Reactions of Oxide Radical Ion ($\cdot O^-$) with Pyrimidine and Purine Derivatives

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The reaction of oxide radical ion ($\cdot O^-$) with a variety of pyrimidine and purine derivatives, in strong alkaline aqueous solution (pH 13.7), has been investigated by means of the pulse radiolysis technique. Rate constants for the reaction of $\cdot O^-$ species with various substrates are in the range of $(2.8-7.8) \times 10^8$ M^{-1} s^{-1}. Absorption spectra of the transients and information on their decay reactions have also been obtained. For comparison, the one-electron oxidation of the same substrates with $Br_2^{\bullet -}$ have also been carried out at the same pH when it was necessary. With uracil, cytosine, adenine, and guanine, the oxide radical ion effects a one-electron oxidation, whereas in the presence of extractable hydrogens (e.g., with methyl-substituted derivatives) it abstracts a hydrogen atom from the methyl group. The transient species derived from uracil and adenine decay by first-order kinetics ($k_{decay} = 1.0 \times 10^4$ s^{-1}), whereas the transient species derived from cytosine and guanine as well as those obtained after hydrogen abstraction from the methyl-substituted derivatives decay by second-order kinetics ($2k = (3-9) \times 10^8$ M^{-1} s^{-1}).

Introduction

In strongly alkaline solutions $\cdot OH$ radical is rapidly converted to its conjugate base $\cdot O^-$ (oxide radical ion).[1] Differences between the reactivities of these two species have been observed; e.g., inorganic anions react more slowly with $\cdot O^-$ than with $\cdot OH$, whereas cations react faster.[2] Furthermore, $\cdot O^-$ (in contrast with $\cdot OH$) adds to molecular oxygen with a rate of 3.6×10^9 M^{-1} s^{-1}.[2b] With organic molecules, $\cdot O^-$ radical has a low tendency to add to double bonds or to aromatic rings (2−3 orders of magnitude less than $\cdot OH$) although its hydrogen abstracting ability does not significantly differ from that of $\cdot OH$.[1,3−5] Neta and Schuler[4] have also found that $\cdot O^-$ reacts with the phenoxide anion to produce the phenoxyl radical, with a rate constant of 6.5×10^8 M^{-1} s^{-1}, which is 1 order of magnitude greater than the expected value for the addition of $\cdot O^-$ to aromatic rings.[3−5] They suggested a mechanism in which a direct one-electron transfer from phenoxide to $\cdot O^-$ radical takes place.[4] This indicates that $\cdot O^-$ species can also behave as an oxidant, since $E°(\cdot O^-, H^+/OH^-) = 1.77$ V,[6] and one-electron oxidation must be considered when the substrate does not contain any extractable hydrogens. Surprisingly, the electron-transfer path with organic molecules has been reported only in the phenoxide anion case.

The reaction of the $\cdot O^-$ radical with pyrimidine derivatives is not without precedent, but there are no reports available in the literature with purine derivatives. Early pulse radiolysis[7,8] and EPR[9] studies have explicitly reported that $\cdot O^-$ reacts with uracil and cytosine by adding to the pyrimidine ring, and with thymine and 5-methylcytosine by abstracting a hydrogen from the methyl group to form an allyl-type radical. The $\cdot OH$ radical adduct of uracil in basic solution undergoes first-order decay to produce a new radical whose spectrum[7−9] was later[10,11] identified as the uracil radical anion obtained by one-electron oxidation of uracil with the powerful oxidant $SO_4^{\bullet -}$, i.e., $E°(SO_4^{\bullet -}/SO_4^{2-}) = 2.43$ V.[12] EPR[10] and pulse radiolysis[11] studies suggested as well that a base-catalyzed dehydration reaction takes place from the $\cdot OH$ adduct of uracil. Similar results were obtained for cytosine by pulse radiolysis.[13] However, in these studies[11,13] the addition of the $\cdot O^-$ species to uracil or cytosine is implicitly assumed since some experiments were performed at pH 13. In this article we wish to report a detailed study of the reactions of $\cdot O^-$ radical generated at pH 13.7 with a variety of pyrimidine (uracil, cytosine, thymine, 1-methyluracil, and 1-methylcytosine) and purine (adenine, guanine, and 9-methylguanine) derivatives. For comparison, the one-electron oxidation of these substrates with $Br_2^{\bullet -}$, which has a redox potential similar to that of the $\cdot O^-$ radical [$E°(Br_2^{\bullet -}/2Br^-) = 1.66$ V],[12] has been carried out at the same pH when it was necessary.[14,15]

Experimental Section

Materials. The substrates were obtained from Aldrich, Fluka, and Sigma and were used as received. All these compounds were stable for at least 6 h under all the concentrations and pH conditions used in this study. NaOH and KBr (Merck Suprapur) were employed. Water was purified with a Millipore (Milli-Q) system.

Pulse Radiolysis. Pulse radiolysis with optical absorption detection was performed at the Consiglio Nazionale delle Ricerche (Bologna) by using the 12 MeV linear accelerator, which delivered 20−200 ns electron pulses with doses between 5 and 50 Gy, by which $OH^{\bullet}$, $H^{\bullet}$, and e_{aq}^- are generated with 1−20 μM concentrations.[16] The pulse irradiations were performed at room temperature (22 ± 2 °C) on samples contained in Spectrosil quartz cells of 2 cm optical path length. Solutions were protected from the analyzing light by means of a shutter and appropriate cutoff filters. The bandwith used throughout the pulse radiolysis experiments was 5 nm. The radiation dose per pulse was monitored by means of a charge collector placed

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behind the irradiation cell and calibrated with an N_2O-saturated solution containing 0.1 M HCO_2^- and 0.5 mM methyl viologen, using $G\epsilon = 9.32 \times 10^4$ at 602 nm.[17] $G(X)$ represents the number of molecules of species formed per 100 eV of energy absorbed by the system. All the experiments in highly alkaline solutions were performed with freshly prepared solutions. Typically, N_2O was bubbled in the solvent for at least 10 min before adding NaOH, whereas the substrate was added immediately before the irradiation. The solutions, if not otherwise stated, typically contained 0.5−1 mM substrate and 0.5 M NaOH.

