac}(\text{Y})[\text{YMP}])[^3\text{acetone*}] \quad (5)$$

$$\frac{d[^3\text{XMP*}]}{dt} = k_{Ac}(\text{X})[\text{XMP}][^3\text{acetone*}](1 - \Phi_{rad}(\text{X})) - k_d(\text{X})[^3\text{XMP*}] - k_{sq}(\text{X})[^3\text{XMP*}][\text{XMP}] - k_f[^3\text{XMP*}][\text{YMP}] + k_r[^3\text{YMP*}][\text{XMP}] \quad (6)$$

$$\frac{d[^3\text{YMP*}]}{dt} = k_{Ac}(\text{Y})[\text{YMP}][^3\text{acetone*}](1 - \Phi_{rad}(\text{Y})) - k_d(\text{Y})[^3\text{YMP*}] - k_{sq}(\text{Y})[^3\text{YMP*}][\text{YMP}] + k_f[^3\text{XMP*}][\text{YMP}] - k_r[^3\text{YMP*}][\text{XMP}] \quad (7)$$

$$\frac{d[\text{X}_{rad}]}{dt} = k_{Ac}(\text{X})[\text{XMP}][^3\text{acetone*}]\Phi_{rad}(\text{X}) \quad (8)$$

$$\frac{d[\text{Y}_{rad}]}{dt} = k_{Ac}(\text{Y})[\text{YMP}][^3\text{acetone*}]\Phi_{rad}(\text{Y}) \quad (9)$$

We have previously determined the quantum yields of purine radical cation formation *via* reaction with acetone triplet, $\phi_{rad}$, as 0.31 for GMP and 0.09 for AMP.[22] To account for radical formation in the model, it was assumed that the quantum yield of triplet formation, $\phi_T$, is equal to $1 - \phi_{rad}$. Appropriate terms were included in the differential equations for [$^3$XMP*] and [$^3$YMP*] (eqs 6 and 7). The corresponding formation of purine radical is similarly taken into consideration through eqs 8 and 9. This species exhibits negligible decay on the time scale of interest and so this process is not included in the model. The self-quenching reaction of the bases does not give rise to any additional absorption.

Rate constants, $k_{Ac}$ and $k_{sq}$, were previously determined for all mononucleotides[22] and these values were used for the model. The observed decays (i.e. ($k_d + k_{sq}$[XMP])) of the isolated mononucleotide triplet states were measured and the values were used in the model. The program also requires values to be input for mononucleotide ground state concentrations (usually 2 mM), decay rate of $^3$acetone* (measured as $1.4 \times 10^5$ s$^{-1}$), and an estimation of the energy gap between the two triplet states, $\Delta E_T$.

Despite its apparent complexity, the only unknown parameters in Scheme 3 are $k_f$ and $k_r$. If 1:1 mixtures of mononucleotide are used, these depend only on $\Delta E_T$ and can be calculated for a range of hypothetical energy gaps using the Sandros equation[37] (eq 3) as described above. Therefore, the only variable in the model of Scheme 3 is $\Delta E_T$.

The *observed* transient absorption in mixed mononucleotide systems is simply the sum of the individual transient absorption contributions. Starting with a known value for the acetone triplet concentration, and all others set to zero, the equations determine the *changes* in the populations which occur between successive 10-ns intervals (the formation of acetone triplet state is assumed to occur prior to any of the steps in Scheme 3). In this way, arrays of transient state populations versus time are constructed. The theoretical transient absorption spectra can thus be derived, at any time after the laser pulse, by summing the product of the population and absorption coefficient of each transient, *j*, at each wavelength (eq 10).

