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The reactions of cytidine and 2′-deoxycytidine with $SO_4^{\bullet -}$ revisited. Pulse radiolysis and product studies

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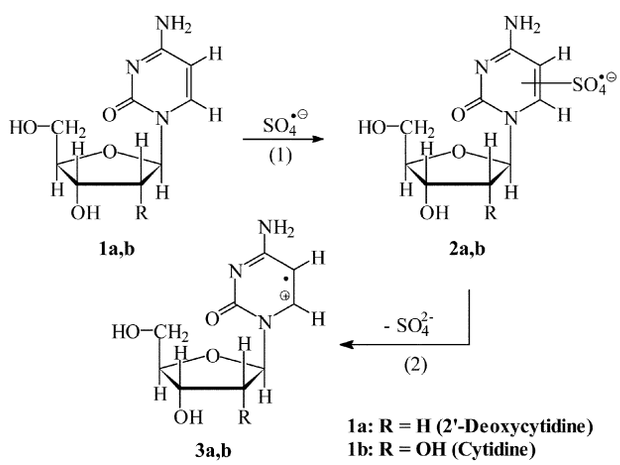
The reactions of $SO_4^{\bullet -}$ with 2′-deoxycytidine **1a** and cytidine **1b** lead to very different intermediates (base radicals with **1a**, sugar radicals with **1b**). The present study provides spectral and kinetic data for the various intermediates by pulse radiolysis as well as information on final product yields (free cytosine). Taking these and literature data into account allows us to substantiate but also modify in essential aspects the current mechanistic concept (H. Catterall, M. J. Davies and B. C. Gilbert, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1379). $SO_4^{\bullet -}$ radicals have been generated radiolytically in the reaction of peroxodisulfate with the hydrated electron (and the $H^{\bullet}$ atom). In the reaction of $SO_4^{\bullet -}$ with **1a** ($k = 1.6 \times 10^9$ dm^3 mol^{-1} s^{-1}), a transient ($\lambda_{max} = 400$ nm, shifted to 450 nm at pH 3) is observed. This absorption is due to two intermediates. The major component ($\lambda_{max} \approx 385$ nm) does not react with O_2 and has been attributed to an *N*-centered radical **4a** formed upon sulfate release and deprotonation at nitrogen. The minor component, rapidly wiped out by O_2, must be due to *C*-centered OH-adduct radical(s) **6a** and/or **7a** suggested to be formed by a water-induced nucleophilic replacement. These radicals decay by second-order kinetics. Free cytosine is only formed in low yields ($G = 0.14 \times 10^{-7}$ mol J^{-1} upon electron-beam irradiation). In contrast, **1b** gives rise to an intermediate absorbing at $\lambda_{max} = 530$ nm (shifted to 600 nm in acid solution) which rapidly decays ($k = 6 \times 10^4$ s^{-1}). In the presence of O_2, the decay is much faster ($k \approx 1.3 \times 10^9$ dm^3 mol^{-1} s^{-1}) indicating that this species must be a *C*-centered radical. This has been attributed to the C(5)-yl radical **8** formed upon the reaction of the C(2′)-OH group with the cytidine $SO_4^{\bullet -}$-adduct radical **2b**. This reaction competes very effectively with the corresponding reaction of water and the release of sulfate and a proton generating the *N*-centered radical. Upon the decay of **8**, sugar radical **11** is formed with the release of cytosine. The latter is formed with a *G* value of 2.8×10^{-7} mol J^{-1} (85% of primary $SO_4^{\bullet -}$) at high dose rates (electron beam irradiation). At low dose rates (γ-radiolysis) its yield is increased to 7×10^{-7} mol J^{-1} due to a chain reaction involving peroxodisulfate and reducing free radicals. Phosphate buffer prevents the formation of the sugar radical at the $SO_4^{\bullet -}$-adduct stage by enhancing the rate of sulfate release by deprotonation of **2b** and also by speeding up the decay of the C(5)-yl radical into another (base) radical. Cytosine release in cytidine is mechanistically related to strand breakage in poly(C). Literature data on the effect of dioxygen on strand breakage yields in poly(C) induced by $SO_4^{\bullet -}$ (suppressed) and upon photoionisation (unaltered) lead us to conclude that in poly(C) and also in the present system free radical cations are not involved to a major extent. This conclusion modifies an essential aspect of the current mechanistic concept.

In the free-radical chemistry of DNA, the properties of nucleobase radical cations are of considerable interest. They are formed as short-lived intermediates upon photoionization[1] and by the direct effect of ionising radiation[2] on DNA. Besides photoionisation,[3] a number of methods have been employed to generate radical cations of DNA model compounds using strong oxidants such as radical cations having a higher oxidation state,[4] photoexcited quinones[5–7] or the $SO_4^{\bullet -}$ radical anion.[4,8–22] The latter has the advantage that it can be readily generated from peroxodisulfate either photolytically[23] or radiolytically[24] (see below).

In the reaction of $SO_4^{\bullet -}$ with pyrimidine nucleosides, it is generally inferred that $SO_4^{\bullet -}$ adds to the olefinic double bonds of the base moieties [*cf.* reaction (1), R = H, 2′-deoxycytidine **1a**, R = OH, cytidine **1b**].[16] These adducts are very short-lived intermediates and are considered to dissociate in the nanosecond time scale to give the base radical cations and SO_4^{2-} [*cf.* reaction (2)].[17,18,25]



These base radical cation species then rapidly decay (life time estimated at < 200 ns)[10] either by deprotonation,[10,18,25] by the reaction of water at C(5) and C(6),[10,26,27] or by addition of phosphate.[23] Alternatively, the $SO_4^{\bullet -}$-adduct radical may release sulfate concomitantly with a proton and/or it may

