Free Rad. Res. Comms., Vol. 11, Nos. 4–5, pp. 195–206
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ESR SPECTRA OF RADICALS OF SINGLE-STRANDED AND DOUBLE-STRANDED DNA IN AQUEOUS SOLUTION. IMPLICATIONS FOR ·OH-INDUCED STRAND BREAKAGE+

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(Received, March 2, 1990; in final form, May 4, 1990)

In situ photolysis at 20°C (argon plasma light source, $\lambda \gtrsim 200$ mm) of oxygen-free solutions containing 2 mM H_2O_2 and heat-denatured, single-stranded (ss)DNA from calf-thymus resulted in the ESR spectra of the 6-hydroxy-5,6-dihydro-thymin-5-yl {**1**} and 5-methyleneuracil {**3**} radicals linked to the sugar-phosphate backbone. They were generated by reaction of OH radicals with DNA. By comparison of the decay characteristics of the ESR signals with rate constants from pulse-conductivity measurements [E. Bothe, G.A. Qureshi and D. Schulte-Frohlinde, *Z. Naturforsch.*, **38c**, 1030, (1983)] the thymine-derived radicals {**1**} and {**3**} can be excluded as precursors of the fast, dominating component of strand breakage of ssDNA. In the absence of H_2O_2 from native, double-stranded (ds)DNA an ESR signal was obtained (singlet, $g \sim 2.004$, $\Delta\nu_{1/2} \sim 0.8$ mT) which was assigned to the deprotonated guanine radical cation, {G˙(-H)} of a DNA subunit. It is assumed that by the UV irradiation the guanine radical cation, {$G^{+\cdot}$}, is generated, either by monophotonic photoionisation or by electron transfer to pyrimidine bases. By rapid transfer of the bridging proton from {$G^{+\cdot}$} to the hydrogen bonded cytosine {G˙(-H)} is formed. When photolysis of dsDNA was carried out in the presence of H_2O_2, reaction of photolytically generated ˙OH resulted in peroxyl radicals and purine radicals. The oxygen for formation of the peroxyl radicals is probably produced by reaction of {G˙(-H)} with H_2O_2. Photolysis of N_2O-saturated solutions containing dsDNA or ssDNA provided another possibility of generation of OH radicals. Under those conditions the ˙OH-induced radicals {**1**} and {**3**} were obtained not only from ssDNA but also from dsDNA.

KEY WORDS: Free Radicals, ESR, DNA, thymine, guanine.

INTRODUCTION

It is well-known that reaction of radiation-produced hydroxyl radicals with DNA in aqueous solution leads to formation of strand breaks in the sugar-phosphate backbone.[1,2] There is evidence that in the absence of oxygen cleavage of the main chain originates mainly from the C4′ radical of deoxyribose.[3–5] This radical may be produced directly by H abstraction from the sugar moieties by ˙OH or indirectly via a base radical. For polyuridylic acid [poly(U)] the main reaction pathway occurs via the uracilyl base radicals which by H abstraction from the sugar moiety induce strand breakage (sb).[6,7] As a result of sb counterions (Na^+ or H^+) are released from the polynucleotide- or DNA-strands and the kinetics of conductivity increase upon

Dedicated to Prof. Dr. J. Kroh on the occasion of his 65th birthday

+ Paper presented at the 23rd Annual International Conference of the Electron Spin Resonance Group of the Royal Society of Chemistry, held from 26th to 30th March 1990 at Royal Holloway and Bedford New College, University of London.

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pulse-irradiation with an electron beam is identical with sb kinetics.[8,9] Valuable information on the sb mechanism of poly(U) in fluid aqueous solution was obtained by comparing the decay kinetics of ESR signals[10] with pulse conductivity data.[8] In this way it was shown that in poly(U) at neutral pH the decay of the 5,6-dihydro-5-hydroxy-uracil-6-yl radical was rate determing for ·OH-induced sb of the main chain.[10] To this purpose, in the ESR experiments OH radicals were produced by photolysis of H_2O_2 added to the polynucleotide solution.

