Electron Spin Resonance Study of the Temperature Dependence of Electron Transfer in DNA: Competitive Processes of Tunneling, Protonation at Carbon, and Hopping

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Received: July 20, 2000; In Final Form: September 11, 2000

In this work, we employ electron spin resonance (ESR) spectroscopy to investigate the effects of temperature on excess electron and hole transfer through DNA. The competitive processes of tunneling, protonation at carbon, and hopping are investigated in hydrated DNA solids (hydrated to 14 waters/nucleotide) and frozen glassy aqueous (D_2O) solutions of DNA intercalated with mitoxantrone (MX) at temperatures from 4 to 195 K. Monitoring the changes in the ESR signals of MX radicals, one-electron oxidized guanines ($G^{\bullet+}$), one-electron reduced cytosines [C(N3)$D^{\bullet}$ ($CD^{\bullet}$)], thymine anion radicals ($T^{\bullet-}$), and irreversibly deuterated thymine radicals [T(C6)$D^{\bullet}$ ($TD^{\bullet}$)] with time at different temperatures allows for distinguishing the thermal barriers of each process. The tunneling of electrons from DNA radicals to MX is found to be the dominant process at temperatures less than or equal to 77 K. The value of the average tunneling distance decay constant, β_{avg}, is found to be the same at 4 and 77 K. Working with hydrated DNA allows the distinction between electron adducts to cytosine and those to thymine, a distinction not possible in glassy systems. In the solid hydrated DNA, we find that $CD^{\bullet}$ does not undergo significant electron loss in the time of our experiments below 170K and that electron tunneling in DNA is mainly from $T^{\bullet-}$. Irreversible deuteration of $T^{\bullet-}$ at carbon position 6, which results in $TD^{\bullet}$, begins at 130 K and increases in relative fractions of the radicals as temperature increases. Hole and electron hopping resulting in the recombination of $G^{\bullet+}$ and $CD^{\bullet}$ are not substantial until temperatures near 195 K are reached. Above 130 K, the tunneling processes are not competitive with deuteration of $T^{\bullet-}$, and above 170 K, they are not competitive with recombination, which presumably results via activated excess electron hopping.

Introduction

Due to the biological significance of DNA damage and repair, electron and hole transfer processes in DNA have attracted considerable experimental and theoretical interest.[1−14] Recent work from photochemical studies has shown that DNA channels charge (hole) over long distances via the stacked DNA bases.[10,15−17] Hole migration within DNA in aqueous solution was suggested to be a result of tunneling through the stacked bases with a weak distance dependence. However, this became controversial, and it is now clear that the long-range hole transfer is best explained by a hopping mechanism such as multistep incoherent hopping[3,5] or phonon-like thermally activated hopping.[10] While photochemical studies have extensively addressed hole transfer in DNA, little has been reported on excess electron transfer. The results from the radiation chemistry of DNA have led to estimates of excess electron and hole transfer and have suggested that DNA at low temperatures behaves more like an insulator, with its charge transfer hindered by the competitive interstrand protonation of DNA base anion radicals and the deprotonation of cation radicals.[4,7,9,11,18,19] Long-range electron or hole transfer was questioned in these studies, as it is not found for hydrated DNA at low temperatures.

In our recent efforts, we investigated excess electron transfer for DNA in glasses at low temperatures via ESR.[9,19] These studies probed the conditions where hopping was not effective and only excess electron transfer by tunneling was functional. We reported an overall distance decay constant, β, near 1 $Å^{-1}$.[9] In our most recent work, we showed that at high concentrations of DNA in solution or for solid hydrated DNA samples, electron transfer between DNA double strands (ds's) was competitive with transfer within the ds. The distance decay constant between ds's was suggested to be only slightly higher than that along the ds.[19] These previous works assumed tunneling to be the active process at 77 K; however, no work at lower or higher temperatures was performed to investigate the temperature dependence of the electron-transfer process. A pure tunneling mechanism would be expected to have little or no temperature effect, whereas activated hopping should show significant temperature dependence (Arrhenius behavior). In our previous studies on electron transfer within DNA in glassy media, the individual DNA radical anions, ESR spectra could not be separated;[9,19] as a consequence, only the overall transfer from the combined DNA anion species was observed.