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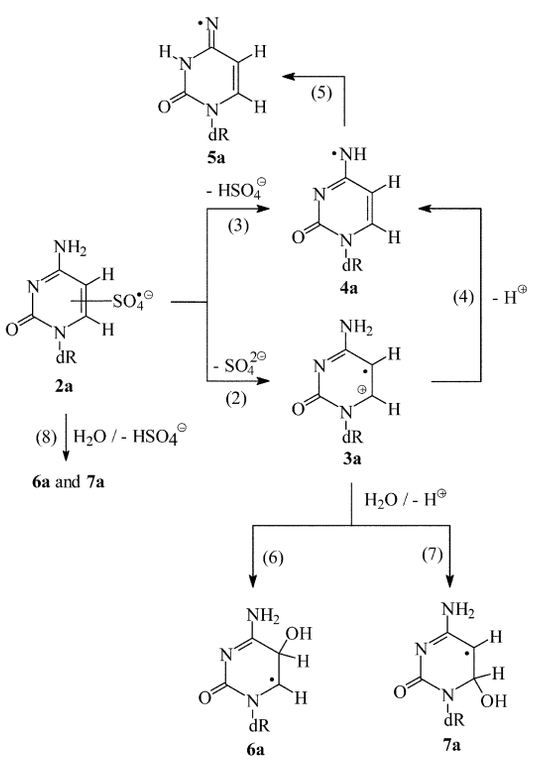
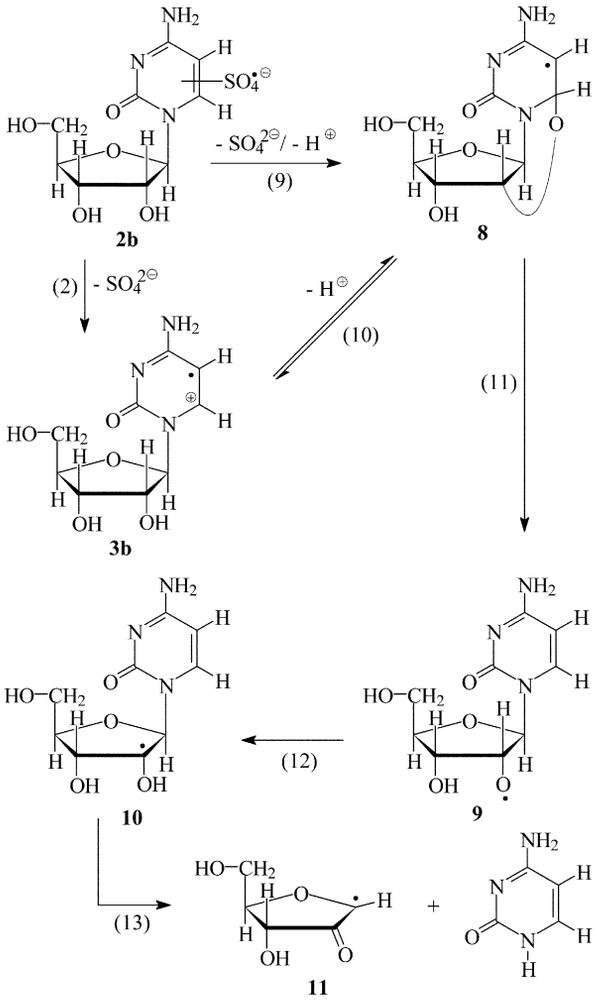
undergo sulfate release upon nucleophilic attack (see below). Although the initial site of the attack of $SO_4^{\bullet -}$ is definitely the base moiety, very striking differences between ribo- and 2′-deoxyribonucleosides have been observed as the results of $SO_4^{\bullet -}$ reactions.[14,17,26] In the case of uridine and cytidine, $SO_4^{\bullet -}$ reactions lead to the transfer of the radical site from the base to the sugar moiety, as observed by EPR studies,[15,19,25] as well as to be concluded from the release of the free base in high yields.[14] These phenomena were not observed in their 2′-deoxyribosyl counterparts. This interesting problem was elegantly solved by Gilbert and co-workers.[27] Their mechanistic proposal is shown in reactions (1)–(12) with some additions in order to accommodate more recent interpretations[28] and our present view.

Common to both systems is the primary step, the addition of $SO_4^{\bullet -}$ to the cytosine moiety and the subsequent release of sulfate [reactions (1) and (2)]. With 2′-deoxycytidine, subsequent deprotonation occurs leading to the *N*-centered radical **5a** *via* **4a** [reactions (4) and (5)].[28] As an alternative, without a free radical cation as intermediate, reactions (3) and (5) may be considered as well. In competition, reactions of the postulated radical cation **3a** with water may give rise to the OH-adduct radicals **6a** and **7a** [reactions (6) and (7)]. The latter may also arise from a nucleophilic attack by water on the $SO_4^{\bullet -}$-adduct radical **2a** [reaction (8)].



With cytidine **1b** as substrate in contrast, nucleophilic attack by the OH group at C(2′) leads to the C(5)-yl radical **8** [reactions (9) and/or (10)] which undergoes reaction (11) and subsequently leads to the formation of the sugar centered radical **11** observed by EPR and to concomitant base release [reactions (12) and (13)].

At about the time when Gilbert and co-workers[27] had done their EPR studies, we had approached the same problem by pulse radiolysis.[29] Yet, our then available pulse radiolysis data were inadequate to correlate them with the various intermediates, that must be formed[27] at the various stages of these reactions. In the meantime, the interesting observation was published that the addition of phosphate buffer to the cytidine system suppresses the formation of the sugar radical,[23] and quantum-mechanical calculations allowed us the assignment of the *N*-centered base radical derived from 2′-deoxycytidine.[28] Compared to *C*-centered radicals, *N*-centered radicals do not



(or very slowly) react with O_2,[30] and in the present study use will be made of this assay to arrive at the UV-spectrum of the *N*-centered radical by eliminating with O_2 *C*-centered radicals formed in competing reactions. Moreover, the base release data reported[13] on the cytidine and the related uridine system were difficult to reconcile with the EPR data. We thus decided to resume our earlier pulse radiolysis studies and supplement them by a more detailed base release study. These results will also be relevant for the understanding of the marked mechanistic differences of strand break formation in poly(C) induced by $SO_4^{\bullet -}$ radicals[31] and by photoionisation.[32] The now available information will allow us to support but also to modify in some essential aspects the present view[23,27] on the two so differently behaving systems, **1a** and **1b**.

Experimental

Cytidine, 2′-deoxycytidine, 1-methylcytosine, uridine, 2′-deoxyuridine, 5-methyluridine, thymidine (all Sigma) and potassium peroxodisulfate (Merck) were used as received. The solutions, prepared in water purified with the Millipore Milli-Q system, were saturated with argon (Messer Griesheim) prior to irradiation. A 4:1 v/v mixture of Ar and O_2 was used to study the reactions of oxygen with the intermediates formed by $SO_4^{\bullet -}$ attack. For the conversion of the hydrated electron into $^{\bullet}OH$, solutions were saturated with N_2O, and a 4:1 N_2O/O_2 mixture was used to follow the reactions with O_2. The peroxodisulfate concentration was typically 1×10^{-2} mol dm^{-3} while that of the substrates was 10- to 20-times lower.

Pulse radiolysis experiments at the MPI were carried out using high-energy electron pulses (2.8 MeV, 0.4 μs pulse width) from a Van de Graaff electron accelerator. The optical and DC

conductance detection techniques and data processing methods have been described previously.[33] For the shorter time scale, the 11 MeV linear electron accelerator at the IOM (Electronika 003, Thorium, Moscow) delivering 43 Gy pulses of 7 ns duration was used. For the optical detection method, the thiocyanate system was used for dosimetry taking $G \times \varepsilon = 5.2 \times 10^{-4}$ m^2 J^{-1} for the formation of $(SCN)_2^{\bullet -}$ in N_2O-saturated solutions.[34] The dosimetry for the DC conductance measurements was performed using an Ar-saturated solution of methanol (0.05 mol dm^{-3}), $K_2S_2O_8$ (0.01 mol dm^{-3}), and tertiary butanol (0.1 mol dm^{-3}) at pH ~5 and taking $G(H^+) = 6.2 \times 10^{-7}$ mol J^{-1}.[18] Steady-state γ-radiolyses were carried out in a ^{60}Co-γ-source at three different dose rates (0.013, 0.1 and 1.3 Gy s^{-1}) to total absorbed doses of up to 250 Gy. For very high dose rates, 2 μs electron pulses of 6 Gy per pulse were employed.

Base release (uracil, cytosine, thymine) in irradiated samples were analysed by HPLC (Merck-Hitachi L-4000 with UV detector) using a 4 × 200 mm 5-C-18 Nucleosil column with a 4 × 50 mm pre-column. The eluent used was 5% methanol in 10^{-3} mol dm^{-3} KH_2PO_4 aqueous solution at a flow rate of 1 cm^3 min^{-1}. The retention times (min) were: cytosine (2.5), uracil (2.8), cytidine (3.5), uridine (4.2), thymine (4.5), 2′-deoxycytidine (5.0) 2′-deoxyuridine (5.6), thymidine (8.0); water was used as eluent for the separation of thymine (6.7) from 5-methyluridine (10).