For ssDNA information on the rate of sb in fluid aqueous phase is also available from conductivity measurements.[9] However, ESR spectra on DNA have been reported for the solid state, only.[11-14] Therefore, the aim of the present paper was to generate ESR spectra of DNA radicals in aqueous solution under the conditions of *in situ* UV-irradiation and to compare the decay behaviour of OH-induced DNA radicals with sb kinetics.[9]

MATERIALS AND METHODS

DNA from calf-thymus was obtained from Merck, Darmstadt. Purification by treating the solution with trypsin and precipitating from water/ethanol mixtures containing NaCl had no effect on the ESR results. For ESR measurements DNA was dissolved in water (1 mg/ml, corresponding to ~3 mM with reference to subunits) from a Millipore ion exchange unit (Milli-Q). Single-stranded DNA was obtained by heating the solution to 90°C, keeping it at this temperature for 15 min. and then chilling in an ice bath. The optical densities at 260 nm, OD_{260}, were ~1.0 and ~1.5 for ssDNA and dsDNA (0.5 mg/ml, $d = 0.1$ cm). No change in OD_{260} was observed upon addition of $NaClO_4$ (10 mM) to the solution of dsDNA. In order to produce OH radicals, H_2O_2 (2 mM) was added to the DNA solutions. From the absorption spectra of DNA and H_2O_2 it is estimated that ~1% of the incident light is absorbed by H_2O_2. After adjusting the pH to 7.5 the solutions were pumped through the ESR quartz cuvette (8 × 40 × 0.3 mm) with a flow rate of ~2 ml/min. The temperature in the cuvette was kept at 20°C. *In situ* irradiation was achieved with an argon plasma light source (GAT-PB 1500 from GAT Gamma Analysentechnik, Bremerhaven) which emits a continuum with $\lambda > 200$ nm. In the region of 200–225 nm the intensity of this light source is larger by a factor of 20–30 as compared to that of a high pressure xenon lamp XBO 501.[15] In some experiments the spectral distribution was changed by placing WG filters (Schott-Jena) in the optical pathway. Twenty min. prior to the measurements and during the measurements the solutions were gassed either with Ar, N_2O, N_2O/O_2 (4:1) or O_2. Details of the ESR spectrometer (X band with 100 kHz modulation) and the photolytic flow system are described elsewhere.[16,17] Base-line subtraction was achieved with a Nicolet NIC 1170 signal averager. For time-resolved measurements a mechanical chopper device was used.[18] The time-resolution of the set-up was ~0.1 s as determined from control measurements with the rapidly decaying 6-OH adduct radical of 1-methyluracil in aqueous solution.

RESULTS AND DISCUSSION

1) Photolysis of Solutions containing ssDNA and H_2O_2

In situ photolysis of an argon-saturated solution containing ssDNA and H_2O_2 resulted in the ESR spectrum shown in Figure 1a. From the linewidth of ~0.2 mT a rigid,

