Proton-Coupled Electron-Transfer Reactions at a Distance in DNA Duplexes: Kinetic Deuterium Isotope Effect

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Kinetic solvent isotope effects on the electron-transfer kinetics associated with the oxidation of guanine by a 2-aminopurine (2AP) neutral radical separated by 2 or 3 thymine, or 6 adenine residues on the same strand in 15-mer double-stranded oligonucleotides in H_2O or D_2O were measured. The evolution in time of the oxidized form of guanine, the neutral radical G(−H)•, and the electron acceptor, the neutral 2AP(−H)• radical, were followed directly by a spectroscopic laser pulse-induced transient absorption technique on a 1−200 μs time scale. About 70−100% of this one-electron-transfer reaction occurs on this relatively slow time scale in the three oligonucleotide duplexes studied. The rate constants of formation of G(−H)• in the oligonucleotides are larger in H_2O than in D_2O by a factor of 1.3−1.7. This kinetic isotope effect suggests that the electron-transfer reaction from G to 2AP(−H)• is coupled to a deprotonation of $G^{\bullet+}$, and a protonation of $2AP^-$, the primary products of the electron-transfer reaction. Thus, electron-transfer reactions occurring at a distance in these DNA duplexes can be considered in terms of proton-coupled electron-transfer reactions.

Introduction

DNA chemistry at a distance associated with the transfer of electrons (or holes) in double-stranded DNA is a subject of intense current interest.[1−3] The oxidative modifications of nucleic acid bases, especially guanines, can be detected at sites up to ∼200 Å from the site of generation of the initial oxidants.[4,5] Theoretical analysis of the propagation of reactive intermediates indicates that the long-distance transfer can occur via both the superexchange and the hole-hopping mechanisms of charge transport between energetically appropriate charge traps.[6−8] In these models, guanine radical cations, $G^{\bullet+}$, are considered to be the mobile holes that can migrate by the hole hopping mechanism over great distances between energetically appropriate intermediate hole traps, like GG and GGG sites.[9−11] The superexchange mechanism is operative at much shorter distances, *r*. The rate constant, *k*, is attenuated exponentially with increasing distance ($k \sim \alpha \exp[-\beta r]$), and values of β in the range ∼0.7−1.5 Å have been reported.[12−17] However, purine radical cations are strong Brønsted acids that rapidly deprotonate in neutral aqueous solutions[18,19] and in double-stranded DNA as well.[20,21] The neutral radicals of G and A nucleic acid bases remain strong one-electron oxidants.[22] We have shown that the neutral guanine radicals can also undergo electron-transfer reaction as nucleotides in solution,[23,24] and at a distance in oligonucleotide duplexes.[25]

Recently, we demonstrated that the excitation of DNA duplexes containing 2-aminopurine (2AP), a DNA base analogue, with intense 308 nm XeCl excimer laser pulses, results in the site-selective two-photon photoionization of the 2AP residues.[23,25] The photoionized 2AP residues are capable of oxidizing G residues separated by one or more A and T residues on the same strand by one-electron-transfer mechanisms.[25] A fast (<100 ns), distance-dependent component has been interpreted in terms of an oxidation of guanines by the initially generated 2AP radical cations, $2AP^{\bullet+}$. We proposed that the oxidation of G by $2AP^{\bullet+}$ competes with a rapid (<100 ns) deprotonation of $2AP^{\bullet+}$ to the 2AP neutral radical, 2AP(−H)•. This neutral radical is a one-electron oxidant responsible for the generation of the neutral guanine radicals, G(−H)•, on the microsecond to millisecond time scale, thus constituting the second, slower electron-transfer component. The efficiency of the oxidation of G by $2AP^{\bullet+}$ rapidly decreases strongly with increasing distance between the 2AP and the G residues (1−2 bases). On the other hand, the oxidation of guanines by the neutral 2AP(−H)• radical is dominant at longer distances (2−4 bases) and occurs on slower time scales.[25]

A kinetic isotope effect on the rates of electron transfer between the nucleosides or nucleotides 2AP(−H)• and dG or dGMP in H_2O and D_2O, and the concomitant formation of the guanine radicals, G(−H)•, has been reported.[24] These observations suggest that electron transfer from G to 2AP(−H)• is coupled to a proton-transfer process that results in the formation of the neutral products, G(−H)• and 2AP when these products of the electron-transfer reaction are monitored on microsecond or longer time scales.