Results and discussion

The free-radical generating system

In the radiolysis of water, $^{\bullet}OH$, solvated electrons (e_{aq}^-) and some $H^{\bullet}$ are formed [reaction (14), $G(^{\bullet}OH) = G(e_{aq}^-) = 2.9 \times 10^{-7}$ mol J^{-1}, $G(H^{\bullet}) = 0.6 \times 10^{-7}$ mol J^{-1}].[1] In the presence of tertiary butanol (0.2 mol dm^{-3}) and peroxodisulfate (10^{-2} mol dm^{-3}) $^{\bullet}OH$ is scavenged by the tertiary butanol [reaction (15)] and e_{aq}^- and $H^{\bullet}$ react with peroxodisulfate [reactions (16) and (17); $k_{16} = 1.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}, $k_{17} = 2.5 \times 10^7$ dm^3 mol^{-1} s^{-1}][35] yielding $SO_4^{\bullet -}$ which may further react with the solutes present at lower concentrations (≤ 1×10^{-3} mol dm^{-3}) [reaction (18)]. If the reaction of the solute with $^{\bullet}OH$ (plus 10% $H^{\bullet}$) is to be studied the solution is saturated with N_2O [reaction (19)] and tertiary butanol is omitted. For studying the reactions of the solute radicals with O_2 [reaction (20)], the saturating gas typically contains 20% O_2, unless a dioxygen concentration variation is carried out to determine the rate of reaction in detail.

$$H_2O \xrightarrow{\text{ionizing radiation}} e_{aq}^-,\ ^{\bullet}OH,\ H^{\bullet},\ H^+,\ OH^-,\ H_2O_2,\ H_2 \quad (14)$$

$$^{\bullet}OH + (CH_3)_3COH \longrightarrow {}^{\bullet}CH_2C(CH_3)_2OH + H_2O \quad (15)$$

$$e_{aq}^- + S_2O_8^{2-} \longrightarrow SO_4^{\bullet -} + SO_4^{2-} \quad (16)$$

$$H^{\bullet} + S_2O_8^{2-} \longrightarrow H^+ + SO_4^{\bullet -} + SO_4^{2-} \quad (17)$$

$$e_{aq}^- + N_2O + H_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^- \quad (18)$$

$$SO_4^{\bullet -}\ (^{\bullet}OH, H^{\bullet}) + \text{solute} \longrightarrow \text{solute radicals} \quad (19)$$

$$\text{solute radicals} + O_2 \longrightarrow \text{solute peroxyl radicals} \quad (20)$$

The reaction of cytosine with $H^{\bullet}$ has been reported at ~9.2×10^8 dm^3 mol^{-1} s^{-1}.[35] Taking a similar value for its nucleosides, it is estimated that 70% of $H^{\bullet}$ undergoes reaction (17) under our conditions, *i.e.* the total $SO_4^{\bullet -}$ yield is $G = 3.3 \times 10^{-7}$ mol J^{-1}.

Rate of $SO_4^{\bullet -}$ addition to 2′-deoxycytidine and cytidine

The rate constant of $SO_4^{\bullet -}$ addition to 2′-deoxycytidine **1a** was determined by monitoring the decay of $SO_4^{\bullet -}$ at 470 nm as a function of 2′-deoxycytidine concentration [(2–6) × 10^{-4} mol dm^{-3}, data not shown] in Ar-saturated solution in the presence of peroxodisulfate (1×10^{-2} mol dm^{-3}) and tertiary butanol (0.2 mol dm^{-3}) at pH 5.6 (doses ~8 Gy per pulse). From the slope of the linear plot, the rate constant was calculated to be 1.6×10^9 dm^3 mol^{-1} s^{-1}. The rate constant of $SO_4^{\bullet -}$ with cytidine **1b** was determined under similar conditions from the absorbance build-up of the transient at 520 nm to be 3×10^9 dm^3 mol^{-1} s^{-1}. These values are considerably higher than the value of 2.5×10^8 dm^3 mol^{-1} s^{-1} obtained[10] for **1a** based on a competition with 1,3,5-trimethoxybenzene. The present values, measured directly, are likely to be more reliable. The value reported for the free base cytosine is 7.5×10^8 dm^3 mol^{-1} s^{-1}.[36]

Due to solubility limitations, the concentration of peroxodisulfate cannot be raised above 2×10^{-2} mol dm^{-3}, and the competition for the solvated electron requires that the concentrations of the cytosine nucleosides should be not higher than ~1×10^{-3} mol dm^{-3}. The formation of $SO_4^{\bullet -}$ then occurs practically within the few ns pulse length of our faster pulse radiolysis setup. The decay of its 470 nm absorption and the buildup of the absorptions governing the later ns and early μs time scale coincide. Since the latter can no longer be attributed to $SO_4^{\bullet -}$-adduct radicals **2a**,**b** (see below), their lifetime must be shorter than ~20 ns.

Proton formation

Conductometric detection was used to monitor the proton plus corresponding anion yields in the course of the reactions of $SO_4^{\bullet -}$ with 2′-deoxycytidine **1a** and cytidine **1b**. Immediately following the pulse in an Ar-saturated solution of **1a** (or **1b**) and $K_2S_2O_8$ at pH 5.4, a prompt conductance increase was observed [$G(H^+) \approx 2.1 \times 10^{-7}$ mol J^{-1}]. No further increase in conductance was observed up to 80 μs after the pulse. The pK_a of the protonated cytosine nucleosides is 4.15, and it can be shown that under these conditions 93% of H^+ formed in reactions (3), (4), (8) and (9) are immediately (within 400 ns) taken up by **1a/1b** to their protonated forms. Since the equivalence conductance of protonated cytosine nucleosides ($\lambda_0 \approx 40$ cm^2 Ω^{-1} equiv.$^{-1}$) is much lower than that of H^+ ($\lambda_0 = 324$ cm^2 Ω^{-1} equiv.$^{-1}$ at 20 °C), the conductivity change made up of H^+, the protonated cytosine nucleosides, and sulfate ions ($\lambda_0 = 70$ cm^2 Ω^{-1} equiv.$^{-1}$), is reduced to one-third of the original change made up by the full amount of H^+ and sulfate. Thus, the observed conductance increase of $G(H^+) \approx 2.1 \times 10^{-7}$ mol J^{-1} reflects the full yield of protons in this buffered system. The buffering effect of the cytosine nucleosides would not allow us to monitor a delayed decay of a potential radical cation [*cf.* reactions (4) and (9)] on the basis of the slight difference in the conductance signal (≈ 7% of the reduced signal; the radical cations of the cytosine nucleosides and the protonated cytosine nucleosides can be assumed to have practically the same equivalence conductance).

This difficulty is no longer relevant in basic solution, where the cytosine nucleosides do not exert any buffering effect. The conductance of a cytosine nucleoside solution (1×10^{-3} mol dm^{-3}) containing tertiary butanol (0.2 mol dm^{-3}) and $K_2S_2O_8$ (1×10^{-2} mol dm^{-3}) at pH 9.5 (adjusted with NaOH) was observed to decrease [in basic solution the proton removes one OH^- ($\lambda_0 = 180$ cm^2 Ω^{-1} equiv.$^{-1}$ at 20 °C)] in the course of a few μs to a value corresponding to 100% of proton formation ($G(H^+) \approx 7 \times 10^{-7}$ mol J^{-1}). The data (not shown) are compatible with a rapid release of protons, *i.e.* there is no evidence for a proton-releasing species with a lifetime of a few μs. These results agree with the previous report, based on the change in its absorption spectrum with pH, that the life time of the relevant intermediate (attributed to the radical cation of 2′-deoxycytidine **3a**) is ≤ 200 ns.[37] Thus, these data do not allow us to ascertain whether or not free radical cations are short-lived intermediates in these systems.

Properties of the $^{\bullet}$OH-adduct radicals of 2′deoxycytidine and cytidine

Upon sulfate release of the $SO_4^{\bullet-}$-adduct radicals, $^{\bullet}$OH-adduct radicals may be formed [*cf.* reactions (6)–(8)]. For this reason, $^{\bullet}$OH (plus 10% H$^{\bullet}$, here largely neglected) were generated in N_2O-saturated solution [*cf.* reaction (18)]. They react fast ($k = 5.6 \times 10^9$ dm^3 mol^{-1} s^{-1})[35] with cytidine **1b** and give rise to a spectrum which is characterised by maxima at 340 nm and 430 nm (Fig. 1).

