The Kinetic Deuterium Isotope Effect as a Probe of a Proton Coupled Electron Transfer Mechanism in the Oxidation of Guanine by 2-Aminopurine Radicals

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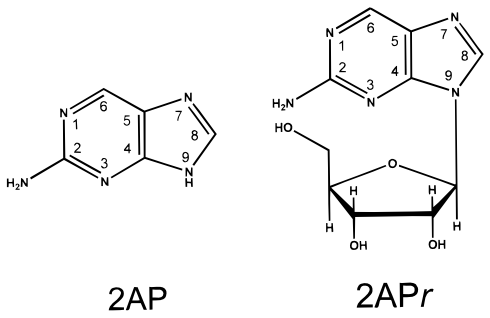
Photoexcitation of 2-aminopurine riboside (2AP*r*, 2-amino-9-β-D-ribofuranosylpurine) and 2-aminopurine (2AP) in oxygenated aqueous buffer solutions (pH 7.0) with 308 nm XeCl excimer laser pulses (fwhm = 12 ns, ca. 70 mJ/pulse/cm^2) results in the consecutive two-photon ionization of the aromatic 2AP*r* (or 2AP) residues. In neutral solutions, the 2AP*r* (or 2AP) radical cations rapidly deprotonate (<100 ns). The 2AP*r*(−H)$^\bullet$ (or 2AP(−H)$^\bullet$ neutral radicals thus formed reversibly oxidize 2-deoxyguanosine 5′-monophosphate (dGMP) on μs time scales, resulting in the formation of dGMP(−H)$^\bullet$ neutral radicals. Transient absorption measurements show that a remarkable solvent isotope effect is observed on the kinetics of oxidation of dGMP by 2AP*r*(−H)$^\bullet$ (or 2AP(−H)$^\bullet$) radicals in H_2O and D_2O solutions. In H_2O, the rate constants of dGMP(−H)$^\bullet$ formation, as well as the rate constants of the reverse reaction of the 2AP*r* (or 2AP) oxidation by dGMP(−H)$^\bullet$ is larger than in D_2O by a factor of 1.5−2. This kinetic isotope effect indicates that the electron-transfer reaction from dGMP to 2AP*r*(−H)$^\bullet$ (or to 2AP(−H)$^\bullet$), and the reverse electron transfer from 2AP*r* (or 2AP) to dGMP(−H)$^\bullet$, is coupled to a deprotonation of the primary electron-transfer radical cation products, dGMP$^{\bullet+}$ and 2AP*r*$^{\bullet+}$ (or 2AP$^{\bullet+}$). Therefore, these reactions, involving redox equilibria between different nucleobases, can be considered in terms of proton-coupled electron-transfer reactions.

Introduction

Irradiation of DNA with intense UV laser pulses of 266 nm or shorter wavelengths can induce photoionization of the aromatic bases by sequential two-photon excitation mechanisms.[1] The base analogue 2-aminopurine (2AP) has a lower energy electronic absorption band than do the normal nucleic acid bases,[2] and therefore can be selectively excited with light of wavelengths >300 nm when incorporated as a single base in an oligonucleotide containing A, C, T, and G.[2f,3] We recently demonstrated that the excitation of oligonucleotides containing single 2AP residues with intense 308 nm XeCl excimer laser pulses results in the site-selective two-photon photoionization of the 2AP residues to form radical cations.[3] Purine radical cations generated by photoexcitation, or by reaction with strong oxidants such as $SO_4^{\bullet-}$, Tl(II), and $Br_2^{\bullet-}$, are strong Brønsted acids and deprotonate rapidly in neutral aqueous solutions.[4] Thus at pH 7.0, the 2AP$^{\bullet+}$ radical cations formed deprotonate to the 2AP neutral radicals, 2AP(−H)$^\bullet$, on time scales <100 ns. We have shown[3] that the 2AP(−H)$^\bullet$ radicals selectively oxidize guanines resulting in the formation of the neutral radicals, G(−H)$^\bullet$, but do not oxidize any of the other three nucleic acid bases (A, C, and T). Here, employing transient absorption spectroscopic techniques, we report that a *solvent isotope effect on the kinetics of oxidation of 2′-deoxyguanosine 5′-monophosphate (dGMP) by 2AP(−H)$^\bullet$ radicals is observed in H_2O and D_2O solutions.* This kinetic isotope effect[5] suggests that electron transfer from dGMP to 2AP(−H)$^\bullet$ is coupled with a proton transfer process[8] which leads to the formation of the neutral products, dGMP(−H)$^\bullet$ and 2AP. Here, we focus on the reactions of 2-amino-9-β-D-ribofuranosylpurine (2AP*r*) with dGMP.