Here, employing transient absorption spectroscopy techniques to monitor the evolution in time of both the electron acceptor and the electron donor on microsecond time scales, we report that *a solvent deuterium isotope effect is observed on the rates of oxidation of guanine at a distance by the neutral 2AP(−H)• radical in oligonucleotide duplexes in H_2O/D_2O solutions.* This kinetic isotope effect, observed on time scales of 1−200 μs, indicates that the electron-transfer reaction from G to 2AP(−H)• in double-stranded DNA is coupled to a proton-transfer step. These reactions involve the deprotonation of $G^{\bullet+}$ and the protonation of $2AP(-H)^-$, the primary products of the electron-transfer reaction. Thus, the charge-transfer reactions occurring

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at a distance in DNA duplexes on time scales of microseconds can be considered in terms of proton-coupled electron-transfer reactions.

Results and Discussion

Three different oligonucleotides, each containing a single 2AP base at the 5′-end, and a single GG doublet separated by two (**1**) or three (**2**) thymidines, or six (**3**) adenine bases, were constructed.

(**1**) 5′-**[2AP]**TT**[GG]**TTTTTTTTTT-3′

or, (**[2AP]**T_2**[GG]**T_{10})

(**2**) 5′-**[2AP]**TTT**[GG]**TTTTTTTTT-3′

or, (**[2AP]**T_3**[GG]**T_9)

(**3**) 5′-**[2AP]**AAAAAA**[GG]**AAAAAA-3′

or, (**[2AP]**A_6**[GG]**A_6)

All experiments were conducted with the oligonucleotides in the double-stranded form in complexes with their complementary strands, with T opposite the 2AP. In 20 mM phosphate buffer solutions (pH 7) containing 100 mM NaCl, these duplexes exhibit well-defined cooperative melting behavior with melting temperatures, $T_m = 41-43$ °C.[25]

Electron-transfer rates in the double-stranded oligonucleotides (**1**, **2**) have been previously studied by transient absorption techniques in oxygenated H_2O buffer solutions[25] (pH 7). In oxygenated solutions, the hydrated electrons released by the site-selective photoionization of the 2AP residues are rapidly scavenged by molecular oxygen,[26] and the 2AP radicals are sufficiently long-lived to induce oxidation of guanines at a distance. The transient absorption spectra recorded at the delay times, $\Delta t \geq 100$ ns, are characterized by a bleaching of the 2AP absorption band at 310 nm, and the appearance of stronger (365 nm) and weaker (510 nm) absorption bands attributed to the 2AP(−H)• radical (Figure 1, panel A). The decay of the transient signals at 365 and 510 nm are accompanied by a buildup of the characteristic narrow absorption band of the G(−H)• radicals at 310 nm. A detailed analysis of the microsecond to millisecond transient absorption spectra showed that in the double-stranded oligonucleotides (**1**, **2**), the oxidation of G by 2AP(−H)• was dominant.[25] In the oligonucleotide duplexes with adenine rather than thymidine bridges between 2AP and GG, the electron-transfer rates are significantly greater.[25] In the case of the oligonucleotide (**3**) with six bridging adenine bases, transient absorption measurements indicate that the rate of oxidation of G by 2AP(−H)• is dominant and that the electron-transfer rate constants are of the same order of magnitude as in duplexes (**1**, **2**) having only 2 and 3 bridging thymidines. In all cases, the first-order electron-transfer processes from GG to 2AP(−H)• are *intraduplex processes* since these rate constants of electron transfer are independent of the concentrations of duplexes in the 10−100 μM range (data not shown). These systems are therefore excellent candidates for establishing the existence of kinetic isotope effects in intraduplex electron-transfer reactions at a distance induced by neutral base analogue radicals in double-stranded DNA.