Results

Generation of $\cdot O^-$ and $Br_2^{\bullet -}$ Radicals.[1] Radiolysis of neutral water leads to the species e_{aq}^-, ·OH, and ·H as shown in eq 1 in which the values in parentheses represent the yields expressed in terms of *G*-values (molecules/100 eV of absorbed radiation). In basic solutions, ·H are converted into solvated electrons with a rate constant of 2.2×10^7 M^{-1} s^{-1} (eq 2), whereas the presence of N_2O transforms efficiently solvated electrons into the $\cdot O^-$ species (eq 3, $k_3 = 9.1 \times 10^9$ M^{-1} s^{-1}). The ·OH radical [$pK_a(OH^{\bullet}) = 11.9$] is in equilibrium with its basic form, the $\cdot O^-$ radical. The rate constants of the forward and reverse reactions (eq 4/−4) are 1.2×10^{10} M^{-1} s^{-1} and 1×10^8 s^{-1}, respectively.[18] Thus, at high pH both the ·OH and the $\cdot O^-$ must be considered as the reacting species.

$$H_2O \rightsquigarrow e_{aq}^- (2.6),\ \cdot OH\ (2.7),\ \cdot H\ (0.6) \qquad (1)$$

$$\cdot H + OH^- \longrightarrow e_{aq}^- + H_2O \qquad (2)$$

$$e_{aq}^- + N_2O \longrightarrow \cdot O^- + N_2 \qquad (3)$$

$$\cdot OH + OH^- \rightleftharpoons \cdot O^- + H_2O \qquad (4/-4)$$

In the present study, transformation of ·OH into $\cdot O^-$ was accomplished at pH 13.7 in N_2O-saturated aqueous solutions. Under these conditions we assumed that $G(\cdot O^-) = G(\cdot OH) + G(\cdot H) + G(e_{aq}^-) = 5.9$.

The dibromine radical anion was produced by irradiating N_2O-saturated solutions containing 0.1 M KBr and 0.1−0.5 M NaOH. Under such conditions both ·OH and $\cdot O^-$ species are converted into the dibromine radical anion through processes shown in eqs 5 and 6 with rate constants of 1.1×10^{10} and 2.2×10^8 M^{-1} s^{-1}, respectively.[1]

$$Br^- + \cdot OH \xrightarrow{Br^-} Br_2^{-\bullet} + OH^- \qquad (5)$$

$$Br^- + \cdot O^- \xrightarrow{Br^-,\ H_2O} Br_2^{-\bullet} + 2\,OH^- \qquad (6)$$

Uracil. The optical absorption spectrum obtained after reaction of $\cdot O^-$ radical with uracil, taken 15 μs after the pulse, is shown in Figure 1a. The spectrum contains a sharp band with $\lambda_{max} = 400$ nm and a broader band at longer wavelengths. The time profile of the formation of this transient (see Figure 2, inset) leads to a pseudo-first-order rate constant, $k_{buildup}$, and

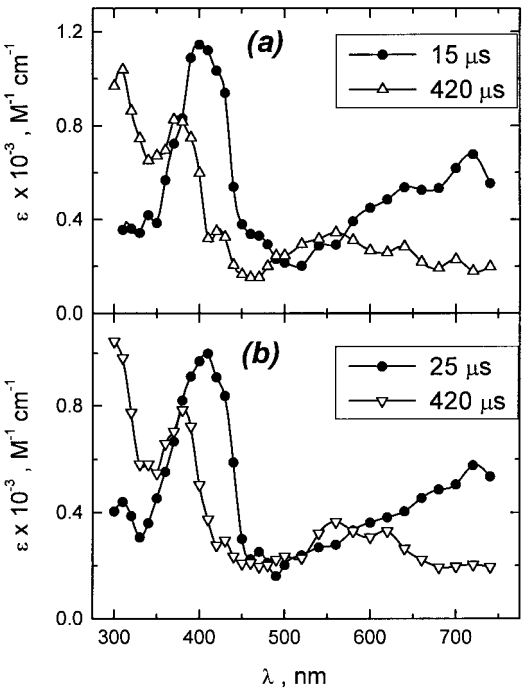


Figure 1. Comparison between the absorption spectra obtained after reaction of uracil with $\cdot O^-$ (a) and $Br_2^{\bullet -}$ (b) at pH 13.7. Data from pulse radiolysis of N_2O-saturated aqueous solutions (dose = 20 Gy) of (a) uracil (1 mM) and NaOH (0.5 M); (b) uracil (1 mM), NaOH (0.5 M), and KBr (0.1 M).