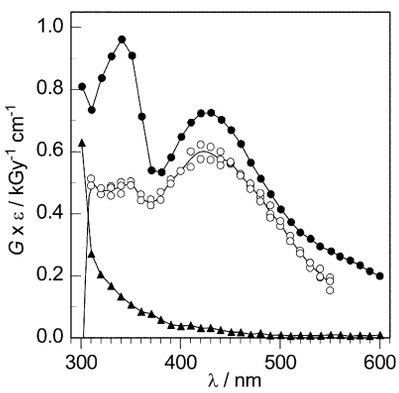
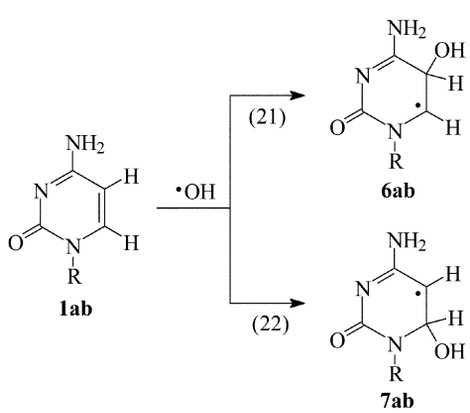


Fig. 1 Pulse radiolysis of N_2O-saturated aqueous solutions of cytidine (1×10^{-3} mol dm^{-3}) in the absence (pH 6.5: ●; pH 3: ○, [cytidine] = 5×10^{-4} mol dm^{-3}, at 300 nm bleaching is observed, data point not shown) and in the presence of dioxygen (2.5×10^{-4} mol dm^{-3}, ▲, pH 6.5). The spectra were taken 15 μs after the pulse. 1 kGy^{-1} cm^{-1} equals 10^{-4} m^2 J^{-1}.

The spectrum shown in Fig. 1 agrees well with those reported for cytosine and other cytosine derivatives, *e.g.* 2′-deoxycytidine **1a**.[38] This spectrum is due to $^{\bullet}$OH-adduct-radicals (with some contribution of $^{\bullet}$H-adduct-radicals). With cytosine, the reactions of $^{\bullet}$OH are rather selective, and addition mainly occurs at C(5) (~87%) [*cf.* reaction (21)], and only ~10% add to the C(6)-position [*cf.* reaction (22)] as derived from redox-titration.[36] In the nucleosides, some 10% may abstract an H-atom from the sugar moiety, but the resulting radicals do not absorb in the wavelength region of interest to any significant extent. Thus, the strong absorptions at 340 and 430 nm may be attributed to the 5-OH-6-yl radicals **6a**,**b** formed in reaction (21).



With other pyrimidines, uracil and thymine, it was possible to disentangle the absorption spectra of the C(5)- and C(6)-OH-adducts. While in the case of 1,3-dimethylthymine, for example, the spectrum of the 5-OH-6-yl radical has only one maximum at ~390 nm, the 6-OH-5-yl radical shows maxima at 350 nm and 440 nm.[39] Such an assignment cannot be made here, but the overall spectrum is sufficiently characteristic to distinguish it from the one obtained by $SO_4^{\bullet-}$ attack (*cf.* Figs. 2–4).

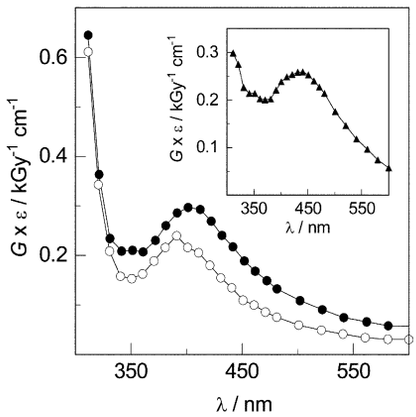


Fig. 2 Pulse radiolysis of Ar-saturated aqueous solutions of 2′-deoxycytidine (1×10^{-3} mol dm^{-3}) in the presence of peroxodisulfate (1×10^{-2} mol dm^{-3}) and tertiary butanol (0.2 mol dm^{-3}) in the absence (●) and presence of dioxygen (○, 2.5×10^{-4} mol dm^{-3}) at pH 5.6, 15 μs after the pulse. Inset: spectrum in the absence of dioxygen at pH 3, ~10 μs after the pulse.

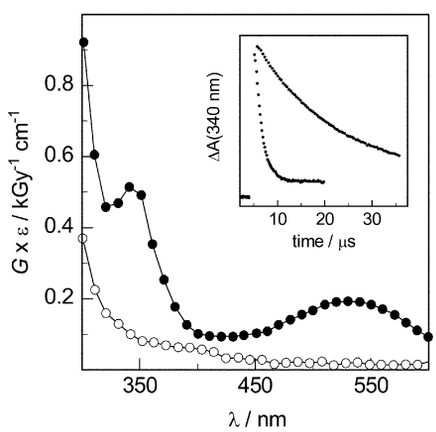


Fig. 3 Transient spectra obtained in the pulse radiolysis of Ar-saturated aqueous solutions of cytidine (1×10^{-3} mol dm^{-3}) in the presence of peroxodisulfate (10×10^{-3} mol dm^{-3}) and tertiary butanol (0.2 mol dm^{-3}) in the absence (●, 2 μs after the pulse) and presence of dioxygen (○, 2.5×10^{-4} mol dm^{-3}, 15 μs after the pulse) at pH 5.6. Inset: time dependence of the absorbance decay at 340 nm in the absence (slow trace) and in the presence of dioxygen (fast trace).

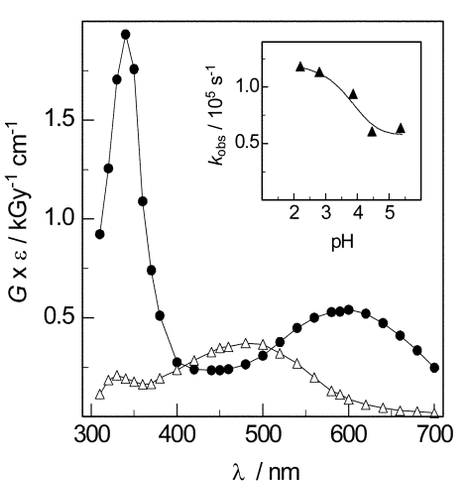


Fig. 4 Transient spectra (●, 1μs, and △, 40 μs after the pulse) obtained in the pulse radiolysis of Ar-saturated aqueous solutions of cytidine (1×10^{-3} mol dm^{-3}) in the presence of peroxodisulfate (1.5×10^{-2} mol dm^{-3}) and tertiary butanol (0.2 mol dm^{-3}) at pH 2.8 (~ 4 Gy per pulse). Inset: k_{obs} (extrapolation to zero dose) of absorption decay at 350 nm as a function of pH.

A typical feature of *C*-centered radicals is their rapid reaction with dioxygen (for a review on peroxyl radicals in aqueous solution see ref. 40). In the presence of dioxygen, the long-wavelength absorption decays practically completely, and only a tail extending to the UV remains. This feature is typical for most peroxyl radicals (for exceptions see ref. 40). The rate of decay of the 430 nm absorption has been followed as a function of the dioxygen concentration, and from the linear k_{obs} *vs.* [O_2] plot (data not shown) the rate constant for the addition of dioxygen to the $^{\bullet}$OH-adduct-radicals is calculated at $k = 1.3 \times 10^9$ dm^3 mol^{-1} s^{-1}. This value is very typical for such a reaction.[41] In the absence of dioxygen, the 2′-deoxycytidine $^{\bullet}$OH-adduct radicals **6a/7a** decay by second-order kinetics without any noticeable change in the spectrum.[26] This also holds for the cytidine-derived radicals **6b/7b** (data not shown).