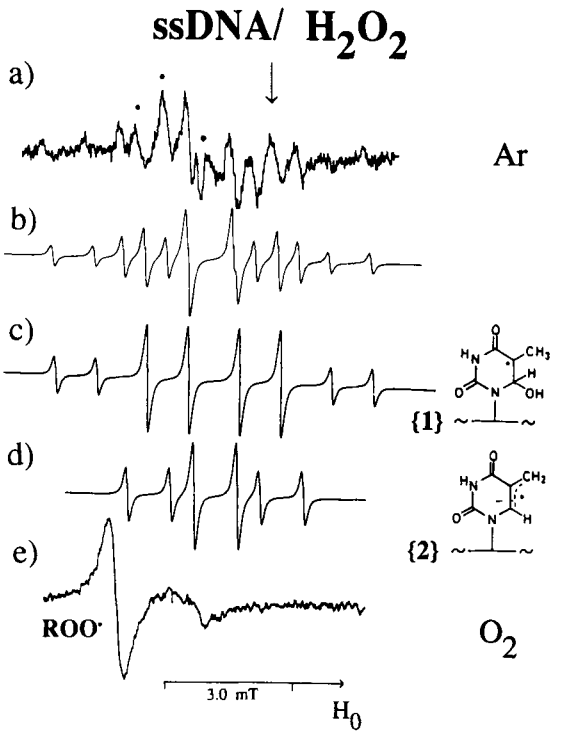


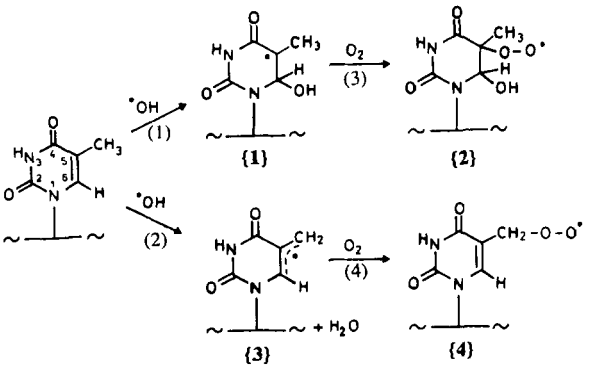
FIGURE 1a) ESR spectrum obtained by *in situ* photolysis of an aqueous solution of ss DNA (1 mg/ml) containing 2 mM H_2O_2, pH 7.5, 20°C, Ar-saturated. The base-line was subtracted. Microwave power: 2 mW, modulation amplitude: 0.16 mT, receiver gain: 5×10^6; b) Simulated spectrum obtained by addition of the spectra of radicals {**1**} and {**3**} with an intensity ratio of 1:2, g = 2.0032; c) Simulated spectrum of 6-hydroxy-5,6-dihydro-thymin-5-yl ({**1**}) (a_{CH_3} = 2.23 mT, a_{H6} = 1.00 mT). (For the corresponding radical derived from thymidine in aqueous solution a_{CH_3} = 2.23 mT, a_{H6} = 1.125 and g = 2.0032 were obtained[23]). d) Simulated spectrum of 5-methyleneuracil ({**3**}) with a_{CH_2} = 1.60 mT and a_{H6} = 1.05 mT. (For the corresponding radical derived from thymidine-5′-phosphate in aqueous solution a_{CH_2} = 1.62 and 1.54 mT, a_{H6} = 1.05 mT and g = 2.0023 were obtained[25]). e) Spectrum obtained by *in situ* photolysis of an aqueous solution of SSDNA (1 mg/ml) containing 1 ml H_2O_2, pH 7.5, 20°C, O_2-saturated; receiver gain: 2.5×10^6

powder-type arrangement of the paramagnetic species was excluded. As shown by the simulations in Figure 1b–1d the spectrum can be assigned to a superposition of the signals of isotropically tumbling thymine-derived radicals {**1**} and {**3**}* generated in reactions (1) and (2). In the low-field part of the spectrum additional signals (marked with dots) of unknown origin lead to slight distortions of the intensities.

In the presence of O_2, instead of the signals of {**1**} and {**3**} a single broad resonance was detected ($g \sim 2.015$, $\Delta\nu_{1/2} \sim 0.4$ mT, Figure 1e). It probably originates from the thymine-derived peroxyl radicals {**2**} and {**4**} and from contributions of peroxyl radicals generated from other nucleobases and from the sugar moieties

Spectral parameters of the 6-hydroxy-5,6-dihydro-thymin-5-yl and of the 5-methyle-

*The thymine-derived radicals as well as peroxyl and purine radicals described below are linked to the sugar-phosphate backbone. This situation is indicated by brackets { }. In the same way the parent nucleobases of DNA subunits are characterized.



neuracil radical derived from thymine, 1-methylthymine and thymidine in the solid state[19-21] and in solution[22-25] have been reported in literature. Isotropic values for hyperfine couplings and g factors were close to those used for the simulations of the DNA spectra in Figure 1b–1d. Evidence for the attack of the thymine residues in DNA by OH radicals has recently been presented. Upon X-irradiation of DNA in aqueous solution radicals {**1**} and {**3**} and also the 5-hydroxy-5,6-dihydro-thymin-6-yl radical have been detected by Kuwabara *et al.*[26] using spin-trapping in combination with ESR. γ-rradiation of cultured mammalian cells yielded oxidative conversion products of thymine-derived radicals, namely thymine glycol, 6-hydroxy-5,6-dihydrothymine and 5-(hydroxymethyl)uracil as major sources of DNA damage.[27-29] It should be mentioned that the intensity of steady-state ESR signals depends on the extent of radical production and on the rate of radical decay. This means that the most intense signals are not necessarily due to the major species originally produced. Detection of radicals {**1**} and {**3**} by ESR might be possible because of their relativley low reactivity as compared to other OH-induced DNA radicals. In line with this suggestion are the results of time-resolved ESR measurements. As an example the plot of signal intensity vs. time of one of the peaks (marked with an arrow in Figure 1a) is given in Figure 2a. The semilogarithmic plot in Figure 2b shows that the decay may be approached by two exponential functions with lifetimes of 0.4 s and 1.6 s.