Results and Discussion

The transient absorption spectra of the 2AP*r* photolysis products generated by 308 nm laser pulses were first determined in the absence of dGMP. The 2AP*r* (0.1 mM) phosphate buffer solutions (pH 7.0) were saturated with O_2 (1 atm partial pressure) to scavenge the hydrated electrons formed in the primary photoionization reaction.[3] The transient absorption spectrum of 2AP*r*(−H)$^\bullet$ neutral radicals recorded at a delay time of $\Delta t = 1$ μs after an ~12 ns 308 nm XeCl excimer laser pulse excitation is shown in Figure 1A (trace 1). Two absorption maxima due to the 2AP*r*(−H)$^\bullet$ radical[9] at 365 and at 515 nm and a decrease in the absorbance at 305 nm due to the laser-induced bleaching of the 2AP absorption band are evident.

The addition of dGMP to the 2AP*r* solutions results in the appearance of the known narrow absorption band of the dGMP(−H)$^\bullet$ neutral radical near 310 nm.[4] The transient absorption spectra of 2AP*r* solutions containing two different concentrations of dGMP at delay times $\Delta t = 25$ μs ([dGMP] = 1.2 mM, trace 2) and $\Delta t = 15$ μs ([dGMP] = 5 mM, trace 3) after the laser pulse excitation are also shown in Figure 1A. At these long delay times, the absorbances at 310 nm, and hence

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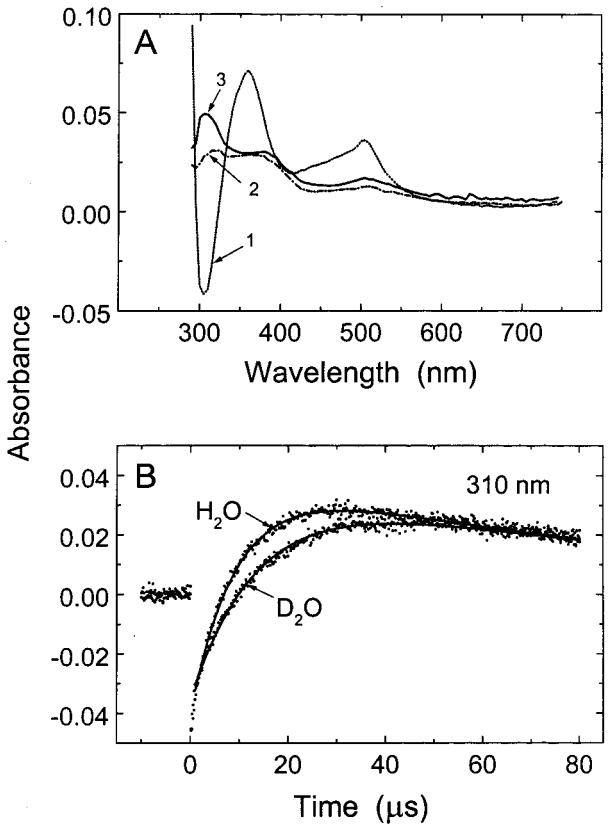
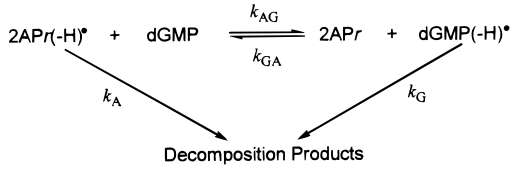