In previous low-temperature studies on hydrated (deuterated) solid DNA samples, Wang et. al. and Yan et. al. were able to separate the ESR spectra of the various radical species.[20,21] They reported that at 77 K, the one-electron reduced radicals formed in irradiated DNA were one-electron reduced cytosine C(N3)-$D^{\bullet}$ ($CD^{\bullet}$) with a smaller fraction of the thymine anion radical, $T^{\bullet-}$, while one-electron oxidized radicals resided on guanine, $G^{\bullet+}$. When DNA samples were annealed to higher temperatures, the reversible electron migration between $CD^{\bullet}$ and $T^{\bullet-}$and the irreversible protonation of $T^{\bullet-}$ into T(C6)$H^{\bullet}$ ($TH^{\bullet}$) progressed in competition with electron and hole recombination. These steps were thermally activated and slow; however, the study by Wang

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10.1021/jp0025882 CCC: $19.00 © 2000 American Chemical Society
Published on Web 10/19/2000

et al. did not probe into the time dependence of the radical composition or electron-transfer mechanisms.

In this work, we employ mitoxantrone (MX)-intercalated DNA and electron spin resonance spectroscopy to follow the time dependence of individual radical fractions as a function of temperature in solid hydrated DNA samples. Our samples are prepared in D_2O instead of H_2O, since the ESR spectra of $CD^{\bullet}$ and $T^{\bullet-}$ are considerably more resolved and distinguishable in D_2O than in H_2O matrixes.[20,21] The deuteration reactions are slower than the protonation reactions at the low temperatures employed in our work, but the overall processes are unchanged.[21] The effect of temperature is observed from 4 to 195 K and allows assigning the range of temperature in which tunneling, protonation, hopping, or recombination is dominant. These studies allow a better understanding of the relative importance of these processes. Comparison of the overall ET distance in both glassy and solid samples at 4 and 77 K as a function of time shows that the distance decay constant for tunneling, β, is the same at 4 and 77 K.

Experimental Section

Sample Preparation. *Preparation of Mitoxantrone-Intercalated DNA Solids.* Various molar ratios of salmon sperm DNA with MX were prepared by slowly adding ca. 1 mg/mL of MX aqueous solution into an aqueous solution of 100 mg/mL of DNA while stirring under nitrogen. The mixture sat in the dark for long periods (days to weeks); it was stirred periodically with a vortex mixer and then freeze-dried when the solutions appeared to be uniform.

Glassy Samples. Aqueous solutions containing 7 M LiBr were prepared by adding 0.3 mL of D_2O to 20 mg of freeze-dried MX-DNA solid, followed by 0.7 mL of 10 M LiBr. The resulting mixture was kept in the dark for several days and stirred daily with a vortex mixer until the solution became homogeneous. The solutions were drawn into thin-wall Suprasil quartz tubes 4 mm in diameter and then cooled to 77 K by immersion into liquid nitrogen, resulting in glassy homogeneous samples.

The glassy samples were γ-irradiated for the absorbed dose of 0.88 kGy (20 min). Upon the irradiation of the 7 M LiBr glass, the majority of initial ionization occurs in the solution and creates electrons and holes. The electrons are scavenged by the solutes, and the holes remain in the glass as $Br_2^{\bullet-}$. This species has a very broad ESR spectrum extending over many hundreds of gauss and does not interfere with the DNA and MX radical signals, which extend over less than 75 G at 77 K.

Hydrated Samples. About 100 mg of freeze-dried MX-DNA solid was kept for 7 days in a desiccator containing D_2O under N_2 gas. The hydrated solids were pressed into solid plugs 4 mm in diameter with an aluminum press, freeze-dried, and then kept in a desiccator containing saturated KCl aqueous (D_2O) solutions under N_2 gas for 14 days to attain a final hydration level of 14 D_2O/nucleotide based on mass measurements. The saturated KCl solution controls the humidity and the level of hydration of DNA. These samples were then placed in liquid nitrogen.

Hydrated DNA samples were γ-irradiated for a dose of 2.6 kGy (60 min). Irradiation of the hydrated solids produces both electron adducts ($CD^{\bullet}$ and $T^{\bullet-}$) and holes ($G^{\bullet+}$) within DNA.