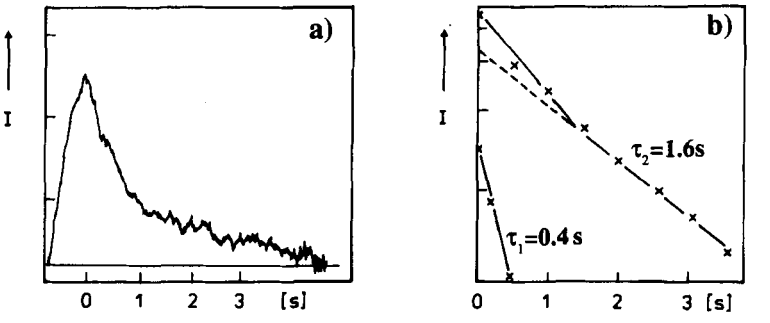


FIGURE 2a) Time-resolved ESR signal of one of the peaks of radical {**1**} marked in Fig. 1a with an arrow. b) Semilogarithmic plot of the signal intensity vs. time. The decay was fitted by two exponential functions with lifetimes of 0.4 s and 1.6 s with amplitudes of 40% and 60%.

Similar decay curves were obtained for the other 7 intense resonances in the center of the spectrum. The 4 peaks in the wings were too weak for time-resolved measurements. The deviation from monoexponential decay behaviour may be due to contributions of second order reactions and due to the fact that signals of several radical species are overlapping. Pulse-conductivity measurements[9] have shown rather complex strand break kinetics for ssDNA. The conductivity increased rapidly within ~ 0.02 s after the pulse. This fast increase was responsible $\sim 50\%$–70% of sb formation. After 0.02 s there was a second increase which was complete after ~ 0.5 s. In some experiments a third component was found with a much longer lifetime of several seconds. On the other hand, the ESR measurements on radicals {**1**} and {**3**} showed that the decay-times of the fast components were significantly larger than the time-resolution of the instrumentation of ~ 0.1 s. Therefore, both thymine-derived radicals can be excluded as precursors of the fast, dominating component of sb. However, as far as the slower sb components in the range of 0.5 s and in the range of several seconds are concerned, contributions of thymine radicals {**1**} and {**3**} by attack of the sugar phosphate backbone can not be ruled out.

Adinarayana *et al.*[30] showed that the pH dependence of the rate constant for ssb formation was very similar for ssDNA and for poly(dA). Moreover, the yield of ssb formation (in μmolJ^{-1}) was much larger for poly (dA) (0.23) than for poly (dC) (0.14), poly(dT) (0.057), and poly(dG) (0.009). From these observations it was concluded that adenine radicals might act as precursors of the fast component of sb in ssDNA. The long lifetimes of {**1**} and {**3**} in the range of seconds clearly indicate that the radicals are integral parts of the DNA strand. The isotropic behaviour of their ESR spectrum is in agreement with ^{13}C-NMR relaxation data which indicate isotropic overall motion for ssDNA as opposed to native DNA.[31] In addition, $^{\cdot}$OH-induced perturbations of the DNA structure like e.g. strand breakage or loss of base stacking might lead to further increase in motional freedom.

2) Photolysis of Solutions of dsDNA.

a) Photolysis in the absence of H_2O_2 Although data on sb kinetics of dsDNA are not available it was of interest to study the ESR spectra under the conditions applied in the experiments with ssDNA. A major difference between dsDNA and ssDNA was observed upon *in situ* irradiation in the absence of H_2O_2. Whereas no ESR signals were obtained from ssDNA and also from mononucleosides (guanosine, adenosine, thymidine and cytidine) photolysis of solutions of dsDNA resulted in a single broad resonance with $g \sim 2.004 \pm 0.0004$ and a line-width of $\Delta\nu_{1/2} = 0.8$ mT (Figure 3a).