$$\Delta A(\lambda) = \sum_1^j c_j \epsilon_j(\lambda) \quad (10)$$

This process was repeated for various $\Delta E_T$ values to produce a series of $\Delta E_T$-dependent theoretical spectra. Correlation of the best-fit theoretical spectra with the experimentally obtained spectra enables an estimation of the actual triplet energy gap. The logarithmic nature of the relationship between the energy gap and the corresponding equilibrium constant (eq 4) results in a higher rate of change in the latter as the energy gap approaches zero. Consequently, for small energy gaps (0−10 kJ mol$^{-1}$, as are thought to be involved here), the spectral differences are more pronounced and thus easier to resolve with a small experimental error. For energy differences of greater than ∼12.6 kJ mol$^{-1}$ (3 kcal mol$^{-1}$), the equilibrium constant is greater than 100. In such cases, the energy transfer is effectively irreversible and the spectrum at equilibrium will be that of the acceptor triplet alone. In such cases, it is only possible to place a lower limit on the energy gap. For systems in which the triplet states have absorption maxima which are well separated and have comparable $\epsilon$ values, it should be possible to resolve gaps up to this limit. For the current systems, we estimate that gaps which are smaller than 10 kJ mol$^{-1}$ can be resolved.

The modeling program was initially validated by generating theoretical spectra for the individual mononucleotide triplet states and comparing these to the experimental spectra. In the cases of GMP and AMP, the known significance of electron transfer from purine to acetone triplet was included in the model. The conversion of the initial radical cation into the deprotonated neutral radical is rapid[25,26] and has negligible contribution to the absorptions. This was confirmed by the correlations obtained for GMP and AMP; theoretical and experimental spectra show very similar levels of absorption and time-dependent changes therein. For all five mononucleotides, agreement between predicted and experimental spectra was excellent as shown by $^3$GMP* and $^3$TMP* in Figure 4.[41] It should be emphasized that the experimental spectra were obtained independently of the measurements of triplet absorption coefficients and $\Phi_{rad}$ values used in the model. Therefore, the excellent agreement between the two sets of spectra is strong evidence for the validity of the modeling program.

**Correlation of Mixed Mononucleotide Transient Absorption Spectra with Theoretical Models.** Each of the experimental absorption spectra for mixed mononucleotide systems was compared to a range of model spectra obtained by variation

(41) $^3$GMP* was used to normalize the acetone triplet state concentration for the correlations. The value of [$^3$Ac*] used in the model was varied until the amplitudes of the theoretical $^3$GMP* absorption spectra matched experimental amplitudes (varying [$^3$Ac*] did not affect the *shape* of the spectra). This value of [$^3$Ac*] (47 $\mu$M) was then kept constant for the generation of the other four triplet absorption spectra, since samples were optically matched and the same number of acetone triplet states would be formed in each solution.

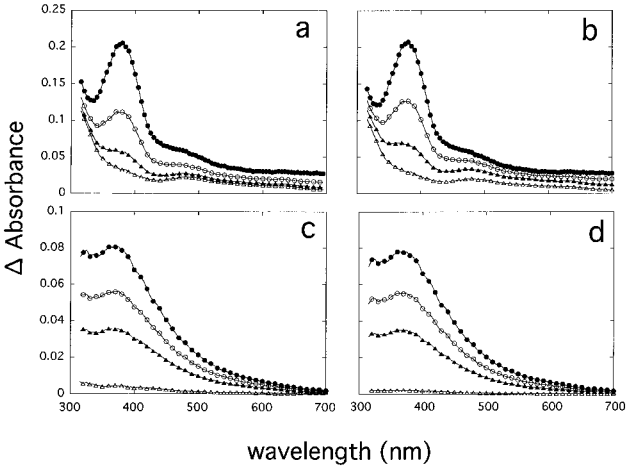



**Figure 4.** (a) Transient absorption spectra obtained following 308-nm excitation of a deaerated, aqueous solution of acetone (OD = 0.68) containing GMP (2 mM) at delays of 1.6 (●), 2.8 (○), 4.6 (▲), and 15.6 $\mu$s (△) following the laser pulse, and (b) the corresponding theoretical spectra obtained *via* computer simulation of acetone triplet sensitization of GMP (2 mM) at the same time delays. (c) Transient absorption spectra obtained following 308-nm excitation of a deaerated, aqueous solution of acetone (OD = 0.68) containing TMP (2 mM) at delays of 1.4 (●), 2.8 (○), 4.6 (▲), and 15.6 $\mu$s (△) following the laser pulse, and (d) the corresponding theoretical spectra obtained *via* computer simulation of acetone triplet sensitization of TMP (2 mM) at the same time delays.

of $\Delta E_T$ in the model. The individual base pairings fell into two distinct groups: those which could be modeled accurately by considering only triplet energy transfer between bases, and those for which a good correlation could not be obtained using this model.