All preparations were performed in a nitrogen atmosphere. The samples were kept in liquid nitrogen during irradiation and ESR measurements. Irradiated samples were kept in the dark throughout all experiments. For the experiments at 4, 77, and 195 K, irradiated samples were stored in liquid helium, liquid nitrogen, and dry ice, respectively. For the experiments at 130, 150, and 170 K, the samples were held in a regulated thermostat in which the temperature was controlled and kept constant to within ± 2 K by varying the flow of cooled nitrogen gas.

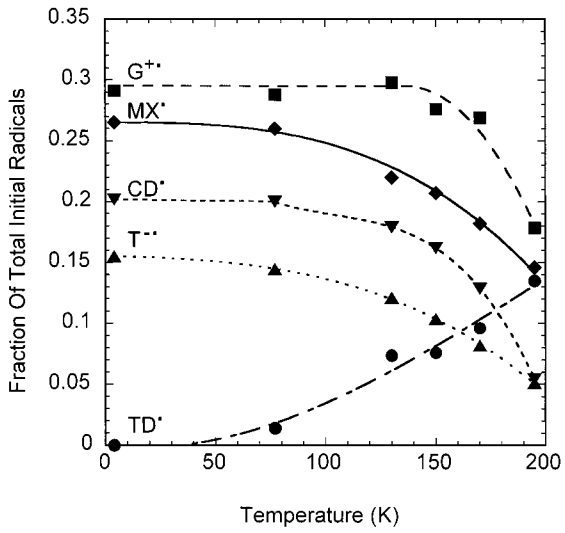

Figure 1. Temperature dependence of the relative radical composition of irradiated hydrated solid samples of MX-DNA (14 D_2O/nucleotide, $\nu = 1/214$). The fraction of MX radicals (◆), $G^{\bullet+}$ (■), $CD^{\bullet}$ (▼), $T^{\bullet-}$ (▲), and $TD^{\bullet}$ (●) relative to the total initial radicals are plotted vs temperature. The data points at each temperature are from a separate sample at 400 min after γ-irradiation. This plot shows the temperature dependence in the radical composition. The time dependencies at each temperature are shown in subsequent plots.

Methods of Analysis. *Electron Spin Resonance*. ESR spectra were taken on a Varian Century Series X-band EPR spectrometer with a dual cavity and a 200 mW klystron, with Fremy's salt ($g = 2.0056$, $A_N = 13.09$ G) as a reference. All ESR spectra were recorded at 77 K within a few minutes after irradiation and at increasing time intervals thereafter up to 12 days (for the 4 and 77 K tunneling experiments). The time of exposure of the 4 K samples to 77 K was 0.6% of the total time.

Benchmark Spectra. The methods of analyses were similar to those in our previous work.[9,19] The benchmark spectra of DNA and MX radicals in 7 M LiBr glass,[9] MX radicals in DNA solid[19] and $G^{\bullet+}$, $CD^{\bullet}$, $T^{\bullet-}$, $TD^{\bullet}$, and $TH^{\bullet}$ [21] were used in the analysis of each experimental spectrum. Linear least-squares fitting of benchmark spectra to experimental spectra is employed to determine the fractional composition of DNA and MX radicals in 7 M LiBr glass and MX radicals, $G^{\bullet+}$, $CD^{\bullet}$, $T^{\bullet-}$, $TD^{\bullet}$, and $TH^{\bullet}$ in hydrated solid.

Results

Competitive Processes at 4−195 K. ESR spectra for irradiated hydrated MX-DNA (the mole ratio of MX to DNA base pairs, $\nu = 1/214$, $\Gamma = 14$ D_2O/nucleotide) were followed at increasing time intervals at 4, 77, 130, 150, 170, and 195 K. The linear least-squares fits of benchmark spectra to experimental spectra give the fractional composition of $G^{\bullet+}$, $CD^{\bullet}$, $T^{\bullet-}$, $TD^{\bullet}$, $TH^{\bullet}$, and MX radicals. Since D_2O is employed for solutions, DNA is fully exchanged, and $TD^{\bullet}$, instead of $TH^{\bullet}$, is predominantly formed. We see little evidence for $TH^{\bullet}$ in our spectra.