Relevant for the present study is the fact that not only do cytosine and its derivatives protonate in moderately acid solutions (pK_a values around 4.3–4.6) with concomitant changes in the UV spectra. This is also observed with the $^{\bullet}$OH-adduct radicals (a case in point is cytidine, Fig. 1; similar spectral changes are observed with cytosine and 1-methylcytosine, spectra not shown).

While in neutral solution the $^{\bullet}$OH-adduct radicals are characterised by UV spectra with maxima at 340 nm and 430 nm (with a shoulder near 480 nm, *cf*. Fig. 1), the spectrum in acid solution does not display a strong absorption near 340 nm, and at 300 nm even bleaching occurs. Whether the pH effect on the $^{\bullet}$OH-adduct spectra is due to protonation of the $^{\bullet}$OH-adduct radicals or due to a difference in the relative importance of the site of $^{\bullet}$OH-attack [C(5) *vs.* C(6)] cannot be decided on the basis of our data. However, if our assignment of the long-wavelength absorption (530 nm in neutral and 600 nm in acid solution) to a C(5)-yl radical (see below) is correct, the spectral changes observed here are likely to be due to protonation reactions. We report this pH effect, because an even stronger pH dependence of the UV spectra is observed with some of the radicals formed upon $SO_4^{\bullet-}$ attack (see below).

Formation of the *N*-centered radical upon $SO_4^{\bullet-}$-attack on 2′-deoxycytidine

The transient absorption spectrum obtained in the reaction of $SO_4^{\bullet-}$ with 2′-deoxycytidine **1a** as shown in Fig. 2 is similar to the reported[10] one. The absorption coefficient at the maximum (400 nm) is 1100 dm^3 mol^{-1} cm^{-1}, in agreement with the reported[26] value of 1000 dm^3 mol^{-1} cm^{-1} at λ_{max} 405 nm.

In the presence of dioxygen, a fraction of the absorption is eliminated, but the major part remains (Fig. 2). This indicates that we deal with two types of radicals, *C*-centered radicals [**6a** and **7a**, minor, reactions (6)–(8)] which are readily converted to their corresponding peroxyl radicals with no significant absorptions near 400 nm (*cf*. Fig. 1) and an *N*-centered radical (**4a**, major) which is expected[30] to react with dioxygen only very slowly or not at all. Thus, it seems that deprotonation at nitrogen [reaction (3) or reaction (2) followed by (4)] is the dominating process.

This conclusion is supported by EPR studies. Here, a very pronounced spectrum is observed[23] which has more recently been attributed[28] to the *N*(3)-protonated tautomer **5a** [*cf*. reaction (5)]. When the dioxygen-sensitive contributions have decayed, the final spectrum has a maximum at 400 nm. The position of the maximum is sensitive to pH, and at pH 3 it is red-shifted to 450 nm (data taken in the absence of dioxygen, *cf*. inset in Fig. 2). Red-shifts of UV spectra upon protonation are not common, but the parent, cytosine, also shows this effect.

As it stands, the question whether a free radical cation is an intermediate [reactions (2) and (4)] or concerted release of H^+ and SO_4^{2-} [reaction (3)] leads to the *N*-centered radical must remain an open question, but below circumstantial evidence will be given that is in favour of reaction (3).

The fact that dioxygen eliminates some of the absorption points to the formation of minor amounts of *C*-centered radicals [reactions (6) and (7)] in competition with the formation of the *N*-centered radical discussed above.

Formation of a sugar-centered radical upon $SO_4^{\bullet-}$-attack on cytidine

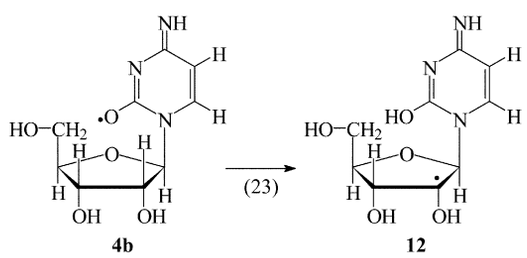
Similar to the previous report,[26] the spectrum of the radicals formed upon $SO_4^{\bullet-}$ attack on cytidine **1b** shows maxima at 340 nm and 530 nm (Fig. 3), and is markedly different from that of 2′-deoxycytidine **1a** under the same conditions (*cf*. Fig. 2). In acidic solution, the corresponding spectrum still has one maximum at 340 nm, but the one at 530 nm shifts to 600 nm (Fig. 4). These spectra are quite different from those observed in the reaction of $SO_4^{\bullet-}$ with 2′-deoxycytidine (Fig. 2) but are also different from the spectrum obtained by $^{\bullet}$OH-attack on cytidine (Fig. 1).

The 530 nm absorption and those at other wavelengths decay fast in a unimolecular reaction ($k = 6 \times 10^4$ s^{-1}; *cf*. inset in Fig. 3), in agreement with a half-life of 11 μs reported[26] earlier. In basic solution, the rate of decay is speeded up, and from the data at different OH^- concentrations (not shown) a rate constant of ~1×10^9 dm^3 mol^{-1} s^{-1} is calculated for the OH^--induced reaction.

In contrast to the major 2′-deoxycytidine-derived radical, the cytidine-derived radical reacts readily with dioxygen ($k \approx 1.3 \times 10^9$ dm^3 mol^{-1} s^{-1}) leaving barely any absorption behind (Fig. 3). Due to this high rate constant, the reaction with dioxygen becomes sufficiently fast at a dioxygen concentration of 2.5×10^{-4} mol dm^{-3} to compete successfully with its unimolecular decay (*cf*. inset in Fig. 3). For its assignment, some additional information is required.

In EPR studies using a flow system, the directly detectable radical is the sugar radical **11**.[26] It has been considered[27] to be formed in reactions (2) and (10)–(13), but as an alternative reaction (8) has to be considered as well. The precursors of **11**, radicals **9** and **10** formed in reactions (11) and (12), are not visible directly.[15,26] Especially, the oxyl radical **9** would undergo the 1,2-H shift reaction (12) too fast to be detectable.[42–44]

There is an another potential route to the sugar radical. One may consider that the *N*-centered radical **4b** formed in a reaction analogous to reaction (3) [or reactions (2) and (4)] abstracts very rapidly an H-atom from the sugar moiety [reaction (23)].



4b **12**

For the assignment of the 530 nm species shown in Fig. 3, we have to consider not only its spectral properties, but also its decay kinetics. Important for this assignment is the observation that it reacts rapidly with dioxygen. This reactivity towards dioxygen rules out the isocytosine **12** species formed in reaction (23) as the 530 nm intermediate (although isocytosines absorb at longer wavelengths than cytosine,[45] the red-shift is not as dramatic). On spectral grounds, the oxyl radical **9** [formed in reaction (11)] and the hydroxyalkyl radical **10** [formed in reaction (12)] are also ruled out. This leaves us with the 5-yl radical **8** formed in reactions (9) and/or (10).