**(a) Group I: GMP/TMP; GMP/AMP; AMP/TMP; CMP/TMP; CMP/UMP.** For these combinations, good correlations were achieved for energy transfer alone, allowing $\Delta E_T$ to be determined, as described. Due to the logarithmic relationship between the energy gap and the equilibrium constant (eq 4) small energy gaps can be more accurately (±1 kJ) determined; in all cases it was clear that a small deviation from the correct energy gap led to a significant mismatch of the theoretical spectra. Figure 5 illustrates the case of GMP/TMP. The values obtained for the Group I energy gaps are shown Figure 6.

**(b) Group II: CMP/GMP; CMP/AMP; UMP/GMP; UMP/AMP.** The best spectral correlations which could be obtained for these combinations all displayed the same trend in their mismatch with the experimental data: the experimental spectra exhibited more purine radical absorption (<350 nm) and less purine triplet absorption (>350 nm) than could be accounted for by the model. This strongly suggested that base triplet states were being replaced by radicals in the system. In other words, interbase reactions also resulted in radical formation, a process not accounted for in the computer model (Scheme 3).

**Confirmation of Electron Transfer from Purine to Pyrimidine.** All five mononucleotides quench acetone triplet with a similar rate constant. For the purines, some of this quenching involves electron transfer. Experiments were carried out in which solutions of a single mononucleotide (4.0 mM) were compared to pairs of mononucleotides (2.0 mM each, i.e. 4 mM total), in terms of the absorption amplitude produced. For purines, in the absence of additional radical formation steps, the amplitude of radical absorption in a 1:1 mixture should depend on the relative values of $k_{Ac}$. This was found to be the case for the GMP/AMP (Figure 7a) and GMP/TMP systems. However, for a 1:1 mixture of GMP and UMP, the residual

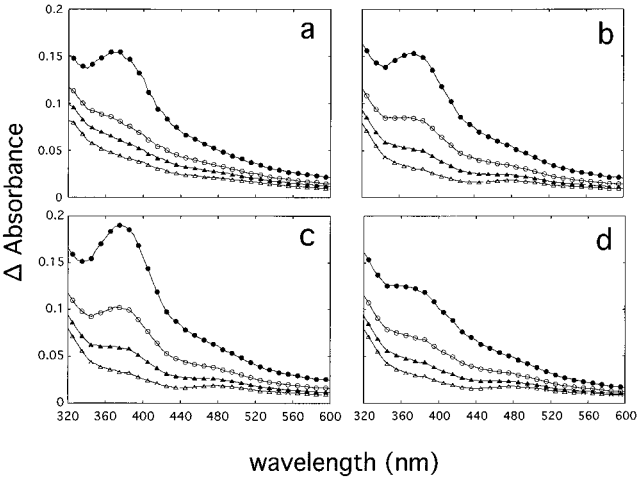



**Figure 5.** (a) Transient absorption spectra obtained following 308-nm excitation of a deaerated, aqueous solution of acetone (OD = 0.68) containing GMP and TMP (2 mM each) at delays of 1.6 (●), 5.2 (○), 9.0 (▲), and 15.6 $\mu$s (△) following the laser pulse. These are compared to theoretical spectra for the same time delays which correspond to triplet energy gaps of (b) 6, (c) 4, and (d) 11 kJ mol$^{-1}$. The latter represents the value previously determined at low temperature.[21]