Figure 1 shows the fractions of the various radicals found 400 min after irradiation relative to the total radicals formed initially after irradiation. The fractions of MX radicals (P_{MX}) and the various DNA radicals—$G^{\bullet+}$ (P_G), $CD^{\bullet}$(P_{CD}), $T^{\bullet-}$(P_T),

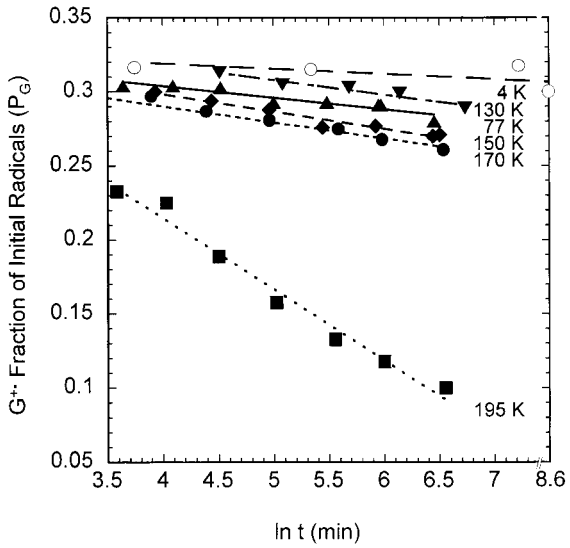

Figure 2. Plot of the $G^{\bullet+}$ fraction (P_G) vs the natural logarithm of time in minutes as a function of temperature. The fraction is relative to the total initial radicals formed in irradiated hydrated solid samples of MX-DNA (14 D_2O/nucleotide, $\nu = 1/214$) at 4 K (○), 77 K (▲), 130 K (▼), 150 K (◆), 170 K (●), and 195 K (■). The last 4 K point shown on the right axis is at $\ln t = 8.6$.

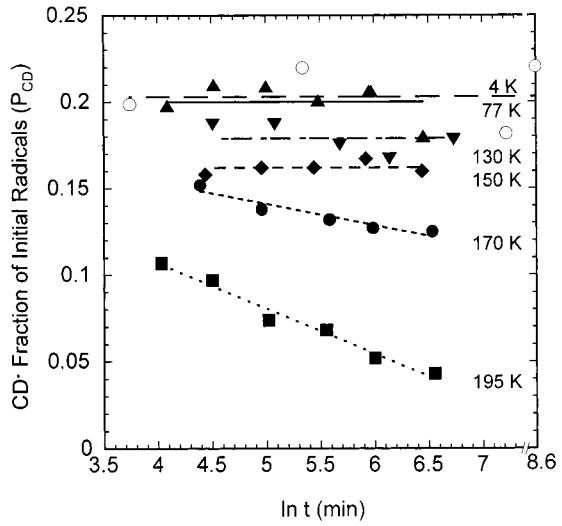

Figure 3. Plot of the $CD^{\bullet}$ fraction (P_{CD}) vs the natural logarithm of time in minutes as a function of temperature. The fraction is relative to the total initial radicals formed in irradiated hydrated solid samples of MX-DNA (14 D_2O/nucleotide, $\nu = 1/214$) at 4 K (○), 77 K (▲), 130 K (▼), 150 K (◆), 170 K (●), and 195 K (■). The last 4 K point shown on the right axis is at $\ln t = 8.6$.

and $TD^{\bullet}$(P_{TD})—are plotted with temperature. As temperature rises, P_{TD} increases, while the other fractions all remain constant below 77 K and then fall. The dependence of P_{TD} on temperature is nearly linear above 77 K; however, there is a turning point near 150 K for the other curves of P vs temperature.