The kinetic profiles of the oxidation G by 2AP(−H)• radicals in H_2O or D_2O buffer solutions (pH 7) of the **[2AP]**T_2**[GG]**T_{10} duplex were recorded at two representative wavelengths, 320 and 365 nm (Figure 1, panel A). We selected 320 nm because the buildup of the transient absorption due to the formation of the G(−H)• radicals can be best observed at this wavelength (Figure 1, insert B). On the other hand, the decay of the 2AP(−H)• transient absorption can be observed at 365 nm (Figure 1, insert C); this signal decays to a nonzero level after ~200 μs because of the residual absorbance of the G(−H)• radical, which, in turn, decays on time intervals of ~1−4 ms.[27] Thus, the decay of the G(−H)• radicals in the duplexes does not contribute significantly to the kinetics of the buildup of the G(−H)• absorbance on the time scale of interest here (≤200 μs, Figure

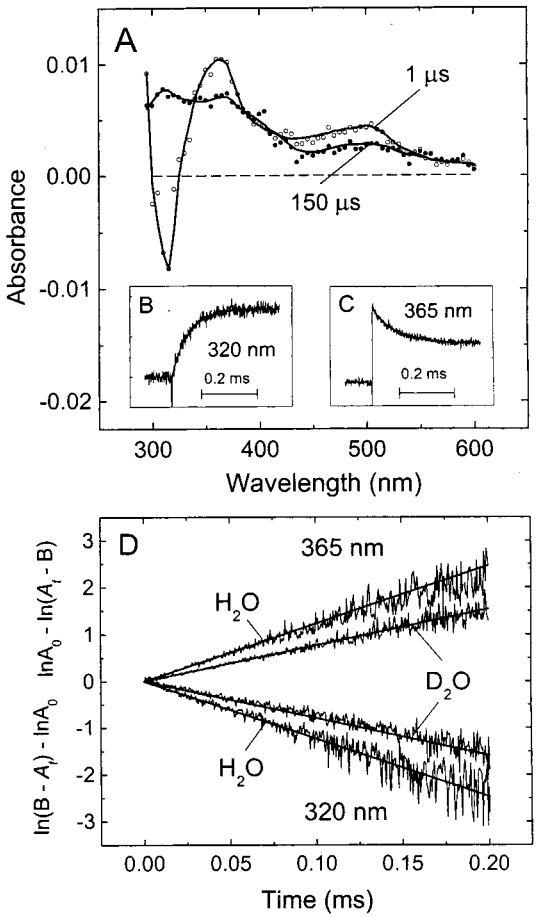


Figure 1. Deuterium isotope effect on the kinetics of oxidation of G by 2AP(−H)• radicals in the double-stranded oligonucleotide d([2AP]-T_2GGT_{10})•d($A_{10}CCA_2T$) in oxygenated H_2O/D_2O buffer solutions (pH 7.0). Panel A shows the transient absorption spectra of the duplexes at time intervals $\Delta t = 1$ and 150 μs after the actinic 308 nm laser pulse. Panel B depicts the rising absorbance at 320 nm, which is attributed mainly to the formation of the G(−H)• radical. Panel C depicts the decay of the absorbance at 365 nm, which is mainly due to the decay of the 2AP(−H)• radical. The resolution in time of these kinetic profiles is 1 μs/point. Insert D shows the kinetic profiles (resolution of 0.5 μs/point) according to a semilogarithmic form of eq 1 (the solid lines are the best linear fits to the experimental data points with the values of k_1 summarized in Table 1). The DNA samples were dissolved in buffer solutions (20 mM $NaHPO_4/Na_2HPO_4$, pH 7.0) and were photoexcited with 308 nm XeCl excimer laser pulses (80 mJ/pulse/cm^2, fwhm = 12 ns, 0.5 Hz). The transient absorption spectra were recorded using a computer-controlled kinetic spectrometer system.[23−25] To maximize the signal/noise ratio in the recording of the absorbance signals on millisecond time scale, the Xe arc lamp probe source was operated in the cw mode, and the photomultiplier output was terminated by a 3 kΩ resistor, instead of 50 Ω signal output as in the recording of the signals on the 50 ns to 50 μs time scales.[25] Typically, 50 kinetic curves were accumulated and averaged in order to improve the signal/noise ratio.