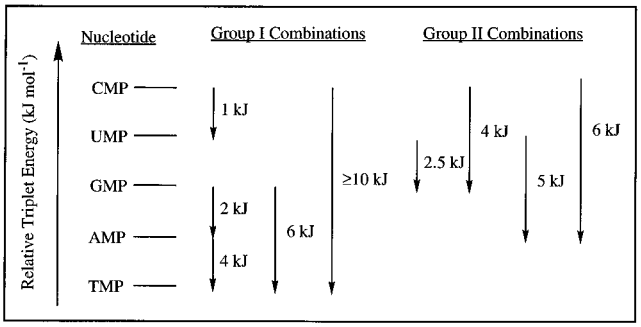



**Figure 6.** Relative triplet energy diagram showing the triplet energy gaps deduced in this study for Group I (i.e. interbase triplet energy transfer only) and Group II (i.e. interbase triplet energy and electron transfer) combinations of mononucleotides.

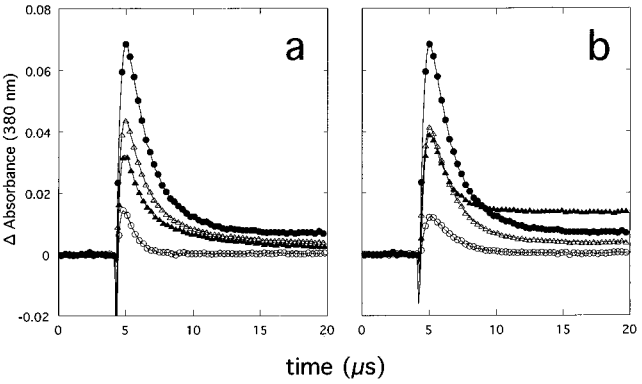



**Figure 7.** Transient decay profiles at 380 nm obtained following 308-nm excitation of a deaerated aqueous solution of acetone (OD = 0.68) containing (a) GMP (4 mM; ●), AMP (4 mM; ○), and GMP and AMP (2 mM each; ▲) and (b) GMP (4 mM; ●), UMP (4 mM; ○), and GMP and UMP (2 mM each; ▲). A theoretical combination of the two mononucleotide absorptions in the ratio of the respective values of $k_{Ac}$[22] is shown for both cases (△).

absorption amplitude was approximately double that seen for 4.0 mM GMP alone ($^3$UMP* has negligible absorption at the monitoring wavelength; Figure 7b). The enhanced residual absorption results from a triplet state mediated electron transfer reaction between GMP and UMP.

A similar result was obtained with GMP and CMP. The level of absorption at 380 nm was found to remain approximately constant for the 1:1 mixture ($^3$CMP* alone gave rise to negligible residual absorption; Figure 3). Again, the explanation is electron transfer, this time from GMP to CMP, although the smaller magnitude of the observed absorption changes suggests that the process is more favorable in the GMP/UMP system.

In experiments involving AMP with UMP or CMP, enhanced radical formation was also observed but the effect was not as large as that observed for GMP. This is consistent with both the relative quantum yields for electron transfer from GMP and AMP to acetone triplet and the conclusion of Steenken *et al.*[4] that adenosine is less easily semi-oxidized than guanosine.

**Modification of the Computer Model to Include Interbase Electron Transfer.** Having confirmed that additional electron transfer is significant for the purine−pyrimidine systems (thymine excepted), it was necessary to incorporate this reaction into the computer model. For this purpose, it was assumed that the additional electron transfer occurred from purine triplet to pyrimidine ground state (eq 11; see Discussion). Pyrimidine radical anions are also formed in this reaction, which are likely rapidly protonated, but the absorption coefficient of these species is more than a factor of 5 lower than that of the deprotonated purine radical cations in the spectral region of interest ($\lambda >$ 320 nm)[4,42] and the absorption arising from pyrimidine-derived radicals is assumed to be negligible compared to the absorption of the various triplet states and purine-derived radicals in the system. In all of the Group II combinations, the purine triplet state is lower in energy and corresponds to $^3$YMP* in Scheme 3. Additional terms were added to the differential equations to account for depletion of $^3$YMP* and formation of purine radical cation *via* this route; eqs 7 and 9 in the model were replaced with eqs 12 and 13.