Several interesting changes in radical fractions with time are observed. The dependence of radical fractions of $G^{\bullet+}$, $CD^{\bullet}$, and $T^{\bullet-}$—i.e., P_G, P_{CD}, and P_T—on time as a function of temperature is shown in Figures2−4, respectively. Figure 2 shows that P_G decreases dramatically with time above 170 K; in contrast, P_G only slightly decreases with time in the range of 4−170 K. Figure 3 demonstrates that at temperatures in the range of 4−150 K, P_{CD} is relatively constant with time; however, at 170 K, P_{CD} begins to decrease with time, and at 195 K, the decrease becomes more pronounced. Figure 4 shows that P_T decreases

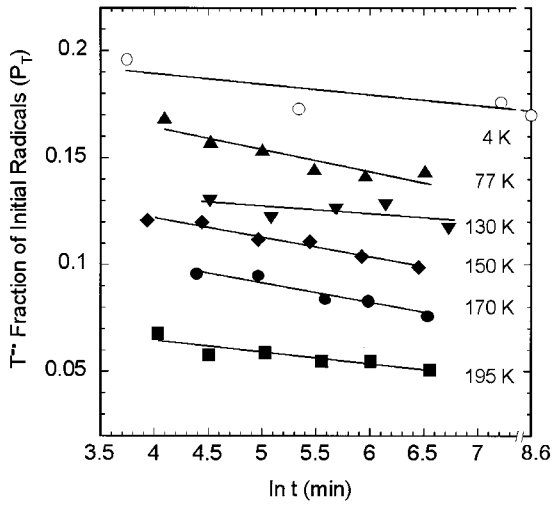

Figure 4. Plot of the $T^{\bullet-}$ fraction (P_T) vs the natural logarithm of time in minutes as a function of temperature. The fraction is relative to the total initial radicals formed in irradiated hydrated solid samples of MX-DNA (14 D_2O/nucleotide, $\nu = 1/214$) at 4 K (○), 77 K (▲), 130 K (▼), 150 K (◆), 170 K (●), and 195 K (■). The last 4 K point shown on the right axis is at $\ln t = 8.6$.

with ln(t) with roughly similar slopes (-0.007 ± 0.003) at each temperature. The slope at 4 K (0.004 ± 0.002) is less than that at 77 K (0.011 ± 0.002). If real, the increased slope at 77 K would likely reflect some transfer of electron from $T^{\bullet-}$ to C. This is not unexpected, as it has been previously reported that the fraction of electron adducts of T and C change with temperature to favor C.[21a,b] Such a change would not show up as a transfer to MX in our overall analysis for β_{avg} because we compare only DNA anion and MX electron adducts with time.

At 4 and 77 K, we find statistically indistinguishable increases in P_{MX} with time, which we assign to tunneling processes[9] and which will be discussed in more detail below. This suggests little difference in the overall rate of electron transfer from $T^{\bullet-}$ and $CD^{\bullet}$ to MX between 4 and 77 K even though some ET likely occurs from $T^{\bullet-}$ to $CD^{\bullet}$ at 77K. For MX and $TD^{\bullet}$, we find little time dependence in the temperature range of 130−195 K after a rapid initial change at each new temperature.

Comparison of Tunneling Processes at 4 and 77K. ESR spectra for MX-DNA in the 7 M LiBr D_2O glassy solution containing 20 mg/mL of DNA with $\nu = 1/52$ and for hydrated MX-DNA (14 D_2O/nucleotide) with $\nu = 1/214$ were followed at increasing time intervals after irradiation at 4 and 77 K, respectively. The linear least-squares fits of benchmark spectra to the experimental spectra show that the fraction of MX radicals increases while the fraction of DNA radicals decreases at 4 and 77 K. The apparent tunneling distances are derived from eq 1[9]

$$D_a(t) = \frac{\ln(1 - P_{MX}(t))}{2\ln(1 - \upsilon)} \quad (1)$$

For samples in frozen aqueous solutions at low concentrations, the value of β_α is obtained from plots of D vs ln t and eq 2[9 22]

$$D_a = 1/\beta_{avg} \ln(k_o t) \quad (2)$$

Since the increase in MX (P_{MX}) with time represents the transfer of all DNA radicals to MX—i.e., DNA anions ($CD^{\bullet}$ and $T^{\bullet-}$) in glasses or DNA anions and holes in solid—the values are averages of the individual transfers (β_{avg})[23,24] and do not distinguish between the individual radicals. Later, we suggest that the transfer in these systems is predominately from thymine