Figure 1. Panel A: Transient absorption spectra of 2AP*r* (0.1 mM) and dGMP in *oxygenated* buffer solutions (pH 7.0) measured at different delay times, Δt, after a laser pulse excitation. Trace 1: [dGMP] = 0, $\Delta t = 1$ μs. Trace 2: [dGMP] = 1.2 mM, $\Delta t = 20$ μs. Trace 3: [dGMP] = 5.0 mM, $\Delta t = 15$ μs. Panel B: Deuterium isotope effect on the kinetics of the transient absorption at 310 nm induced by a laser pulse excitation of 2AP*r* (0.1 mM) and dGMP (1.2 mM) in *oxygenated* H_2O/D_2O buffer solutions (pH 7.0). The solid lines are fits of eqs 1 and 2 to the experimental data points. The samples in buffer solutions (20 mM $NaHPO_4/Na_2HPO_4$, pH 7.0) were photoexcited with 308 nm XeCl excimer laser pulses (70 mJ/pulse/cm^2, fwhm = 12 ns, 10 Hz) in a quartz flow cell, and a solution flow rate of 2.5 mL/min. The transient absorption spectra were recorded using a computer-controlled kinetic spectrometer system[3] capable of automatically advancing the wavelength settings of the monochromator stepwise, at 5 nm intervals.

the yields of dGMP(−H)$^\bullet$ radicals, attain their maximum values. The dGMP(−H)$^\bullet$ yields increase with increasing dGMP concentrations (compare traces 2 and 3) since the oxidation of dGMP by 2AP*r*(−H)$^\bullet$ radicals is a reversible reaction, and the formation of dGMP(−H)$^\bullet$ radicals is favored at the higher dGMP concentrations.[3]

The kinetics of dGMP oxidation by 2AP*r*(−H)$^\bullet$ radicals occurring on a microsecond time scale were recorded at 310 nm because, at this wavelength, both the 2AP(−H)$^\bullet$ radicals and the dGMP(−H)$^\bullet$ radicals contribute to the transient absorbance signals (Figure 1A). The time course of the laser pulse-induced transient absorbance measured at 310 nm of H_2O and D_2O solutions of 2AP*r* and dGMP are depicted in Figure 1B. In H_2O, the rise of the 310 nm absorbance signal is markedly faster than in D_2O. The maximum of the transient absorbance in H_2O is observed at an earlier time than in D_2O. A similar deuterium isotope effect was also observed in the kinetics of oxidation of dGMP by 2AP(H)$^\bullet$ neutral radicals (data not shown).

SCHEME 1



The kinetic isotope effect on the electron-transfer equilibrium between 2AP*r* and dGMP may be interpreted in terms of Scheme 1. In this scheme, k_{AG} and k_{GA} are bimolecular constants of dGMP and 2AP*r* oxidation by 2AR*r*(−H)$^\bullet$ and dGMP(−H)$^\bullet$ radicals, while k_A and k_G represent pseudo first-order rate constants that specify competitive decay channels.

On time scales ≥ 1 μs, the 310 nm transient absorbance is a function of: (i) the concentrations of the neutral radicals [2AP*r*(−H)$^\bullet$], [dGMP(−H)$^\bullet$], and ground-state molecules [2AP*r*], and (ii) their molar extinction coeffiecients ϵ_A, ϵ_G, and ϵ_0, respectively, as follows:

$$A_{310}(t) = (\epsilon_A - \epsilon_0)\,[2\mathrm{AP}r(-\mathrm{H})^\bullet] + \epsilon_G\,[\mathrm{dGMP}(-\mathrm{H})^\bullet] \quad (1)$$