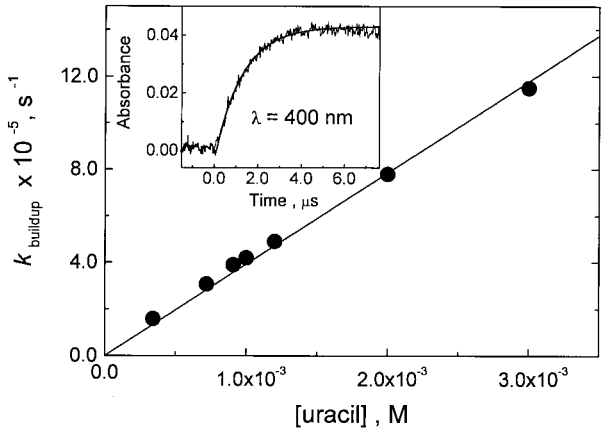


Figure 2. Plot of $k_{buildup}$ vs [uracil] for the reaction of $\cdot O^-$ with uracil at pH 13.7 in N_2O-saturated solution. Inset: representative buildup trace for [uracil] = 2 mM, dose = 20 Gy, optical path 2 cm; the solid line represents a first-order fit to the data.

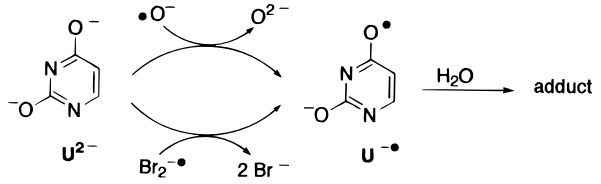
was measured at a number of different uracil concentrations. From the slope of the linear plot, the bimolecular rate constant, k_{obs}, was found to be 3.9×10^8 M^{-1} s^{-1} (Figure 2 and Table 1).[19] By replacing $\cdot O^-$ radical with $Br_2^{\bullet -}$, a similar spectrum is obtained (Figure 1b).[14] The slightly lower ϵ values are probably due to the fact that the spectrum was recorded 25 μs after the pulse, in order to allow most of the $Br_2^{\bullet -}$ radical (λ_{max} = 370 nm) to decay. Therefore, some of the transient with $\lambda_{max} = 400$ nm also disappeared. A bimolecular rate constant for this reaction, obtained in the same manner, was found to be 1.7×10^8 M^{-1} s^{-1}. At pH 13.7, uracil is present as a dianion (see Table 1 for the pK_a values). On the basis of the facts that $\cdot O^-$ and $Br_2^{\bullet -}$ radicals are good one-electron oxidants (E° = 1.77 and 1.66 V, respectively) and that $\cdot O^-$ reacts slightly faster than $Br_2^{\bullet -}$ with uracil to give the same species (see Table 1), we assigned this transient species to the uracil radical anion ($\mathbf{U}^{\bullet -}$), obtained by an electron-transfer oxidation (Scheme 1).[20]

TABLE 1: Absolute Rate Constants for the Reactions of a Variety of Substrates (S) with the $\cdot O^-$ (k_{obs}) and $Br_2^{\bullet -}$ Radicals,[a,b] Together with the Corresponding Reduction Potentials ($S^{\bullet}/S^-$) vs NHE and pK_a Values of S[c]

substrate (S)	E°_{13},[c] V	pK_a[e]	k_{obs},[a,b] M^{-1} s^{-1}	$k(Br_2^{\bullet -})$,[a] M^{-1} s^{-1}	$k(Br_2^{\bullet -})$,[c] M^{-1} s^{-1}
uracil	0.88	9.5, 13[f]	3.9×10^8	1.7×10^8	2×10^8 [h]
cytosine	0.81	4.6, 12.2	7.8×10^8	1.6×10^8	2×10^8 [h]
adenine	0.75	4.15, 9.8	2.8×10^8		4.6×10^7
guanine	0.63	3.3, 9.2, 12.3	4.3×10^8		2.5×10^8
thymine	0.79	9.9, >13[f]	7.2×10^8	1.7×10^8	2×10^8
1-methyluracil	1.6	9.7	5.5×10^8		2.2×10^6
1-methylcytosine	1.6	>13[g]	6.8×10^8		2×10^6
9-methylguanine	0.7[d]	9.2[d]	6.9×10^8	5.0×10^8	

[a] This work; at 22 ± 2 °C and pH 13.7; estimated to be accurate to ±10%. [b] The k_{obs} for reactions of $\cdot O^-$ at pH 13.7, which have been obtained from the linear plot of the rate of buildup of the radicals vs the substrate concentration, contain the contribution of the reaction of the substrates with radical ·OH; see the text. The corrected values are shown in Table 2. [c] From ref 15a; at pH 13.0. [d] Value refers to guanosine. [e] From ref 15a, unless otherwise stated. [f] From ref 7b. [g] From ref 29. [h] The same values obtained in ref 15b at pH 12.0.

SCHEME 1



The decay of $\mathbf{U}^{\bullet -}$ is identical in both experiments and follows first-order kinetics. Concomitantly, the buildup of a long-lived transient with $\lambda_{max} = 380$ and 550 nm was observed and is shown in Figure 1 (spectra taken 420 and 430 μs after the pulse for $\cdot O^-$ and $Br_2^{\bullet -}$, respectively). The measured rates for the decay of $\mathbf{U}^{\bullet -}$ were found to increase slightly with increasing doses, which indicates the presence of second-order contributions, owing to radical−radical reactions. To correct for this effect, rates were measured at different low doses and extrapolated to dose = 0. The experimental first-order rate constant for $\mathbf{U}^{\bullet -}$ decay, $k_{decay} = 1.0 \times 10^4$ s^{-1}, was found to be independent of OH^- concentration in the range 0.2−1 M and of uracil concentration in the range 0.2−1.2 mM. The long-lived transient with $\lambda_{max} = 380$ and 550 nm, which in turn decays with a rate constant of $<10^2$ s^{-1}, resembles, but is not identical to, that of the ·OH adducts of uracil[11] in neutral or slightly basic solutions. One of the possibilities for this reaction could be the addition of water to the C-6,[21] since this less electron-rich position should be the preferred site of attack for a nucleophilic species.[22]