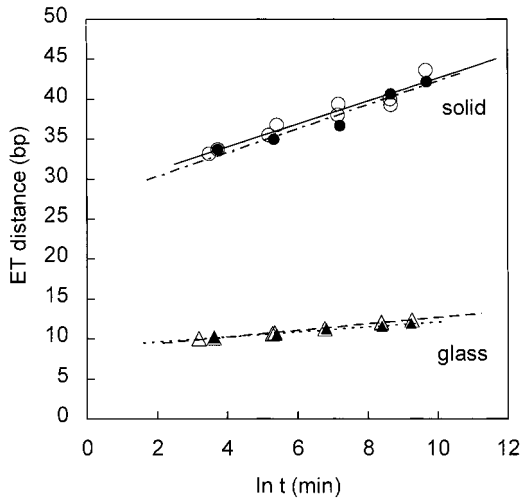

Figure 5. Effect of temperature on the rate of electron transfer through DNA in frozen glassy solutions and hydrated solid DNA. (lower curves) Plot of the electron-transfer distance vs the natural logarithm of time in minutes for frozen glassy solutions of DNA intercalated with mitoxantrone (MX) (7 M LiBr in D_2O solutions, $\nu = 1/52$) at 4 K (▲) and 77 K (△). (upper curves) Plot of the apparent electron-transfer distance vs the natural logarithm of time in minutes for hydrated solid DNA-MX samples (14 D_2O/nucleotide, $\nu = 1/214$) at 4 K (●) and 77 K(○). The transfer rates in both systems are apparently unaffected by temperature in the 4−77 K range.

anion radical ($T^{\bullet-}$) which suggests that the individual radical β's and fundamental rates (k_o) likely differ.

For solid samples, the values from eq 2 must be corrected for interstrand transfer, as described in our previous work.[19]

Figure 5 shows plots of the apparent tunneling distance versus the natural logarithm of time for the above four samples. The two pairs of nearly overlapped lines for two sets of samples at 4 and 77 K are shown in Figure 5. The upper set is for hydrated DNA samples (ν = 1/214, 14 D_2O/nucleotide) in which substantial inter-ds transfer contributes to the capture of radicals by MX.[19] The lower curves are for 7 M LiBr D_2O glassy solution containing 20 mg/mL of DNA with $\nu = 1/52$, in which little inter-ds transfer occurs. The results suggest that the overall tunneling rates of electrons in the glassy system and of electrons and holes in the solid DNA are independent of temperature between 4 and 77 K. The β_{avg} values for the solid samples after correction for inter-ds transfer[19] are found to be identical at 4 and 77 K and somewhat over 1 $Å^{-1}$. The β_{avg} values at both temperatures for the glassy samples are also the same within experimental error and in keeping with our previously reported value of 0.9 $Å^{-1}$.

Discussion

Figure 1 shows that in the temperature range from 4 to 77 K, P_{MX}, P_G, and P_{CD} remain constant and P_T slightly decreases while P_{TD} slightly increases (less than 2%). This result shows no significant $T^{\bullet-}$ protonation to form $TD^{\bullet}$ at 77K or below. We also find that the overall rates of the electron transfer at both 4 and 77 K are nearly identical (Figure 5). The lack of a temperature effect is in accord with a tunneling process from DNA radicals to MX and gives no support for activated processes such as electron or hole hopping.[2,5] We note that at 4 and 77 K, the integrated ESR signal intensity after irradiation remains constant within experimental error for up to 14 days; thus, significant recombination of DNA radicals does not occur at or below 77 K during our time of observation. Transfer to the intercalator MX, however, does occur, as MX is far more abundant than holes.

At temperatures of 130 K and above (Figure 1), we find a linear increase in the fraction of $TD^{\bullet}$ (P_{TD}) with temperature but a paradoxical independence of time immediately after a fast initial increase in $TD^{\bullet}$ at each temperature. For example, at 130 K, the amount of $TD^{\bullet}$ increases by 5.5% over that found at 77 K within the first 10 min but then does not change significantly thereafter. Annealing to 150, 170, and 195 K each time results in an immediate 6.2%, 8.2%, and 12.1% increase in P_{TD}, respectively, without appreciable change with time thereafter. Thus, this irreversible protonation (deuteration) at thymine is a thermally activated process with a peculiar temperature sensitivity. The reason that $TD^{\bullet}$ formation shows a rapid initial increase at each temperature without significant increase with time thereafter is not clear at this time. One possibility is that the protonation (deuteration) of thymine anion radicals depends on an appropriate arrangement of the solvation shell and each arrangement has different activation steps.