This radical must have similar spectral properties as the 6-OH-5-yl radical **7b** formed upon $^{\bullet}$OH attack [reaction (22)]. Unfortunately, the spectrum shown in Fig. 1 is dominated by the 5-OH-6-yl radical **6b** and the former may contribute to the

spectrum only ~10% (assuming similar absorption coefficients). In fact, its expected contribution around 540 nm may be hidden in the long-wavelength tail (*cf*. the shoulder near this wavelength). As has been mentioned above, in the uracil/thymine series the 6-OH-5-yl radicals absorb at considerably longer wavelengths than the 5-OH-6-yl radicals (the corresponding $^{\bullet}$H-adduct radicals[46] seem to show the same tendency).[47,39] Thus, the observed maximum at 540 nm (and at 600 nm in acid solution) is compatible with its assignment to the 5-yl radical **8**. In acid solutions, the reaction seems to follow additional routes; not only increases the rate of decay (*cf*. inset in Fig. 4) but also an absorption builds up at around 475 nm (Fig. 4). This is more pronounced than the featureless absorption remaining in neutral solution which extends from 350–600 nm (continuously one quarter of the maximum at 530 nm, data not shown). It is tempting to attribute the 475 nm species formed in acid solution to a base $^{\bullet}$OH-adduct radical (*cf*. the similarity with the spectrum shown in Fig. 1), *e.g. via* reaction (−10) and subsequent reaction of the radical cation **3b** with water forming the (probably protonated) radicals **6b** and **7b**. The spectrum of the species formed upon $^{\bullet}$OH attack on cytidine at pH 3 is shown in Fig. 1 (○). There are considerable similarities with that of Fig. 4 (△) at short wavelengths. Yet, the absorption maxima do not match. This discrepancy could be accounted for if the relative yields of **6b** and **7b** are different in these two cases.

Effect of phosphate buffer

It has been shown by EPR that in the case of cytidine the addition of phosphate buffer dramatically changes the EPR spectrum.[23] The sugar radical **11** disappears, and instead the same EPR spectrum of a basic radical as observed with 2′-deoxycytidine is formed (assigned[28] to **5b**). While with 2×10^{-3} mol dm^{-3} phosphate buffer pH 7 the conversion is only partial, it is practically complete in the presence of 25×10^{-3} mol dm^{-3} phosphate.[23]

As can be seen from Fig. 5, noticeable changes are also

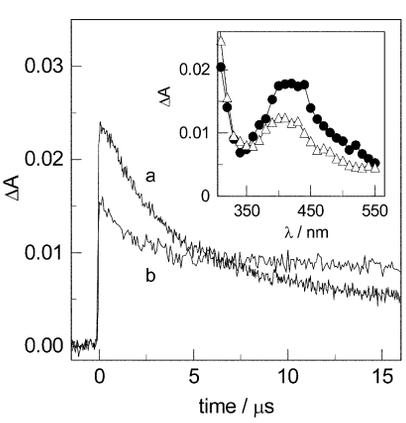


Fig. 5 Pulse radiolysis of aqueous solutions of cytidine (1×10^{-3} mol dm^{-3}) containing peroxodisulfate (10×10^{-3} mol dm^{-3}), tertiary butanol (0.2 mol dm^{-3}) and phosphate buffer pH 7 (a: 2×10^{-3} mol dm^{-3}; b: 25×10^{-3} mol dm^{-3}), 43 Gy per pulse. Absorption at 530 nm as a function of time. Inset: resulting spectra at 15 μs.

observed by pulse radiolysis. Here, the addition of phosphate has a two-fold effect. The formation of the typical 340 nm and 530 nm absorptions are speeded up and their yield reduced on the ns time scale. Once formed, these absorptions also decay increasingly fast, and a new absorption with a broad maximum near 410–440 nm (*cf*. inset in Fig. 5) builds up. Since the rate is faster and the yield of the 410–440 nm species is higher at higher phosphate concentration, there is a crossover of the decay curves (Fig. 5, main graph). In the context of our interpretation of the ongoing processes, this means that the phosphate buffer interferes twice.

At the early stage, it prevents the formation of the 530 nm species (assigned to the 5-yl radical **8**) by interaction with its precursor. This could be the $SO_4^{\bullet-}$-adduct radical **2b** or the radical cation **3b**. If it is the $SO_4^{\bullet-}$-adduct radical **2b** the phosphate buffer may deprotonate it at nitrogen, and the dianion thus formed may lose sulfate (formation of **4b**) more rapidly than without prior deprotonation. If phosphate deprotonates the radical cation **3b** at nitrogen, it also prevents the nucleophilic attack of the C(2′)–OH and the formation of the 530 nm species **8** and consequently also that of the sugar radical **11**.

Once the 530 nm species **8** is formed, the phosphate buffer can protonate the oxygen of its aminal-type bridge [reaction (−9)] (note that the rate of hydrolysis of radicals may be several orders of magnitude faster than that of their parents, *cf*. *e.g.* ref. 48). This would lead to a reformation of the radical cation **3b** which then can further react with phosphate (deprotonation at nitrogen, reaction with water, addition/elimination of phosphate with the consequence of the formation of the *N*-centered radical **4b** as well as $^{\bullet}$OH-adduct radicals **6b** and **7b**).

Base release

One of the major differences between the uridine and 2′-deoxyuridine in their $SO_4^{\bullet-}$-induced reactions is the much higher yield of uracil in the case of uridine.[14] This is paralleled by the observation of base-centered radicals in 2′-deoxyuridine but considerable amounts of sugar-centered radicals in the case of uridine.[15] In the uridine system, the reported uracil yield matched that of the $SO_4^{\bullet-}$ yield.[14] The much higher yield of sugar-derived radicals in cytidine as compared to uridine (K. Hildenbrand, private communication) raised the question, whether in the uridine system the 1:1 correspondence was accidental, due to the neglect of $SO_4^{\bullet-}$-induced chain reactions that occur in the pyrimidine series.[11,16] We therefore decided to study the dose-rate effects on base release comparing the pairs uridine/2′-deoxyuridine, cytosine/2′deoxycytidine and 5-methyluridine/thymidine (Table 1).

It can be seen from this table that in the case of cytidine and under the conditions of electron beam irradiation (high dose rate, negligible contribution of a chain reaction) the base release yield is 85% of the $SO_4^{\bullet-}$ yield. This is much higher than that found for the uridine system (22%), in good agreement with a much stronger EPR signal of the sugar radical in the case of cytidine.[23] All the other systems studied have considerably lower base release yields. Yet in all cases, the base release yields increase with decreasing dose rate (Table 1), a strong indication for the occurrence of chain reactions.

The reaction conditions applied here match well with the assumption of negligible turnover and pseudo-stationary conditions. With these approximations, the ODE-system[49] for a simple and a branching chain reaction leads to the well-known plot of *G*(product) *vs*. the inverse of the square root of the dose rate. Simple chain reactions (bimolecular termination of radicals as the only factor determining the chain length) yield a straight line, while branching leads to the more complex relationship shown by the solid lines in Fig. 6.

The intercept represents the limit of negligible propagation and has been normalised to 1. Eqn. (24) allows one to determine rate constants which are compiled in Table 1.

In eqn. (24), k_{prop} and k_{term} are the propagation and termin-

$$\frac{G}{G_\infty} = 1 + \frac{k_{\mathrm{prop}}(1+b)}{2k_{\mathrm{term}}} x^2 \left(\sqrt{\left(k_{\mathrm{prop}} b\right)^2 + 4\frac{k_{\mathrm{term}}}{x^2}} - k_{\mathrm{prop}} b \right) \qquad (24)$$

ation rate constants, respectively. Upon branching, a radical is generated which is no longer capable of propagating the chain. The branching factor *b* denotes the ratio of the probability of forming a propagating *vs*. a non-propagating radical. The term

Table 1 G(base release) in units of 10^{-7} mol J^{-1} from some pyrimidine nucleosides and 2′-deoxynucleosides induced by the $SO_4^{\bullet -}$ radical [$G(SO_4^{\bullet -})$ = 3.3 × 10^{-7} mol J^{-1}) at different dose rates: pulsed electron beam irradiation (EB, ~6 Gy per 2 μs pulse, high dose rate) and γ-irradiation; the rightmost columns show the parameters of the fit according to eqn. (24)

Nucleoside / 2′deoxynucleoside	EB	1.33 Gy s^{-1}	0.096 Gy s^{-1}	0.013 Gy s^{-1}	k_{prop}/s^{-1}	Rel. branching
Cytidine	2.8	4.9	6.3	7.5	7.2	1.5
Uridine	0.72	1.8	2.9	4.5	22	0.19
5-Methyluridine	n.d.[a]	n.d.[a]	n.d.[a]	<0.05		
2′-Deoxycytidine	0.14	0.76	1.15	1.64	82	0.10
2′-Deoxyuridine	0.06	0.25	0.4	0.6	52	0.11
Thymidine	0.2	0.7	0.85	1.03	60	0.35

[a] n.d., not determined.