Cytosine. The optical absorption spectrum obtained after reaction of $\cdot O^-$ radical with cytosine, taken 10 μs after the pulse, is shown in Figure 3. By replacing $\cdot O^-$ radical with $Br_2^{\bullet -}$, a similar spectrum was obtained (see Supporting Information).[14] The bimolecular rate constants for these reactions were found to be 7.8×10^8 and 1.6×10^8 M^{-1} s^{-1} for $\cdot O^-$ and $Br_2^{\bullet -}$, respectively, by following the buildup of the transient at 700 nm at different cytosine concentrations (Table 1).[19] The absorption spectra strongly resemble in shape and ϵ values those obtained by the reaction of cytosine monoanion with $SO_4^{\bullet -}$, which produces the cytosine radical ($\mathbf{C}^{\bullet}$) followed by a rapid deprotonation to give cytosine radical anion ($\mathbf{C}^{\bullet -}$) (cf. Scheme 2).[13] Therefore, the transient was assigned to $\mathbf{C}^{\bullet -}$. $Br_2^{\bullet -}$ can only oxidize cytosine, and therefore, the mechanism is expected to be the one reported for $SO_4^{\bullet -}$ (Scheme 2). On the other hand, the reaction of $\cdot O^-$ with cytosine could either proceed by

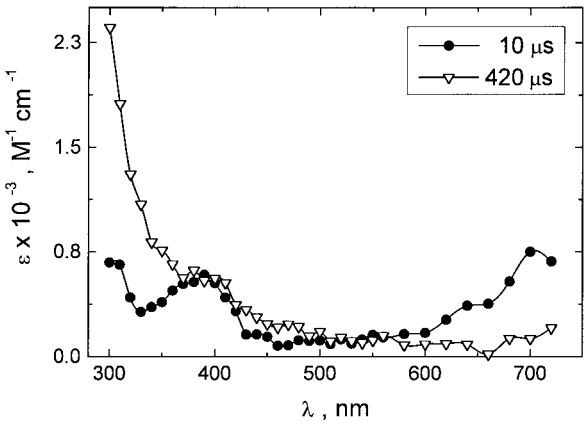
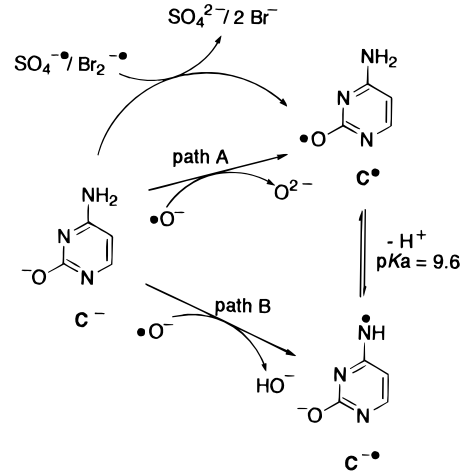


Figure 3. Absorption spectra obtained after reaction of cytosine with $\cdot O^-$ at pH 13.7. Data from pulse radiolysis of N_2O-saturated aqueous solutions (dose = 20 Gy) of cytosine (1 mM) and NaOH (0.5 M).

SCHEME 2



electron transfer followed by deprotonation to give $\mathbf{C}^{\bullet -}$ (Scheme 2, path A) or by a hydrogen abstraction from the NH_2 moiety directly giving the same transient species $\mathbf{C}^{\bullet -}$ (Scheme 2, path B). On the basis of the following observations, (i) $\cdot O^-$ is four times more reactive than $Br_2^{\bullet -}$ toward cytosine, (ii) cytosine is two times more reactive than uracil toward $\cdot O^-$, and (iii) the rate constant for the reaction of cytosine with $\cdot O^-$ is comparable with the rate obtained for the hydrogen abstraction from the methyl-substituted derivatives (vide infra), we suggest that the mechanism could involve direct hydrogen atom abstraction from the amino group to give $\mathbf{C}^{\bullet -}$ (Scheme 2, path B).

In contrast to the $\mathbf{U}^{\bullet -}$ radical, the decay of $\mathbf{C}^{\bullet -}$ approached second-order kinetics ($2k = 4.6 \times 10^8$ M^{-1} s^{-1}, monitored at 700 nm), though it systematically revealed some deviations. However, the disappearance of $\mathbf{C}^{\bullet -}$ gives rise to a new transient with a strong absorption at wavelengths below 350 nm (see Figure 3, spectra taken 420 μs after the pulse).

Adenine. The optical absorption spectrum obtained after reaction of $\cdot O^-$ radical with adenine, taken 15 μs after the pulse, is shown in Figure 4. The spectrum contains a sharp band with $\lambda_{max} = 340$ nm and a low-intensity broad band at longer wavelengths. From the time profile of the formation of this transient at λ_{max} and at a number of different adenine concentrations, the bimolecular rate constant, k_{obs}, was found to be 2.8×10^8 M^{-1} s^{-1} (Table 1). The same spectrum has been reported in the literature[15a] when adenine reacted with $Br_2^{\bullet -}$ at pH = 13.0 which we also reproduced at pH 13.7 (see Supporting Information). At pH 13.7, adenine is present as a monoanion

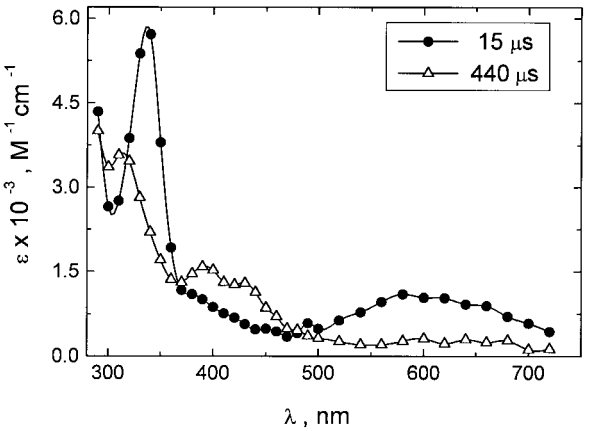


Figure 4. Absorption spectra obtained after reaction of adenine with $\cdot O^-$ at pH 13.7. Data from pulse radiolysis of N_2O-saturated aqueous solutions (dose = 20 Gy) of adenine (1 mM) and NaOH (0.5 M).