We find that at temperatures of 77 K or below, little $TD^{\bullet}$ forms and the tunneling of electrons from thymine anion radicals to MX and cytosine are the predominant mechanisms of loss for $T^{\bullet-}$, with no evidence for significant recombination of $T^{\bullet-}$ and $G^{\bullet+}$. As temperature increases above 130 K, thymine anion radicals are activated and protonate (deuterate) to form $TH^{\bullet}$-($TD^{\bullet}$), the major irreversible sink for the electrons in DNA under these conditions. As a consequence, electron tunneling to MX becomes progressively less competitive with irreversible protonation (deuteration) of the thymine anion radicals. At temperatures above 150 K, the electron transfer from the one-electron reduced cytosine to thymine is activated adding to the formation of $TH^{\bullet}$ ($TD^{\bullet}$).

As shown in Figure 1, from 4 to 130 K, no obvious changes in one-electron oxidized guanine, $G^{\bullet+}$, is observed; on the other hand, the amounts of other species $CD^{\bullet}$, $T^{\bullet-}$, and MX radicals decrease while $TD^{\bullet}$ increases. These results suggest that it is the formation of $TD^{\bullet}$, not the recombination of one-electron reduced species with $G^{\bullet+}$, that accounts for the majority of losses in the ESR signals of $T^{\bullet-}$, $CD^{\bullet}$, and MX radicals from 77 to 130 K.

The change in slopes from 150 to 170 K for the plots of P_{MX}, P_G, P_{CD}, and P_T vs temperature (Figure 1) suggests that the recombination of one-electron reduced species with $G^{\bullet+}$ starts in the range of 150 K to 170 K and accounts for the overall loss of radical signals. Figures 2 and 3 also suggest the recombination of $CD^{\bullet}$ and $G^{\bullet+}$ starts at about 170 K and becomes substantial at 195 K. The recombination clearly involves electron movement and is likely a result of activation of electron hopping and possibly hole hopping.

As shown in Figure 4, for each temperature, an initial drop in $T^{\bullet-}$ occurs, followed by a slow loss with ln(t). The initial drop correlates with a corresponding increase in $TD^{\bullet}$. The later loss of $T^{\bullet-}$ with time at each temperature is likely a result of continued electron tunneling from $T^{\bullet-}$ to MX, but the transfer to cytosine also complicates the system. The 4 K data is less likely to be complicated by the transfer to cytosine. From the slope of the 4K data in Figure 4, we can estimate the tunneling distance decay constant of β for $T^{\bullet-}$ as ca. 0.9 $Å^{-1}$, and the average of the higher temperatures gives a value of ca. 0.6 $Å^{-1}$.[19]

The fraction of the one-electron reduced cytosine (P_{CD}) remains relatively constant within our time scale (from minutes to hours) and temperature range, 4−150 K (Figure 3). Thus, electron tunneling from $CD^{\bullet}$ to MX is not observed in our studies, though we believe that it likely occurs within the much

shorter time scale before $CD^{\bullet}$ is protonated (deuterated) by its paired base, G, to form $CD^{\bullet}$. Our previously reported β_{avg} value of 0.9 ± 0.2 Å^{-1} for the overall electron tunneling along DNA ds in glassy systems assumed that the electrons tunnel from both $CD^{\bullet}$ and $T^{\bullet-}$ to MX at about the same rate. This assumption may overestimate β if electrons tunnel predominantly from $T^{\bullet-}$ to MX in glassy systems. However, electron transfer from $T^{\bullet-}$ to C at 77 K may complicate this further (see Conclusions, point 5 below).

We note that in our present study, deuterated solutions were employed to increase the resolution of the ESR spectra, which allows for the assignment of the individual radicals. Deuteration will also slow the rates of both reversible and irreversible protonation. This likely increases the initial electron-transfer distances, as $C^{\bullet-}$ would protonate more slowly in deuterated systems. In addition, the amount of protonation at carbon would be reduced somewhat versus electron-transfer processes to MX or $G^{\bullet+}$.