By cutting off the wavelength region < 225 nm the ESR peak was reduced to about a third of its original intensity. No signal was observed upon excitation with $\lambda > 280$ nm.

Similar signals have been obtained at 77 K by UV irradiation of nucleotides and of DNA in basic 8 M $NaClO_4$ glass[32] and of oriented DNA containing 30% water.[33] They were assigned to the guanine radical cation produced by biphotonic ionisation via long lived triplet intermediates. In aqueous solutions of purine[34] and 6-methylpurine[34] or of 2′-deoxyadenosine[35] and 2′-deoxyguanosine[35] monophotonic photoionisation has been observed with laser light ($\lambda = 266$ nm or 193 nm, respectively). In view of the latter results it seems possible that under our conditions, i.e. with continuous excitation with $\lambda > 200$ nm in aqueous solution the primary step is monophotonic photoionisation of DNA. It is not known whether the guanine moiety is selectively ionized [eq. (5)]

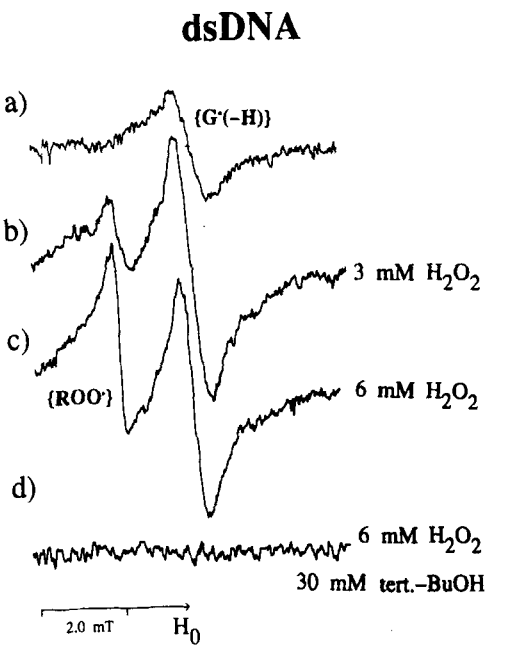


FIGURE 3 ESR spectra obtained by *in situ* photolysis of an aqueous solution of dsDNA (1 mg/ml), pH 7.5, 20°C; a) in the absence of H_2O_2; b) addition of 3 mM H_2O_2; c) addition of 6 mM H_2O_2; d) addition of 6 mM H_2O_2 and 30 mM *tert.*-butanol Microwave power: 2 mW, Modulation amplitude: 0.16 mT, receiver gain: 5×10^6

$$DNA \rightarrow \{G^{+\cdot}\} + e^-_{aq} \quad (5)$$

or whether photoionization generates base radical cations in an unselective way [eq. (6)]

$$DNA \rightarrow DNA^{+\cdot} + e^-_{aq} \quad (6)$$

The electron recombines rapidly with DNA to yield a DNA radical anion, $DNA^{-\cdot}$ [eq. (7)].

$$e^-_{aq} + DNA \rightarrow DNA^{-\cdot} \quad (7)$$

From experiments on oriented DNA fibres[36] and on DNA in frozen aqueous solution[37] it is known that the electron loss centers and electrons are trapped by the guanine moiety and by the pyrimidine bases [eq. (8) and (9)].

$$DNA^{+\cdot} \rightarrow \{G^{+\cdot}\} \quad (8)$$

$$DNA^{-\cdot} \rightarrow \{\text{pyrimidine}^{-\cdot}\} \quad (9)$$

There are two possibilities for decay of the pyrimidine radical anions, namely protonation by external protonating agents[5] such as H_2O or transfer of the electron to $\{G^{+\cdot}\}$ [eq. (10)].

$$\{G^{+\cdot}\} + \{\text{pyrimidine}^{-\cdot}\} \rightarrow \{G\} + \{\text{pyrimidine}\} \quad (10)$$