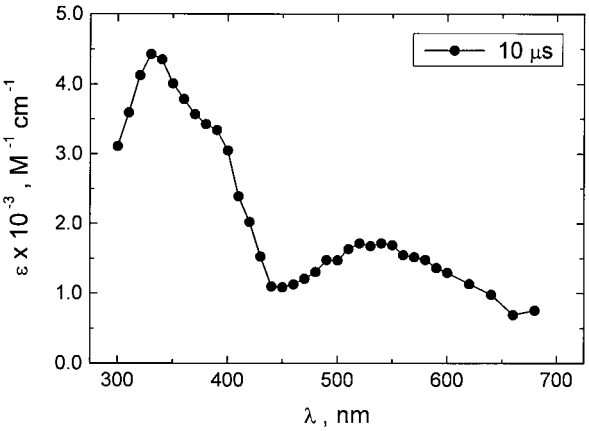
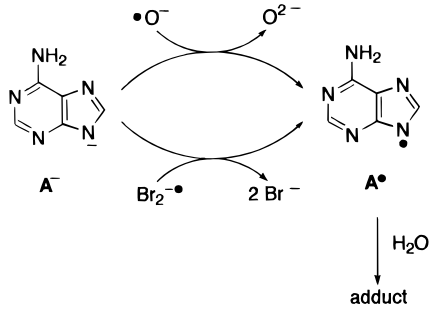


Figure 5. Absorption spectrum obtained after reaction of guanine with $\cdot O^-$ at pH 13.7. Data from pulse radiolysis of N_2O-saturated aqueous solutions (dose = 20 Gy) of guanine (1 mM) and NaOH (0.5 M).

SCHEME 3



(see Table 1 for the pK_a values), and we assigned the transient species to the **A**• radical (Scheme 3).

The behavior of **A**• radical was found to be very similar to that of **U**•− radical; i.e., the decay of **A**• radical approaches first-order kinetics, though it systematically showed some deviation. To correct for this deviation, rates were measured at different low doses and extrapolated to dose = 0. A value of $k_{decay} = 1.0 \times 10^4$ s^{-1} was obtained, which is independent of OH^- concentration in the range 0.2−1 M and adenine concentration in the range 0.2−1.1 mM. As for the **U**•− radical, the decay of **A**• radical occurs simultaneously with the buildup of a long-lived transient with $\lambda_{max} = 310$ and 390 nm (see Figure 4 spectrum taken 440 μs after the pulse), which in turn decays with a rate constant $<10^2$ s^{-1}. This long-lived transient resembles, but is not identical to, that of the •OH adduct of adenine in neutral or slightly basic solutions.[23] Water addition to the C-2 and/or C-8 could be the expected reaction,[21] since those less electron-rich position should be the preferred site of attack for a nucleophile.[24]

Guanine. The optical absorption spectrum obtained after reaction of $\cdot O^-$ radical with guanine, taken 10 μs after the pulse (Figure 5), is similar to the spectra reported in the literature when guanine reacted with $Br_2^{\bullet-}$ at pH 13.0.[15a] In strong alkaline solutions, guanine is present as a dianion (see Table 1 for the pK_a values), and the observed transient was assigned to the **G**•− radical. From the time profile of the formation of this transient at 350 nm and at a number of different guanine concentrations, the bimolecular rate constant, k_{obs}, was found to be 4.3×10^8 M^{-1} s^{-1}. One-electron oxidized guanine (**G**•−) obtained from $\cdot O^-$ radical decays with second-order kinetics ($2k = 2.8 \times 10^8$ M^{-1} s^{-1}, monitored at 350 nm), and its decay does not give rise to absorbing species.

Thymine, 1-Methyluracil, 1-Methylcytosine, and 9-Methylguanine. At pH 13.7, thymine is present as a dianion while the other methyl derivatives are expected to be present as monoanions (see Table 1 for the available pK_a values). The absorption spectra of the transients obtained after reaction of $\cdot O^-$ with thymine (Figure 6a, spectrum taken 10 μs after the pulse), 1-methyluracil, 1-methylcytosine, and 9-methylguanine (Figure 7) are similar and contain a strong band at $\lambda < 320$ nm and a weaker band/shoulder at longer wavelengths. The bimolecular rate constants, k_{obs}, for the reaction of $\cdot O^-$ with these methyl-substituted derivatives were determined by following the buildup of transients at ca. 300 nm at different substrate concentrations (Table 1).[19] All these transients decay with clean second-order kinetics and with rate constants that approach diffusion-controlled processes, i.e., $2k = (5-9) \times 10^8$ M^{-1} s^{-1}. Furthermore, these absorption spectra are very different from those obtained after reaction of the same substrates with $Br_2^{\bullet-}$ and/or with $SO_4^{\bullet-}$.[15a,25] The spectra taken