TABLE 1: Kinetic Parameters of the Proton-Coupled Electron-Transfer Reaction of Guanine Oxidation by 2-Aminopurine Neutral Radicals in Double-Stranded DNA Duplexes

	k_1[b] (10^3 s^{-1})		k_{ag}[c] (10^3 s^{-1})		
sequence[a]	H_2O	D_2O	H_2O	D_2O	H_2O/D_2O
[2AP]T_2**[GG]**T_{10} (**1**)	12.3 ± 1	7.9 ± 0.8	10.3 ± 1	5.9 ± 0.6	1.7 ± 0.2
[2AP]T_3**[GG]**Tg (**2**)	5.3 ± 0.5	4.2 ± 0.4	3.3 ± 0.3	2.2 ± 0.2	1.5 ± 0.2
[2AP]A_6**[GG]**A_6 (**3**)	8.0 ± 0.8	6.6 ± 0.6	6.0 ± 0.6	4.6 ± 0.5	1.3 ± 0.1

[a] Oligodeoxyribonucleotide sequences are written in the 5′ → 3′ direction. [b] The averaged values of k_1 were obtained by the best fits of eq 1 to the kinetic traces (as shown in Figure 1, panel D) recorded at 320 and365 nm. [c] $k_{ag} = k_1 - k_a$. The value of $k_a = 2 \times 10^3$ s^{-1} was taken from ref 25.

1). Therefore, the time dependence of the transient absorption signal, $A(t)$, can be represented by the following general equation:

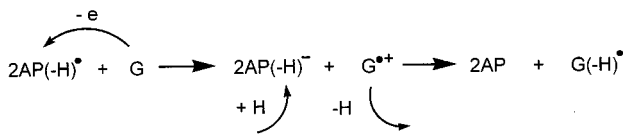
$$A(t) = A \exp(-k_1 t) + B \qquad (1)$$

where the term B is considered to be independent of time on the ≤200 μs time scale. The rate constant k_1 is associated with the appearance of the signal due to the G(−H)• radicals, the concomitant decay of the 2AP(−H)• radicals, and the recovery of the 2AP ground-state molecules. The values of k_1 obtained by the best fits of eq 1 to the experimental kinetic traces are visibly different in H_2O and D_2O buffer solutions (pH 7) of the **[2AP]**T_2**[GG]**T_{10} duplex. This deuterium isotope effect can be best appreciated by plotting the experimental data points according to a linearized form of eq 1 (Figure 1, inset D). Plots of the time-dependent quantities $\ln\{[B - A(t)]/A_0\}$ vs t [the buildup of $A(t)$ at 320 nm] and $\ln\{A_0/[[A(t) - B]\}$ vs t [the decay of $A(t)$ at 365 nm] are shown in Figure 1 (panel D). In these plots, A_0 is the signal amplitude at the time t_0 (∼1 μs), which marks the onset of the transient absorption measurements. The signals shown in panel D are shown without any reduction in the noise levels by software processing. The slopes of these plots are distinctly different in H_2O and D_2O, and the differences are clearly outside the noise levels characterizing these linearized plots. According to eq 1, the slopes of these plots are equal to k_1 (at 365 nm) and $-k_1$ (at 320 nm). In H_2O, the value of k_1 is markedly larger than in D_2O, as shown in Table 1. Similar deuterium isotope effects were also observed on the kinetics of oxidation of G by the 2AP(H)• radical in the **[2AP]**T_3**[GG]**T_9 and **[2AP]**A_6**[GG]**A_6 duplexes (data not shown).

The rate constant, k_1 is composed of two terms, k_a and k_{ag} with $k_1 = k_a + k_{ag}$. We denote the rate constant of oxidation of G by 2AP(−H)• by k_{ag}, while the decay of the 2AP(−H)• radical by other pathways (not involving electron transfer), is denoted by k_a. The rate constant k_a was measured using the oligonucleotide [2AP]T_{14}; in this system, the 2AP(−H)• radical cannot decay by electron-transfer reactions since there are no guanines ($k_{ag} = 0$).[25] The kinetic parameters thus obtained are summarized in Table 1. The values of k_{ag} in H_2O are larger than those in D_2O solutions by factors of ∼1.3−1.7.