$$^3\text{Pur}^* + \text{Pyr} \rightarrow \text{Pur}^{\bullet+} + \text{Pyr}^{\bullet-} \quad (11)$$

$$\frac{\mathrm{d}[^3\text{YMP}^*]}{\mathrm{d}t} = k_{\text{Ac}}(\text{Y})[\text{YMP}][^3\text{acetone}^*](1 - \Phi_{\text{rad}}(\text{Y})) - k_{\text{d}}(\text{Y})[^3\text{YMP}^*] - k_{\text{sq}}(\text{Y})[^3\text{YMP}^*][\text{YMP}] + k_{\text{f}}[^3\text{XMP}^*][\text{YMP}] - (k_{\text{r}} + k_{\text{i}})[^3\text{YMP}^*][\text{XMP}] \quad (12)$$

$$\frac{\mathrm{d}[\text{Y}_{\text{rad}}]}{\mathrm{d}t} = k_{\text{Ac}}(\text{Y})[\text{YMP}][^3\text{acetone}^*]\Phi_{\text{rad}}(\text{Y}) + k_{\text{i}}[^3\text{YMP}^*][\text{XMP}] \quad (13)$$

For these systems, in addition to an estimation of $\Delta E_{\text{T}}$, the model requires the rate constant for interbase electron transfer, $k_{\text{i}}$. For the purpose of the model it was assumed that the latter process involves the purine triplet, since it is the absorption of this species which is quenched and the process was not significant for combinations containing TMP where $^3$TMP* formation is highly favored, e.g. GMP and TMP.

By varying $\Delta E_{\text{T}}$ and $k_{\text{i}}$ for each nucleotide combination, it was possible to obtain good correlations in all cases (the energy gaps are summarized in Figure 6). For example, the correlation for GMP/UMP (Figure 8) is only accurate for an energy gap of 2.5 kJ mol$^{-1}$ (in good agreement with the phosphorescence value) and a rate constant for electron transfer of $\sim 3.5 \times 10^8$ M$^{-1}$ s$^{-1}$. Changing either of these significantly had the result that either the radical or the triplet absorption was inaccurate compared to experiment.

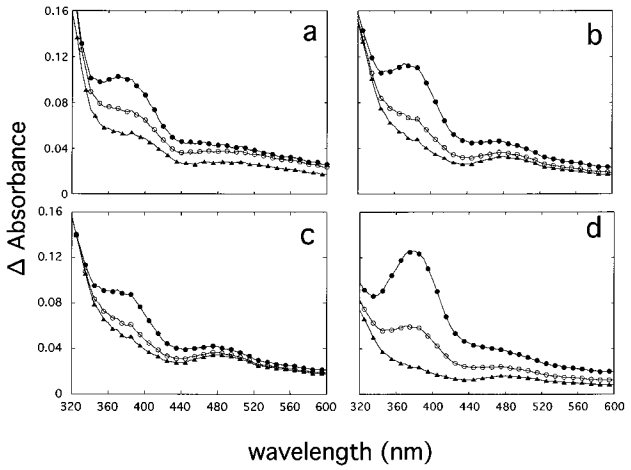



**Figure 8.** (a) Transient absorption spectra obtained following 308-nm excitation of a deaerated, aqueous solution of acetone (OD = 0.68) containing GMP and UMP (2 mM each) at delays of 2.4 (●), 3.8 (○), and 15.6 $\mu$s (▲) following the laser pulse. The spectra are compared to theoretical spectra for the same time delays which correspond to $\Delta E_{\text{T}}/k_{\text{i}}$ combinations of (b) 2 kJ mol$^{-1}$/3.5 × 10$^8$ M$^{-1}$ s$^{-1}$, (c) 0 kJ mol$^{-1}$/3.5 × 10$^8$ M$^{-1}$ s$^{-1}$, and (d) 3 kJ mol$^{-1}$/no electron transfer. The latter represents the value previously determined at low temperature.[21]