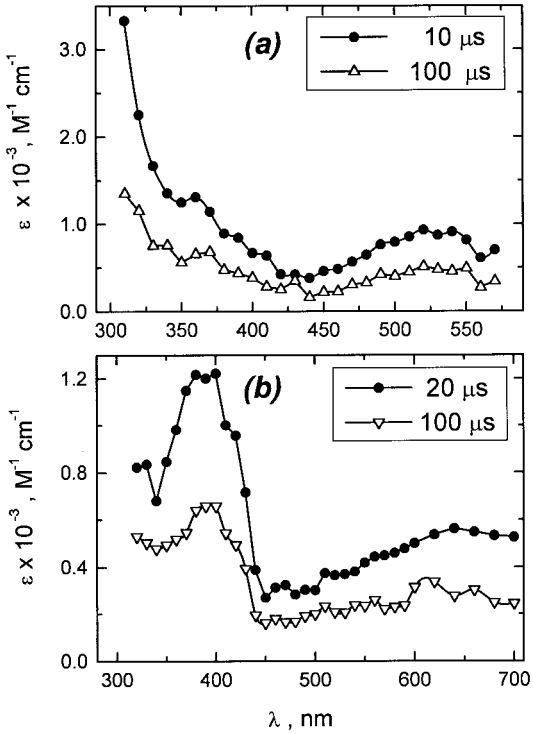
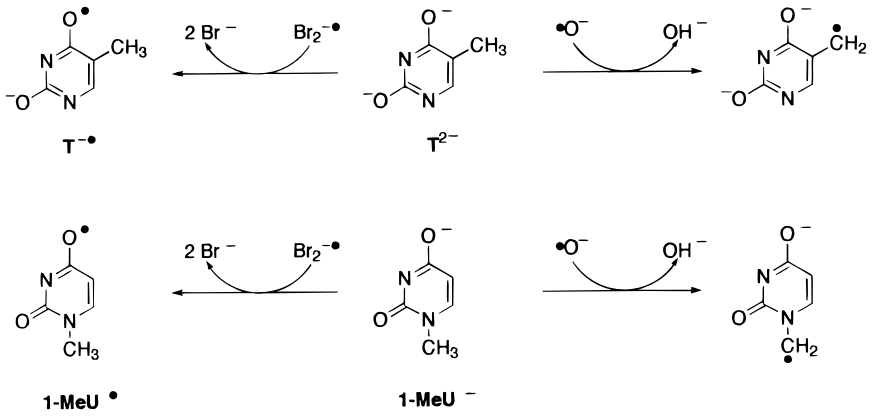


Figure 6. Comparison between the absorption spectra obtained after reaction of thymine with $\cdot O^-$ (a) and $Br_2^{\bullet-}$ (b) at pH 13.7. Data from pulse radiolysis of N_2O-saturated aqueous solutions (dose = 20 Gy) of (a) thymine (1 mM) and NaOH (0.5 M); (b) thymine (1 mM), NaOH (0.5 M), and KBr (0.1 M).

SCHEME 4



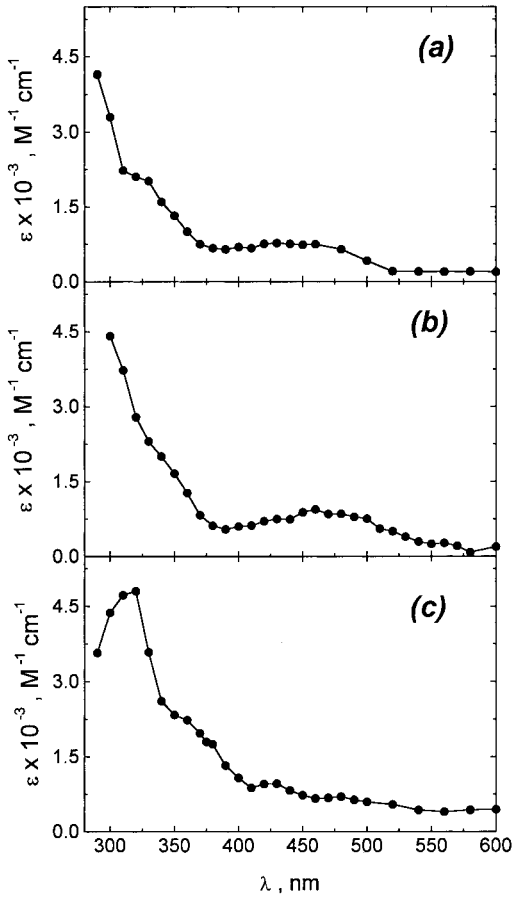


Figure 7. Absorption spectra obtained for the pulse radiolysis (dose = 20 Gy) of N_2O-saturated aqueous solutions containing 1-methyluracil (a), 1-methylcytosine (b), or 9-methylguanine (c) (0.5−1 mM) and NaOH (0.5 M). Spectra were taken 10 μs after the pulse.