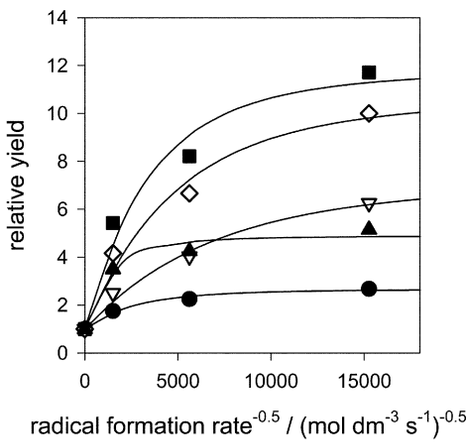


Fig. 6 G(base release) after γ-radiolysis (symbols) divided by G(base release) after pulse radiolysis of peroxodisulfate (2 × 10^{-2} mol dm^{-3}) and tertiary butanol (0.2 mol dm^{-3}) containing solutions of cytidine (●), uridine (▽), 2′-deoxycytidine (■), 2′-deoxyuridine (◇), and thymidine (▲) as a function of (dose rate)$^{-½}$. The solid lines represent the fit to the reduced branching chain reaction approximation, eqn. (24). Data in Table 1.

x is the square root of the reciprocal radical generation rate. In the calculations, k_{term} has been set at 1 × 10^9 dm^3 mol^{-1} s^{-1}.

Note that with no branching (b = 0) the equation reduces to the well known linear relationship of yield and the square root of the reciprocal dose rate [eqn. (25)].

$$\frac{G}{G_\infty} = 1 + \frac{k_{prop}}{2k_{term}} x^2 \left(\sqrt{4 \frac{k_{term}}{x^2}} \right) = 1 + \frac{k_{prop}}{\sqrt{k_{term}}} x \qquad (25)$$

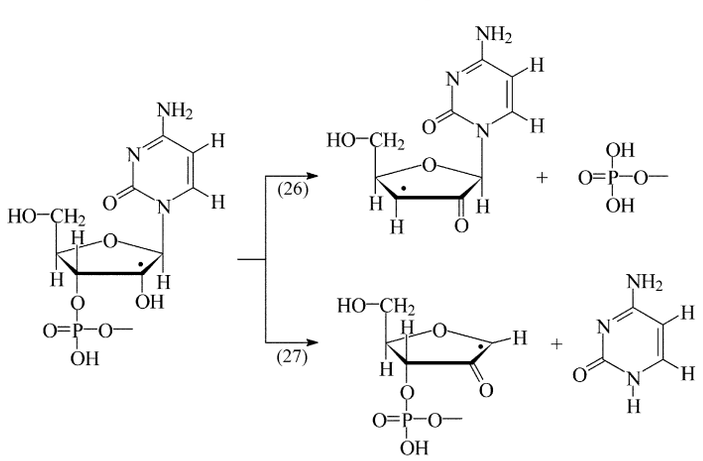
The propagation rate constants (Table 1) should not be considered as rate constants that can be attributed directly to a well-defined mechanistic step. They rather reflect a sum of steps. They are certainly too low to be attributed to the reaction of fairly strongly reducing radicals with peroxodisulfate which typically range in the order of 10^5 dm^3 mol^{-1} s^{-1}.[11,50] In the cytidine system that we are mainly concerned with here, radical **11** may be sufficiently reducing to undergo electron transfer to peroxodisulfate at moderate rates. Yet, in the case of the $SO_4^{\bullet -}$-induced chain reaction with 1,3-dimethyluracil a much slower chain carrying species had to be postulated, and apparently also in the present systems such intermediates could play a role.

As can be seen from Table 1, practically no base release is observed with the thymine derivatives thymidine and 5-methyluridine. The question why 5-methyluridine, the thymine analogue of cytidine and uridine, does not yield any thymine is a most interesting observation that would certainly be worth a detailed study. At present, there is no straightforward explanation for this.

Relevance of the present results to $SO_4^{\bullet -}$-induced strand breakage in poly(C) and poly(U). Are free radical cations important intermediates?

The $SO_4^{\bullet -}$-induced strand breakage yields, measured by pulse radiolysis, have been reported at 23% and 57% for poly(C) and poly(U), respectively (taking $G(SO_4^{\bullet -})$ = 3.15 × 10^{-7} mol J^{-1}).[31] The base release data from the corresponding nucleosides (Table 1) measured under practically the same conditions are 85% and 22%, respectively (taking $G(SO_4^{\bullet -})$ = 3.3 × 10^{-7} mol J^{-1} for our conditions). It is most likely that the same mechanistic principles govern the two systems up to the C(2′) radical. In the polynucleotides this radical can undergo strand breakage [reaction (26)] in competition with base release [reaction (27), see also above].

Phosphate release from α-hydroxy-β-phosphatoalkyl radicals is a well-known reaction[51,52] which occurs[53] at times shorter than a few μs. In agreement with this, the rate of $SO_4^{\bullet -}$-induced strand breakage in poly(C) has been found to be ≥3 × 10^4 s^{-1} (the rise time of the system),[31] *i.e.* certainly not much delayed with respect to the formation of the C(2′) radical determined above for the cytidine system at 6 × 10^4 s^{-1}. With phosphate release being that fast, strand breakage [reaction (26)] is likely to dominate over base release [reaction (27)] in the polynucleotides. If this conclusion is correct the above data are of consequence as to the fate of the $SO_4^{\bullet -}$-adducts in the nucleosides



and polynucleotides. If we now assume that base release in the nucleosides and strand breakage in the polynucleotides is a measure of the C(2′) radical yield in the two systems there must be a considerable decrease of its yield on going from cytosine (85%) to poly(C) (23%) and an increase from 22% in uridine to 57% in poly(U). This could be accounted for if steric conditions are different in the nucleoside and polynucleotide and decide between the two competing reactions, reaction of the 2′-OH group and water with the relevant species [*e.g.,* reactions (9) and/or (10) competing with reactions analogous to reactions (6) and (7)].

The reaction with water yields $^{\bullet}$OH-adducts. From a study on poly(U),[54] we know that it is mainly the C(6) radical that induces base release (and thus also strand breakage). The kinetics of the $^{\bullet}$OH-induced strand breakage in poly(U) is considerably slower than that induced by $SO_4^{\bullet-}$,[31] and a major contribution of an $^{\bullet}$OH-adduct radical to the $SO_4^{\bullet-}$-induced reaction would certainly have been noticed. From this it follows that in poly(U) water reacts at the same position as the 2′-OH group does.

In poly(C), the kinetics of $SO_4^{\bullet-}$-induced strand breakage shows a ~15% slow contribution that is very similar to that of the $^{\bullet}$OH-induced reaction.[31] In the present context, the most important observation is that strand breakage induced by photoionisation[32] which has to proceed *via* a free radical cation is not suppressed by dioxygen in contrast to the $SO_4^{\bullet-}$-induced reaction.[31] This is very strong evidence that a free cytosine radical cation is not involved to a major extent in the $SO_4^{\bullet-}$-induced reaction and that the reaction proceeds mainly by a nucleophilic attack by the C(2′)OH as depicted in reaction (9). In this respect, the current mechanistic concept[27] has to be modified in an interesting and essential aspect.