Conclusions

Our major findings are the following.

(1) At 4−77 K, the overall electron transfer in DNA shows little temperature dependence, in accord with a single-step tunneling process with similar findings in proteins.[5,25] The lack of temperature dependence is evidence that accessible vibronic states in this temperature range do not affect the coupling between donor and acceptor or significantly change the barrier to the transfer.[5,25]

(2) At temperatures above 130 K, the deuteration of $T^{\bullet-}$ at carbon (6) to form $T(C6)D^{\bullet}$ takes place. This process is well-known[20,21,26] and is a thermally activated irreversible process that does not involve the transfer of electrons or holes. The deuteration of $T^{\bullet-}$ successfully competes with electron tunneling through DNA above 130 K and acts as an irreversible sink for the electron, as reported earlier.[20,21]

(3) The formation of $TD^{\bullet}$ occurs in a stepwise fashion with temperature, i.e., rapid initial increase with little time dependence thereafter. This result is somewhat surprising but has been reported previously for $TH^{\bullet}$ formation.[26] It may suggest a conformationally dependent change or an alteration of the water arrangement near the active site with a variety of individual activation energies.

(4) The recombination of $CD^{\bullet}$ and $G^{\bullet+}$ is substantially activated at 195 K and involves ET by electron and possibly hole hopping.

(5) The value of β for electron transfer is likely dependent on the type of radical ion site that acts as donor or acceptor. Our work with hydrated DNA samples suggests that the apparent electron tunneling in DNA is predominantly from $T^{\bullet-}$ during the time of our experiment. This conclusion would be negated if the rate of transfer from $T^{\bullet-}$ to C at 77 K was similar to the rate of electron transfer from $CD^{\bullet}$ to MX. This would make it appear that $CD^{\bullet}$ was not changing while $T^{\bullet-}$ diminished. The data at 4K then becomes the best indicator of tunneling from thymine alone. These data suggests that the β value for electron transfer in hydrated DNA from $T^{\bullet-}$ to MX is ca. 0.9 Å^{-1}. This value could be lower for transfer along a DNA double strand because electorn transfer in the hydrated samples occurrs between ds's as well as along the ds and our correction assumes a similar β for each.[19] Our glassy system does measure the transfer along one ds but does not allow for the differentiation between $T^{\bullet-}$ and $CD^{\bullet}$.[9] Thus, the previously reported β_{avg} value of 0.9 ± 0.2 Å^{-1} for the transfer from DNA anion radicals to MX assumed that both C and T electron adducts tunneled with the same rate.[9] This value would represent an upper limit for β along one double strand if the transfer is predominantly from $T^{\bullet-}$.

(6) Electron transfer from one-electron reduced cytosine was apparently too slow to be observed in our experiment until a temperature (170 K) was reached at which hopping was activated. The prototropic equilibrium between guanine and cytosine[27] results in the protonation of $C^{\bullet-}$ at N3 from its base pair G and provides substantial stabilization energy.[28] This largely prevents electron transfer from this species in the temperature range of 4−150 K, as the reverse process which is estimated to have a ca. 5 kcal barrier must presumably occur before electron transfer can take place.[28] The fact that this proton transfer is reversible and thermally activated suggests that the proton transfer itself poses no limit to the range of electron transfer by hopping. Only the irreversible protonation reactions at $T^{\bullet-}$ or $CD^{\bullet}$ or charge recombination may limit the ultimate ET range in these systems.[2,6,12,20,21] We note that the relative rates of ET and protonation differ with temperature so that the distance of electron transfer is likely to substantially differ with temperature.[2,6,12]

Finally, while our work clearly isolates tunneling from thermally activated processes, the β values and rates are for transfer from each of the individual electron attachment sites, i.e., thymine and cytosine, are not yet known with accuracy. Experiments are underway with various double-stranded polynucleotides of one base pair to shed further light on this problem.[29]

Acknowledgment. This research was supported by NIH NCI Grant RO1CA45424 and by the Oakland University Research Excellence Fund.

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