10 and 20 μs after the pulse for thymine with $\cdot O^-$ and $Br_2^{\bullet -}$ are shown for comparison in parts a and b of Figure 6, respectively. The EPR spectrum[9,26] obtained from reaction of $\cdot O^-$ radical with thymine is consistent with an allyl-type radical, and therefore, we assigned the transients observed by reaction of methyl-substituted derivatives with $\cdot O^-$ to carbon-centered radicals derived from hydrogen abstraction from the methyl moiety.[27]

As shown in the upper part of Scheme 4, the reaction of thymine dianion ($\mathbf{T}^{2-}$) with $\cdot O^-$ ($k_{obs} = 7.2 \times 10^8$ M^{-1} s^{-1}) produces a carbon-centered radical that decays with second-order kinetics ($2k = 6 \times 10^8$ M^{-1} s^{-1}), whereas reaction with $Br_2^{\bullet -}$ ($k = 1.7 \times 10^8$ M^{-1} s^{-1}) produces a transient with $\lambda_{max} = 400$ that resembles $\mathbf{U}^{\bullet -}$ (cf. Figure 1 with Figure 6b). The behavior of one-electron oxidized thymine ($\mathbf{T}^{\bullet -}$) also resembles that of $\mathbf{U}^{\bullet -}$; i.e., the decay of $\mathbf{T}^{\bullet -}$ occurs by first-order kinetics, $k_{decay} = 8 \times 10^3$ s^{-1} (cf. Scheme 1 with Scheme 4).[28]

It is also interesting to compare the rate constants for the reactions of $\cdot O^-$ and $Br_2^{\bullet -}$ with 1-methyluracil (lower part of Scheme 4) and 1-methylcytosine, which have redox potentials two times larger than those of uracil and cytosine (Table 1). As already mentioned, $Br_2^{\bullet -}$ is a one-electron oxidant ($E° = 1.66$ V) that is not able to effect hydrogen abstraction. In fact, in these reactions the rate constants of $\cdot O^-$ are more than 2 orders of magnitude larger than those of $Br_2^{\bullet -}$ (see Table 1).[19] Similarly, the rate constant of thymine [$E°(T^{\bullet -}/T^{2-}) = 0.79$ V] with $Br_2^{\bullet -}$ is 2 orders of magnitude larger than that of 1-methyluracil [$E°$(1-MeU$^{\bullet}$/1-MeU$^-$) = 1.6 V], although the same rate constants were obtained when the two substrates reacted with the radical $\cdot O^-$. Therefore, the rate of hydrogen atom abstraction does not depend on the redox potential of the substrate (Scheme 4).

Discussion

In strong alkaline solutions, both $\cdot O^-$ and $\cdot$OH species must be considered as reactants and the overall reaction rate is expected to depend on pH, since $\cdot$OH is progressively replaced by $\cdot O^-$ with increasing pH.

$$\cdot OH + S \xrightarrow{k(\cdot OH)} \text{Products} \quad (7)$$

$$\cdot O^- + S \xrightarrow{k(\cdot O^-)} \text{Products} \quad (8)$$

Under these conditions, the observed rate constant, k_{obs}, is given by eq 9[1]

$$k_{obs} = \frac{k(\cdot OH) + k(\cdot O^-)\frac{K}{[H^+]}}{1 + \frac{K}{[H^+]}} \quad (9)$$

where the equilibrium constant (K) of eq (4/−4) is 1.26×10^{-12} M^{-1}. Rate constants for the reaction of pyrimidine and purine derivatives with $\cdot O^-$ at pH 13.7 can be obtained from k_{obs} by applying eq 9, provided that the reactivity of the substrates with $\cdot$OH is known at the same pH. The $k(\cdot OH)$ values for uracil, cytosine, adenine, guanine, and thymine at pH 7 are known from the literature and are collected in Table 2. The remaining values for 1-methyluracil, 1-methylcytosine, and 9-methylguanine were

SCHEME 5

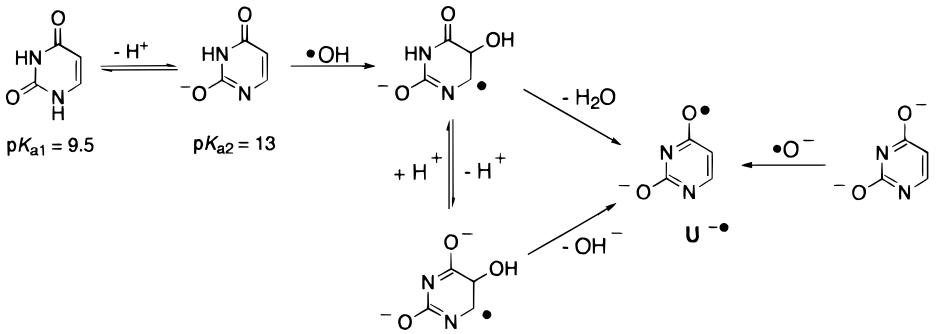


TABLE 2: Rate Constants for Reaction of the Substrates Investigated with the ·OH and ·O⁻ Radicals, Together with the Percentages of ·OH Attack on the Substrates at pH 13.7

substrate (S)	k(·OH),[a] $M^{-1}s^{-1}$	k(·O⁻),[f] $M^{-1}s^{-1}$	%S−OH
uracil	5.7×10^9 [b]	3.1×10^8	23.4
cytosine	6.1×10^9 [b]	7.0×10^8	12.6
	5.6×10^9 [c,d]		
adenine	6.1×10^9 [b]	1.9×10^8	34.7
	5.7×10^9 [c,d]		
guanine	9.2×10^9 [b,e]	2.9×10^8	34.5
thymine	6.4×10^9 [b]	6.3×10^8	14.4
1-methyluracil	7.6×10^9 [d]	4.8×10^8	20.7
1-methylcytosine	6.6×10^9 [d]	5.9×10^8	15.3
9-methylguanine	7.4×10^9 [d]	5.9×10^8	17.0

[a] At pH ∼ 7, unless otherwise indicated. [b] From ref 1. [c] At pH 11. [d] This work; at 22 ± 2 °C; estimated to be accurate to ±10%. [e] Refers to reaction with the guanine anion (pH 10). [f] Obtained from k_{obs} values given in Table 1, by applying eq 9.

determined by us, under identical conditions, and are also reported in Table 2. Furthermore, we have shown that the rate constants for the reaction of ·OH with cytosine and adenine, at pH 11.0, are the same within experimental errors as those previously reported at pH 7 (Table 2). Therefore, it is resonable to assume that the k(·OH) values will be, within experimental error, the same at the alkaline pH under which the reactions were investigated. By applying eq 9, the k(·O⁻) values were evaluated for all substrates (Table 2).