References

1 D. Schulte-Frohlinde, M. G. Simic and H. Görner, *Photochem. Photobiol.*, 1990, **52**, 1137.
2 C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, 1987.
3 M. E. Malone, P. M. Cullis, M. C. R. Symons and A. W. Parker, *J. Phys. Chem.*, 1995, **99**, 9299.
4 R. Lomoth, S. Naumov and O. Brede, *J. Phys. Chem. A*, 1999, **103**, 6571.
5 G. J. Fisher and E. J. Land, *Photochem. Photobiol.*, 1983, **37**, 27.
6 C. Decarroz, J. R. Wagner, J. E. van Lier, C. M. Krishna, P. Riesz and J. Cadet, *Int. J. Radiat. Biol.*, 1986, **50**, 491.
7 J. Geimer and D. Beckert, *J. Phys. Chem. A*, 1999, **103**, 3991.
8 K. M. Bansal and R. W. Fessenden, *Radiat. Res.*, 1978, **75**, 497.
9 S. N. Rustgi and P. Riesz, *Int. J. Radiat. Biol.*, 1978, **34**, 301.
10 P. O'Neill and S. E. Davies, *Int. J. Radiat. Biol.*, 1987, **52**, 577.
11 H.-P. Schuchmann, D. J. Deeble, G. Olbrich and C. von Sonntag, *Int. J. Radiat. Biol.*, 1987, **51**, 441.
12 G. Behrens, K. Hildenbrand, D. Schulte-Frohlinde and J. N. Herak, *J. Chem. Soc., Perkin Trans. 2*, 1988, 9.
13 S.-I. Fujita and Y. Nagata, *Radiat. Res.*, 1987, **114**, 207.
14 S. Fujita, Y. Nagata and T. Dohmaru, *Int. J. Radiat. Biol.*, 1988, **54**, 417.
15 K. Hildenbrand, G. Behrens, D. Schulte-Frohlinde and J. N. Herak, *J. Chem. Soc., Perkin Trans. 2*, 1989, 9.
16 C. von Sonntag, R. Rashid, H.-P. Schuchmann and F. Mark, *Free Radical Res. Commun.*, 1989, **6**, 111.
17 E. Bothe, D. J. Deeble, D. G. E. Lemaire, R. Rashid, M. N. Schuchmann, H.-P. Schuchmann, D. Schulte-Frohlinde, S. Steenken and C. von Sonntag, *Radiat. Phys. Chem.*, 1990, **36**, 149.
18 D. J. Deeble, M. N. Schuchmann, S. Steenken and C. von Sonntag, *J. Phys. Chem.*, 1990, **94**, 8186.
19 K. Hildenbrand, *Z. Naturforsch., C*, 1990, **45**, 47.
20 R. Rashid, F. Mark, H.-P. Schuchmann and C. von Sonntag, *Int. J. Radiat. Biol.*, 1991, **59**, 1081.
21 P. O'Neill and E. M. Fielden, *Adv. Radiat. Biol.*, 1993, **17**, 53.
22 L. P. Candeias and S. Steenken, *J. Am. Chem. Soc.*, 1993, **115**, 2437.
23 H. Niehaus and K. Hildenbrand, *J. Chem. Soc., Perkin Trans. 2*, 2000, 947.
24 W. Roebke, M. Renz and A. Henglein, *Int. J. Radiat. Phys. Chem.*, 1969, **1**, 39.
25 D. J. Deeble, M. N. Schuchmann, H.-P. Schuchmann, S. Steenken and C. von Sonntag, *Int. J. Radiat. Biol.*, 1990, **58**, 46.
26 D. Schulte-Frohlinde and K. Hildenbrand, in *Free Radicals in Synthesis and Biology*, ed. F. Minisci, Kluwer, Dordrecht, 1989, p. 335.
27 H. Catterall, M. J. Davies and B. C. Gilbert, *J. Chem. Soc., Perkin Trans. 2*, 1992, 9.
28 S. Naumov, K. Hildenbrand and C. von Sonntag, *J. Chem. Soc., Perkin Trans. 2*, 2001, 1648.
29 C. T. Aravindakumar, Doctoral Thesis, University of Poona, 1992.
30 H.-P. Schuchmann and C. von Sonntag, in *Peroxyl Radicals*, ed. Z. B. Alfassi, Wiley, Chichester, 1997, p. 439.
31 P. Wolf, G. D. D. Jones, L. P. Candeias and P. O. O'Neill, *Int. J. Radiat. Biol.*, 1993, **64**, 7.
32 H. Görner, M. Wala and D. Schulte-Frohlinde, *Photochem. Photobiol.*, 1992, **55**, 173.
33 C. von Sonntag and H.-P. Schuchmann, *Meth. Enzymol.*, 1994, **233**, 3.
34 G. V. Buxton and C. R. Stuart, *J. Chem. Soc., Faraday Trans.*, 1995, **91**, 279.
35 G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513.
36 D. K. Hazra and S. Steenken, *J. Am. Chem. Soc.*, 1983, **105**, 4380.
37 S. Steenken, *Free Radical Res. Commun.*, 1992, **16**, 349.
38 A. Hissung and C. von Sonntag, *Z. Naturforsch., B*, 1978, **33**, 321.
39 D. J. Deeble and C. von Sonntag, *Z. Naturforsch., C*, 1985, **40**, 925.
40 C. von Sonntag and H.-P. Schuchmann, in *Peroxyl Radicals*, ed. Z. B. Alfassi, Wiley, Chichester, 1997, p. 173.
41 P. Neta, R. E. Huie and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1990, **19**, 413.
42 B. C. Gilbert, R. G. G. Holmes, H. A. H. Laue and R. O. C. Norman, *J. Chem. Soc., Perkin Trans. 2*, 1976, 9.
43 V. M. Berdnikov, N. M. Bazhin, V. K. Fedorov and O. V. Polyakov, *Kinet. Catal. (Engl. Transl.)*, 1972, **13**, 986.
44 H.-P. Schuchmann and C. von Sonntag, *J. Photochem.*, 1981, **16**, 289.
45 C. von Sonntag, *Radiat. Phys. Chem.*, 1987, **30**, 313.
46 S. Das, D. J. Deeble, M. N. Schuchmann and C. von Sonntag, *Int. J. Radiat. Biol.*, 1984, **46**, 7.
47 D. J. Deeble, S. Das and C. von Sonntag, *J. Phys. Chem.*, 1985, **89**, 5784.
48 C. von Sonntag and H.-P. Schuchmann, in *Radiation Chemistry: Present Status and Future Trends*, ed. C. D. Jonah and B. S. M. Rao, Elsevier, Amsterdam, 2001, p. 481.
49 I. N. Bronstein and K. A. Semendjajew, *Taschenbuch der Mathemathik*, Deutsch, Frankfurt am Main, 1979.
50 P. Ulanski and C. von Sonntag, *J. Chem. Soc., Perkin Trans. 2*, 1999, 165.
51 A. Samuni and P. Neta, *J. Phys. Chem.*, 1973, **77**, 2425.
52 S. Steenken, G. Behrens and D. Schulte-Frohlinde, *Int. J. Radiat. Biol.*, 1974, **25**, 205.
53 M. N. Schuchmann, M. L. Scholes, H. Zegota and C. von Sonntag, *Int. J. Radiat. Biol.*, 1995, **68**, 121.
54 D. J. Deeble, D. Schulz and C. von Sonntag, *Int. J. Radiat. Biol.*, 1986, **49**, 915.