The involvement of two independent variables, $\Delta E_{\text{T}}$ and $k_{\text{i}}$, necessitated a way of checking the accuracy of the correlations. This was achieved as follows. Experimental spectra were obtained for 1:3 and 3:1 mixtures of GMP and UMP. Using the values of $\Delta E_{\text{T}}$ and $k_{\text{i}}$ determined for the 1:1 mixture, theoretical spectra were generated for the 3:1 and 1:3 mixtures. The mononucleotide ground state concentrations were the only parameters which were varied in the model. Agreement between theory and experiment for both the 3:1 and the 1:3 mixtures was excellent, thus confirming the accuracy of the $\Delta E_{\text{T}}$ value. This check was also carried out successfully for the Group I combination, GMP and AMP.

**Determination of Absolute Triplet Energies.** Having established the values of $\Delta E_{\text{T}}$ for all mononucleotide combinations, the determination of the absolute value for only one triplet energy allows all the other energies to be put on an absolute scale. Based on triplet energy bracketing experiments,[6] TMP triplet energy was estimated to be between 300 and 310 kJ mol$^{-1}$. Careful quenching experiments confirmed that acetophenone (AP; $E_{\text{T}} = 310$ kJ mol$^{-1}$)[43] transferred triplet energy to TMP with a rate constant of 2.0 × 10$^9$ M$^{-1}$ s$^{-1}$. In contrast, TMP did not quench the triplet state of 3-methoxyacetophenone (3MAP; $E_{\text{T}} = 303$ kJ mol$^{-1}$)[43] at concentrations of TMP up to 10.0 mM. The natural decay of the 3MAP triplet state was 1.2 × 10$^5$ s$^{-1}$. A factor of 2 increase in this decay in the presence of TMP would be easily detectable and so the rate constant for quenching of 3MAP triplet by TMP must be less than 1.2 × 10$^7$ M$^{-1}$ s$^{-1}$.

The triplet energy of $^3$TMP* must be low enough to allow significant energy transfer from AP triplet, but high enough to prevent significant energy transfer from 3MAP triplet. Consideration of the Sandros equation (eq 3) indicates that the only conclusion which is consistent with these observations is TMP triplet state being approximately isoenergetic with that of AP and hence ~7 kJ mol$^{-1}$ above that of 3MAP. Therefore, the triplet energy of TMP is estimated as 310 kJ mol$^{-1}$. Based on the energy gaps obtained by spectral correlation (Figure 6), the triplet energies of CMP, UMP, GMP, AMP, and TMP are

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(43) Carmichael, I.; Hug, G. L. In *Handbook of Organic Photochemistry*; Scaianao, J. C., Ed.; CRC Press: Boca Raton, 1989; Vol. I, pp 369−404.

estimated to be 321, 320, 317, 314, and 310 kJ $mol^{-1}$, respectively.

## Discussion

Consideration of the spectral as well as the kinetic and thermodynamic parameters determined in this study allows some important conclusions to be drawn concerning the dissipation of triplet energy and the relative triplet energies of the nucleic acid bases in solution at room temperature. The observed behavior depends strongly on the nature of the base pair. Triplet energy transfer contributes in all pairs until a triplet energy equilibrium is rapidly attained. The only combination with a triplet energy gap sufficient for ~100% efficient net triplet energy transfer was CMP/TMP, which supports the conclusion that CMP triplet energy is >10 kJ $mol^{-1}$ above that of TMP. In contrast, the gaps between CMP, UMP, GMP, and AMP and those between GMP or AMP with TMP are smaller since in all those cases, spectral changes are observed which are consistent with significant back energy transfer. The energy gaps which have been determined are generally smaller than those obtained at low temperature,[21] although the ordering is the same. The values obtained are supported by the excellent cross-correlation of the various mononucleotide combinations (Figure 6). Our results indicate that there is only a total triplet energy range of ~11 kJ $mol^{-1}$ over all five bases (compared to a range of 19 kJ $mol^{-1}$ in the literature). The low-temperature data puts the thymine triplet 11 kJ $mol^{-1}$ below $^3$GMP*. This corresponds to an equilibrium with a 85:1 ratio in favor of $^3$TMP*. As described earlier, the relationship between energy gap and transient absorption spectrum is such that it is easy to distinguish, for example, a gap of 11 kJ $mol^{-1}$ for GMP/TMP from the gap of 6 kJ $mol^{-1}$ which was determined (Figure 5). Our result corresponds (eq 4) to an equilibrium ratio of 7.5:1. In other words, at room temperature, thymine is not such an effective triplet energy sink as would be expected from the low-temperature energy gaps. If this is extrapolated to DNA, then it permits a more significant contribution from chemistry originating at other triplet states, such as electron transfer.