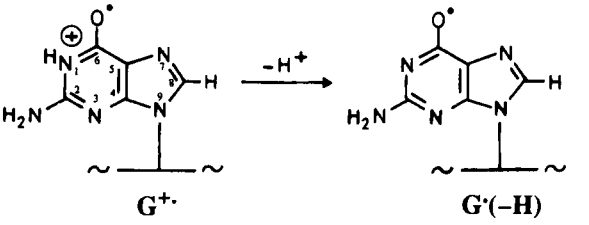
It should be mentioned that photoionisation is not the only possibility to generate guanine radical cations and pyrimidine radical anions. As indicated in eq. (11)–(13), electron transfer between an excited and a second, non-excited nucleobase is an alternative way which cannot be excluded.

$$\{\text{pyrimidine}^*\} + \{\text{purine}\} \rightarrow \{\text{pyrimidine}^{\cdot -}\} + \{\text{purine}^{\cdot +}\} \quad (11)$$

$$\{\text{purine}^*\} + \{\text{pyrimidine}\} \rightarrow \{\text{purine}^{\cdot +}\} + \{\text{pyrimidine}^{\cdot -}\} \quad (12)$$

$$\{\text{purine}^{+\cdot}\} \rightarrow \{G^{+\cdot}\} \quad (13)$$

Due to its low pK_a value[38] of 3.9 the guanine radical cation, $\{G^{+\cdot}\}$, is expected deprotonate rapidly on the time-scale of the ESR experiment to yield $\{G^{\cdot}(-H)\}$.



(14)

Therefore, the spectrum in Figure 3a is assigned to $\{G^{\cdot}(-H)\}$. Eq. (5)–(14) are valid for ssDNA as well as for dsDNA. There are mainly two factors which help to explain the difference in the ESR results between the single and the double helix: i) As was pointed out by Steenken[39] ultrafast proton transfer from $\{G^{+\cdot}\}$ to the hydrogen-bonded cytosine is feasible in dsDNA [eq. (15)].

$$\{G^{+\cdot}\} + \{C\} \rightarrow \{G^{\cdot}(-H)\} + \{C^{+}(+H)\} \quad (15a)$$

or

$$\{G^{+\cdot}\} + \{C^{-\cdot}\} \rightarrow \{G^{\cdot}(-H)\} + \{C^{\cdot}(+H)\} \quad (15b)$$

This process involves only slight displacement of the bridging hydrogen and occurs with an estimated[39] reaction period of < 1 ps. One might imagine that conversion of radical cations and radical anions to neutral radicals by eq. (15) is competing successfully with electron transfer to the guanine moiety [eq. (10)]. ii) Due to higher segmental mobility of ssDNA[31] a reaction channel may exist for rapid conversion of $G^{\cdot}(-H)$ into diamagnetic products which is much less efficient in the immobile, double-stranded form.

Reactions (14) and (15) are in line with the results of recent ESR experiments on γ-irradiated single crystals of 2′-deoxyguanosine-5′-phosphate (5′-dGMP).[40] It was shown that the guanine radical cation decayed at -196°C by deprotonation at N1. Similar results have been described for DNA fibres X-irradiated at -196°C[41]. The successor radical of $\{G^{+\cdot}\}$ observed in the temperature range of -53 to -13°C was also assigned to the N1-deprotonated guanine radical cation, $\{G^{\cdot}(-H)\}$. Its lifetime of ~ 5 s under our conditions, i.e. upon *in situ* irradiation at 20°C of aqueous dsDNA solutions was large enough for ESR detection.

Hüttermann and Voit[41] suggested that the failure to detect a successor radical to $\{G^{+\cdot}\}$ in frozen aqueous solutions[37,42] may be ascribed to the extreme spectral similarity of $\{G^{+\cdot}\}$ and $\{G^{\cdot}(-H)\}$ in powder type systems. We assume that for the

same reasons the spectra of $\{G^{\cdot}(-H)\}$ in our experiments are similar to those obtained by Sevilla *et al.*[32] and Graeslund *et al.*[33] in aqueous glasses and in DNA fibres. It should be mentioned that ESR experiments by Hole *et al.*[43] on single crystals of 5′-dGMP suggest deprotonation at the exocyclic amino group of $G^{+\cdot}$. The resulting neutral radical was characterized by a proton doublet splitting of 0.95 mT which was not observed in our experiments with aqueous DNA solutions.