Comparison of the k(·OH) and k(·O⁻) values in Table 2 reveals that the rate constants for the ·OH reactions are 8−30 times larger than the corresponding reactions with ·O⁻. For a particular substrate, the percentage of contribution of ·OH and ·O⁻ species as a function of pH can be evaluated using computer modeling.[30,31] The results obtained for the uracil case are shown in Figure 8. It is evident that, even at pH 13.7, the contribution of the ·OH reaction is not negligible. We calculated that the contribution of the ·OH and ·O⁻ species at pH 13.7 are 23.4% and 76.6%, respectively. In Table 2 the percentage of contribution of the ·OH reaction with all substates at pH 13.7 is given. It is worth pointing out that for the determination of the ϵ values in Figures 1−7 it was assumed that G(·O⁻) = 5.9. This statement is certainly valid for compounds such as uracil, cytosine, and adenine, since the ·OH adduct rapidly dehydrates to give the same product obtained after oxidation by $O^{\bullet-}$ (vide infra). In all other cases it is necessary to consider that those values are only ϵ apparent.

It is well-known that ·OH radicals add to free bases of nucleic acids preferentially at the more electron-rich positions,[22,24] i.e., C5 of pyrimidines[32] and C4, C5, and C8 of purines.[23] Steenken and co-workers[11,13] have shown that the C5−OH adducts of uracil and cytosine undergo a dehydration reaction, which is accelerated in basic media, e.g., $k_{deh} = 7.6 \times 10^5[OH^-]^{1/2}$ s^{-1} for uracil and $k_{deh} = 2.9 \times 10^6[OH^-]^{1/2}$ s^{-1} for cytosine.[33] Water

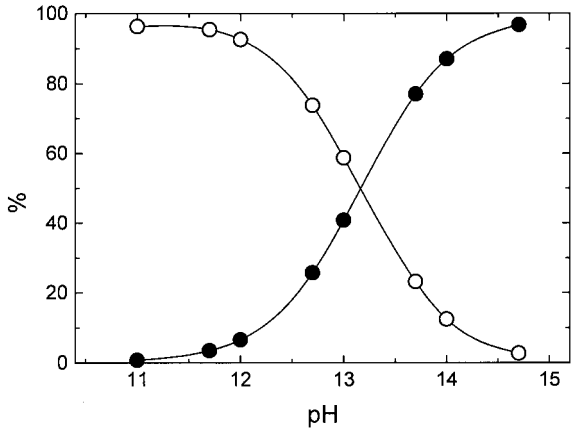


Figure 8. Variation of the percent of electron-transfer oxidation by ·O⁻ (●) and of ·OH addition to the C5−C6 double bond (○) as a function of the pH in pulse radiolysis of N_2O-saturated solutions of 1mM uracil, dose = 20 Gy.

(or OH^-) elimination from the OH adducts of uracil and cytosine produces, depending on the pH, **U**·/ **U**·⁻ and **C**·/**C**·⁻, respectively.

This addition−elimination sequence is illustrated for uracil in Scheme 5 (left side). On the other hand, the ·O⁻ species effects an electron-transfer oxidation, yielding the same **U**·⁻ radical, Scheme 5 (right side). The intermediacy of an ·O⁻ adduct as a precursor of **U**·⁻, as well as of **C**·⁻, **A**·, and **G**·⁻, has to be excluded, since the rates of formation of those species indicate a linear dependence on the substrate even at high concentration. In the case of uracil, for example, this linear dependence also exists with [U] up to 3 mM, for which the $k_{buildup} = 1.2 \times 10^6$ s^{-1}, as shown in Figure 2. We have demonstrated, using computer modeling,[31] that, if an intermediate exists, its decomposition rate must be higher than 10^7 s^{-1}, much higher than the rate obtained for the dehydration of the uracil ·OH adduct at pH 13.7. The overall mechanism of the reaction of ·OH/·O⁻ with uracil is given in Scheme 5, and as indicated in Figure 8, the ·O⁻ species starts to play a role at pH 12.

The spectra in Figure 7 deserve further comment. These transients were assigned to carbon-centered radicals derived from hydrogen abstraction from the methyl moiety. These species can be considered as the prototype C1′ radicals in 2-deoxyuridine, cytosine, and guanosine. Recently, the first spectroscopic data for C1′ radical in 2-deoxyuridine have been obtained selectively by photolysis of the corresponding *tert*-butyl ketone.[34] It is gratifying to see that this optical absorption spectrum is similar to the one obtained in the present study. Furthermore, the similarity of the spectra in Figure 7 between purine and pyrimidine-substituted methyl radicals is in agreement with the findings that C1′ radicals are substantially

stabilized by the presence of the base and that the degree of stabilization is similar for purine and pyrimidine moieties.[35]

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Supporting Information Available: Absorption spectra (4 pages). See any current masthead page for ordering and Internet access information.

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(20) A similar spectrum was obtained from the reaction of $SO_4^{\bullet-}$ with uracil at pH 11.[10,11]

(21) An alternative possibility could be protonation by water in a fashion similar to that observed for the electron adduct of uracil or adenine in neutral pH. See: von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: Philadelphia, PA 1987; pp 118−121. We thank Prof. C. von Sonntag for bringing this alternative pathway to our attention.

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