Triplet state mediated electron transfer from $^3$purine* to pyrimidine has been shown to contribute to triplet energy dissipation between appropriate base pairs. It has been proposed that formation of radical cations in DNA promotes strand breaks.[3,6,44] The current work suggests a mechanism, other than photoionization, by which such radicals can be formed and this route may well contribute to the formation of strand breaks in studies of triplet sensitization of DNA. It is not certain that this triplet mediated electron transfer is the *only* route by which additional purine radical cation is formed; Steenken has described the complexities of purine−pyrimidine radical reactivity in pulse radiolysis and photoionization experiments.[4,25,26,42] However, the evidence leads us to conclude that in these sensitized systems, interbase electron transfer is the predominant route for enhanced purine radical formation. The pair for which this mechanism is most favorable is GMP/UMP. This is consistent with the order of one-electron potential redox potentials obtained by Jovanovic and Simic.[45] However, these values were measured at pH = 13. The current study has been carried out at pH = 7−8 and Steenken[42] concluded that C, T, and U have very similar reduction potentials (~1.1 V versus NHE) at pH = 8.

It is difficult to determine experimentally which base triplet state, purine or pyrimidine, is responsible for inducing the electron transfer. Analogy with the $^3$acetone*/purine reaction suggests that it could be the pyrimidine triplet which abstracts an electron from the purine. However, this being the case, significant electron transfer to $^3$thymine* would be expected in the purine/thymine combinations. The fact that this was not observed leads us to conclude that electron transfer occurs between purine triplet and ground state pyrimidine although involvement of the pyrimidine triplet cannot be ruled out. Despite the uncertainty as to which triplet state is involved, the model remains accurate for one important reason. With the exception of thymine, the triplet energies are close enough to permit establishment of triplet energy transfer equilibria, and regardless of which triplet state is removed *via* electron transfer, in reality it is the *equilibrium* which is quenched. Removal of either triplet state *via* this route simply results in compensation from the other base. If the model is modified to include interbase electron transfer initiated by the *pyrimidine* triplet state rather than purine triplet state, then the triplet concentration in eq 13 changes and a corresponding correction to $k_i$ is required. The energy gap remains constant in the correlation.

The current work and the conclusions derived from it apply to solution chemistry of DNA models. The situation in DNA will be affected by the conformational and structural restrictions. However, the important relationship suggested here between pyrimidines and purines should be relevant. Triplet energy will be dissipated *via* a combination of energy transfer between nucleotides (which is possible in any direction between G, A, and C) and electron transfer (from purine to pyrimidine).

## Conclusions

Several important insights have been gained into the dissipation of triplet energy in any pair of nucleic acid bases. Electron transfer from purine to pyrimidine has been shown to be significant. The order of the triplet energies of the bases which was obtained at low temperature[5] has been confirmed for the first time under more physiologically relevant conditions and the energy gaps have been determined. Our results show that these gaps are smaller than those measured at low temperature and this has important chemical consequences, *viz*. the establishment of triplet energy transfer equilibria. Experiments are in progress involving a range of dinucleotides, i.e. compounds containing the bases together in pairs covalently bonded through a phosphate linkage. This work aims to determine whether the results and conclusions described herein for simple systems can be extrapolated to more biologically relevant molecules.

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