b) Photolysis in the presence of H_2O_2 Argon-saturated solutions containing dsDNA and H_2O_2 showed a peroxyl signal ($g \sim 2.015$, $\Delta\nu_{1/2} \sim 0.8$ mT) and a singlet resonance ($g = 2.004$, $\Delta\nu_{1/2} \sim 0.7$ mT) (Figure 3b and 3c). Upon addition of 30 mM *tert.*-butanol to the H_2O_2 containing solutions, i.e. upon scavenging of the OH radicals, both types of radicals disappeared which proves that they are induced by $^{\cdot}OH$.

Generation of peroxyl radicals: As was already shown reaction of OH radicals with ssDNA results in the ESR spectra of thymine radicals {**1**} and {**3**}. In addition, other reducing radicals, like e.g. OH adducts of adenine, guanine and cytosine or sugar radicals may be formed but not detected by ESR because of their rapid decay. Upon saturating the photolysis solutions with O_2 the reducing radicals are converted to peroxyl radicals (Figure 1e) Photolysis of dsDNA in the presence of H_2O_2 yields peroxyl radicals even in argon-saturated solutions. This means that the oxygen is produced from H_2O_2 in a photolytically induced reaction. As described by Cullis *et al.*[44] there was evidence for reaction of $\{G^{+\cdot}\}$ with H_2O_2 in frozen aqueous solution of γ-irradiated DNA. One may imagine that under our conditions in a similar way reaction (16) takes place which explains the reduced yields of signal intensities of $G^{\cdot}(-H)$ in the presence of H_2O_2.

$$\{G^{\cdot}(-H)\} + H_2O_2 \rightarrow \{G\} + HO_2\cdot \quad (16)$$

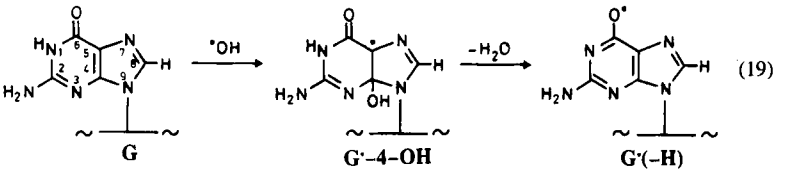
The resulting $HO_2\cdot$ disproportionates.[45]

$$HO_2\cdot \rightarrow H^+ + O_2^{-\cdot} \quad (17)$$

$$O_2^{-\cdot} + HO_2^- \rightarrow O_2 + H_2O_2 \quad (18)$$

It should be mentioned that $\{G^{\cdot}(-H)\}$ proved rather inefficient in H abstraction from 2-propanol, for example. Therefore, we have to be aware of the fact that reaction of $\{G^{\cdot}(-H)\}$ with H_2O_2 may be more complex than one would expect from eq. (16). As a consequence of reactions (16)–(18) signals of carbon-centered radicals, like e.g. {**1**} and {**3**} cannot be detected upon photolysis of solutions of dsDNA in the presence of H_2O_2. They are transformed into peroxyl radicals which are observed instead.

Assignment of the singlet resonance at $g = 2.004$ From pulse radiolysis studies[39] it is known that the OH-adducts of guanine and adenine derivatives undergo two types of fast unimolecular transformation. Dehydration of the 4- and 5-OH adducts results in the deprotonated guanine and adenine radical cations [$G^{\cdot}(-H)$ and $A^{\cdot}(-H)$] whereas the 8-OH adducts decay by opening of the 5-membered ring to yield 5-formamido-6-amino-pyrimidine type radicals. This means that in the presence of H_2O_2 $\{G^{\cdot}(-H)\}$ may be formed not only by photoionisation via $\{G^{+\cdot}\}$ but also in reaction sequence (19) via the 4- and 5-OH adducts.