In principle, when the solvent is changed from H_2O to D_2O, the rates of electron-transfer reactions could be affected by various different factors. We recently considered such possibilities in terms of the traditional theories of nonadiabatic and adiabatic electron-transfer rates.[31,32] The solvent parameters of H_2O and D_2O that are known to affect electron-transfer rates, e.g., the static and dynamic dielectric constants, and the longitudinal dielectric relaxation times, are very close to one another in D_2O and H_2O. Since the differences in the static dielectric constants of H_2O ($\epsilon_s = 78.85$) and D_2O ($\epsilon_s = 77.94$) are very small, the dielectric-continuum models predict essentially similar reorganization energies in H_2O and D_2O solvents. Furthermore, solvation energies of counterions, their interactions with the negatively charged phosphodiester backbone and their positions relative to the DNA backbone, should be similar in the two solvents. Therefore, the impact of counterions on electron-transfer rates are predicted to be similar in H_2O and in D_2O. Overall, differences in the dielectric properties of normal and heavy water cannot account for the kinetic isotope effects on the electron-transfer rate constants in terms of electrostatic effects.

In view of the known protonation/deprotonation reactions of purine radicals and radical cations,[19] the observed kinetic isotope effect is consistent with a proton-coupled electron-transfer mechanism.[33,34] In this effect, the electron transfer from G to 2AP(−H)• is coupled to a deprotonation of the radical cation, $G^{\bullet+}$, and a protonation of the anion, $2AP(-H)^-$:



The kinetic isotope effect reported here is characteristic of the slow, rather than the fast (<100 ns), component of electron transfer described by us earlier.[25] In the oligonucleotide duplexes (**1**−**3**) this slow component accounts for the appearance of 70−100% of the G(−H)• radical signals. Therefore, it should be stressed that the proton-coupled electron-transfer mechanism in these oligonucleotide duplexes is relevant to the major, rather than to a minor electron-transfer component. The GG oxidation by 2AP(−H)• neutral radicals is an intraduplex reaction (see above), but the question can be raised whether the electron transfer occurs at a distance or by a close contact between the 2AP(−H)• radical at the end of the strand and the G residues positioned further from the end of the duplex. This could occur, in principle, via the fraying of the base pairs at the ends of the duplex and a bending of the resulting single-stranded end so that the 2AP(−H)• moiety would come in close contact with the G. To investigate this possibility, we compared the rates of electron transfer in duplex **3** in which there are six adenine base pairs between the 2AP(−H)• and the first G in the GG pair. A fraying and looping mechanism would require the opening of at least 3−4 base pairs and a looping back of the single strand to allow the 2AP(−H)• radical to come in close contact with the G. If these fraying and looping dynamic conformations were to account for the observed electron phenomena, a strong decrease in the electron-transfer rate constants, k_{ag}, would be expected with the increasing length of the bridge separating the electron donor and acceptor. However, k_{ag} in duplex **3** is intermediate in value to the rate constants in duplexes **1** and **2** (Table 1), suggesting that the fraying and looping mechanism allowing close contact between the electron acceptor and donor cannot account for the observed electron-transfer phenomena. Thus, we conclude that intraduplex electron transfer from GG to 2AP(−H)• can be considered as a reaction that occurs at a

distance rather than by close contact between the donor and acceptor moieties.