It is shown in greater detail in the Supporting Information that the time-dependent concentrations of [2AP*r*(−H)$^\bullet$] and [dGMP(−H)$^\bullet$] are given by

$$[2\mathrm{AP}r(-\mathrm{H})^\bullet] = \mathrm{A}\exp(-k_1 t) + \mathrm{B}\exp(-k_2 t)$$

and

$$[\mathrm{dGMP}(-\mathrm{H})^\bullet] = \mathrm{C}\,[\exp(-k_1 t) - \exp(-k_2 t)] \quad (2)$$

where, A, B, and C are constants that depend on the initial concentrations of the 2AP*r*(−H)$^\bullet$ radicals, the concentration of dGMP, and the constants defined in Scheme 1. The solid traces shown in Figure 1B are the best least-squares fits of eqs 1 and 2 to the experimental data points. The values of k_1 and k_2 thus obtained are summarized in Table 1. The values of k_1 in H_2O are significantly larger than those in D_2O solutions by a factor of ~1.5. The k_2 values are, within experimental error, similar in H_2O and in D_2O (Table 1). Further analysis of the values of k_1 and k_2 according to Scheme 1 (see, Supporting Information) indicates that the kinetic isotope effect on the experimental rate constants, k_1, is related to an isotope effect on the rate constants k_{AG} and k_{GA}. In H_2O, the values of k_{AG} and k_{GA} are larger than in D_2O by factors of 1.5−2. This kinetic isotope effect indicates that the electron transfer from dGMP to 2AP*r*(−H)$^\bullet$ (or 2AP(−H)$^\bullet$) radicals, and the reverse electron transfer from 2AP*r* (or 2AP) to dGMP(−H)$^\bullet$ radicals, are coupled to proton transfer processes: (i) a deprotonation of the radical cations, dGMP$^{\bullet+}$ and 2AP*r*$^{\bullet+}$ (or 2AP$^{\bullet+}$), and (ii) protonation of the anions,

TABLE 1: Kinetic Parameters of the Reversible Proton-Coupled Electron Transfer Reaction between 2-Aminopurine Neutral Radical and 2′-Deoxyguanosine 5′-Monophosphate in Neutral Aqueous Solutions

	k_1 (10^5 s^{-1})			k_2[a] (10^5 s^{-1})	k_{AG} (10^7 M^{-1} s^{-1})			k_{GA} (10^7 M^{-1} s^{-1})		
aminopurine radical	H_2O	D_2O	H_2O/D_2O		H_2O	D_2O	H_2O/D_2O	H_2O	D_2O	H_2O/D_2O
2AP*r*(−H)$^\bullet$	1.2 ± 0.1	0.7 ± 0.1	1.7 ± 0.2	0.09 ± 0.01	6.2 ± 0.6	2.9 ± 0.3	2.1 ± 0.3	24 ± 2	14 ± 1	1.7 ± 0.2
2AP(−H)$^\bullet$	2.4 ± 0.2	1.7 ± 0.2	1.4 ± 0.2	0.09 ± 0.01	3.5 ± 0.4	2.3 ± 0.2	1.5 ± 0.2	42 ± 4	29 ± 3	1.4 ± 0.2

[a] Negligible isotope effect.

2AP*r*(−H)$^-$ (or 2AP(−H)$^-$) and dGMP(−H)$^-$. Such a proton-coupled electron transfer step leads to a lowering of the overall free energy of reaction, thus favoring electron transfer.[10] The possibility that an analogous process might occur as an intramolecular reaction in DNA was recently considered in single-stranded oligonucleotides containing a single 2AP residue and GG doublets, and similar proton-coupled electron transfer phenomena were observed.[3]

The recent extensive theoretical analysis of proton-coupled electron transfer reactions between redox sites linked by peptide bridges in proteins has shown that hydrogen bond chains formed in these bridges facilitate a long-distance proton-coupled electron transfer which can occur even on picosecond time scales.[11] The possibility that such processes can occur in DNA as well deserves to be examined.

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Supporting Information Available: The solution of the coupled differential equations based in Scheme 1, the methods of deriving the numerical values of the constants provided in Table 1, and the numerical fits of the data in Figure 1B. This material is available free of charge via the Internet at http://pubs.acs.org.

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