The distance between the donor and acceptor moieties is a very critical parameter in electron-transfer reactions that occur at a distance. Here, we consider whether differences between the electron donor and the electron acceptor due to changes in the distances between adjacent base pairs could account for the differences in rate constants in H_2O and D_2O. In the case of oligonucleotide duplexes **1** and **2**, the value of the distance attenuation parameter β (0.75 $Å^{-1}$) is known.[25] Using this β value and the measured ratios of the electron-transfer rate constants, $k_{ag}(H_2O)/k_{ag}(D_2O) = 1.5-1.7$, the inferred increase in distance between adjacent base pairs in D_2O as compared to H_2O that is needed to account for the smaller value of k_{ag} in D_2O, can be estimated. This increase in distance, ΔR, would have to be equal to $\ln[k_{ag}(H_2O)/k_{ag}(D_2O)]/\beta = 0.64-0.71$ Å. Hence, over 2 or 3 base pairs, the distance between the 2AP and GG should be greater by 0.64−0.71 Å in D_2O solution than in H_2O solution in order to account for the difference in the electron-transfer rates in these two solvents. The interbase distances in the oligonucleotide duplexes are known from X-ray analysis in crystals of oligonucleotides (grown from H_2O solutions) and NMR measurements (measured in D_2O solutions). A recent NMR analysis of the length of the Dickerson dodecamer concludes that the difference in length of this 10-mer oligonucleotide duplex in the crystalline form and in D_2O solution is less than[35] 1 Å. Hence, the difference in distance between two adjacent base pairs in these two environments cannot be greater than 0.1 Å. It is therefore highly unlikely that an increase in the interbase pair distance of 0.2−0.3 Å (=[0.64 − 0.71]/[2 to 3]) would occur in D_2O as compared to H_2O. In fact, it is unreasonable to expect such a large change since it is well-known that the base stacking in DNA results from a hydrophobic effect with no water molecules between the base pairs. In summary, the experimental NMR results[35] suggest that a large increase in the distance between DNA base pairs in D_2O does not occur. Therefore, such a trivial distance effect cannot account for the observed deuterium isotope effects on electron-transfer rates described in this work.

As we have shown elsewhere, the electron-transfer rate constant is significantly greater with adenine rather than with thymine bridging bases.[25] Thus the value of k_{ag} is almost as large in duplex **3** with 6 bridging adenines than in duplex **1** with only two bridging thymidines (Table 1). Nevertheless, the magnitudes of k_{ag} are comparable in duplexes **1**−**3**.

Analogous proton-coupled electron-transfer reactions at a distance have been observed in polypeptides resulting from the oxidation of tyrosine (TyrOH) by tryptophan neutral radical ($Trp^{\bullet}$) linked to oligoproline $(Pro)_n$ bridges.[36−40] In this well-established example of an electron-transfer reaction from a donor to a neutral radical electron acceptor, a kinetic solvent deuterium isotope effect of ∼2 has also been observed.[38] A recent theoretical analysis of models of proton-coupled electron-transfer reactions has shown that a coupling between the electron undergoing transfer and the proton is required for the observation of a kinetic isotope effect, even when this transfer is occurring at a distance.[34] As discussed by Cuiker and Nocera, the electron- and proton-transfer steps can occur either simultaneously or sequentially.[33] The highly ordered structure of hydrated water[41] might favor this coupling at a distance in double-stranded DNA.

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References and Notes

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(27) The decay of the $G(-H)^{\bullet}$ radicals in double-stranded oligonucleotides does not follow simple first-order decay kinetics. The initial decay of the radicals (50−70%) occurs within a characteristic time of $t_e = 2-4$ ms. The remaining 30−50% of $G(-H)^{\bullet}$ radicals decay on a time scale of $t_e \sim 0.1$ s. These experiments were performed in oxygenated buffer solutions, and $G(-H)^{\bullet}$ can decay in reactions with molecular oxygen and/or with $O_2^{\bullet-}$ radicals. However, the reaction of $G(-H)^{\bullet}$ with O_2 is very slow[28] ($k \leq 10^2$ M^{-1} s^{-1}). In contrast, $G(-H)^{\bullet}$ reacts very rapidly with $O_2^{\bullet-}$ with a rate constant[29] of $\sim 3 \times 10^9$ M^{-1} s^{-1}. The $O_2^{\bullet-}$ radicals may disproportionate on millisecond time scales[30] to O_2 and H_2O_2. The latter species can also react[20] with $G(-H)^{\bullet}$. The existence of these multiple reaction pathways can result in the observed nonexponential decay of $G(-H)^{\bullet}$ in DNA duplexes. In the absence of the reactive oxygen species $O_2^{\bullet-}$ and H_2O_2, the lifetimes of $G(-H)^{\bullet}$ in DNA can be as long as 5−30 s.[20,21]
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