(19)

It is expected that in analogous reactions the deprotonated adenine radical cation should be formed. However, this species is known to give rise to a large doublet splitting[46] of 1.21 mT due to the proton of the exocyclic-NH group and therefore its contribution to the singlet resonance of DNA (Figure 3b,c) can be excluded. The 8-OH adducts of adenine derivatives were found to be extremely sensitive to oxygen.[47] Therefore, under our conditions in the presence of O_2 (see Figure 3b, 3c) generation of the purine ring opening products is rather unlikely because of rapid oxidation of the 8-OH adducts. Well-known oxidation products of the 8-OH adducts are 8-hydroxyadenine and 8-hydroxyguanine.[39] Reaction of OH radicals with those species might result in the corresponding 8-oxo-N7-yl purine radicals which could also contribute to the singlet ESR resonance at $g = 2.004$.

3) Photolysis of N_2O-Saturated Solutions of DNA As described in section 2, it was not possible to detect the thymine-derived radicals {**1**} and {**3**} upon photolysis of dsDNA in the presence of H_2O_2. It turned out, however, that the spectra of {**1**} and {**3**} could be obtained in the absence of H_2O_2 by saturating the photolysis solutions with N_2O. The spectrum obtained in this way from ssDNA (Figure 4a) was similar

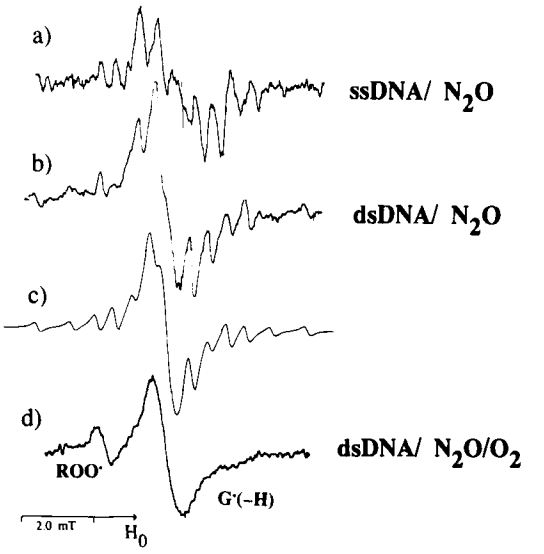


FIGURE 4 ESR spectra obtained upon *in situ* photolysis of solutions of DNA in the presence of N_2O. a) ssDNA (1 mg/ml) b) dsDNA (1 mg/ml) c) computer simulation of a mixture of radicals {**1**}, {**3**} and {G•(-H)} with an intensity ratio of 0.05 : 0.1 : 1, linewidths 0.2, 0.2 and 0.6 mT; d) solution of dsDNA saturated with N_2O/O_2 (4:1)

to the spectrum generated from ssDNA (see Figure 1a). By irradiating a N_2O-saturated solution of dsDNA the spectrum in Figure 4b was obtained. By computer simulation (Figure 4c) the series of narrow signals superimposed on the broad purine resonance was clearly assigned to a mixture of {**1**} and {**3**}. In the presence of O_2, i.e. upon saturating the solution of ssDNA with N_2O/O_2, peroxyl radicals were detected instead of the resonances of {**1**} and {**3**} in agreement with the results in Figure 1e.

We may assume that either the solvated electron, if formed by photoionisation, or the pyrimidine radical anions arising from eq. (11) and (12) react with N_2O. Generation of OH radicals by reaction (20) is a well-known process. In view of results by Al-Sheikhly *et al.*[48] on the reaction of N_2O with the strongly reducing CO_2^- radical the second pathway, [eq. (21)] seems also possible but is probably too slow.

$$e_{aq}^- + N_2O \rightarrow N_2 + {}^{\cdot}OH + OH^- \quad (20)$$

$$\text{pyrimidine}^{\cdot -} + N_2O + H_2O \rightarrow \text{pyrimidine} + N_2 + {}^{\cdot}OH + OH^- \quad (21)$$

In order to explain the isotropic spectra of {**1**} and {**3**} in solutions of dsDNA we have to be aware of the fact that the samples were prepared in the absence of salt, i.e. under conditions where the double-helical DNA structure is destabilized. Therefore we assume that the narrow signals in Figure 4b arise from open-chain, single-stranded regions. In agreement with this hypothesis upon addition of $NaClO_4$ in a final concentration of 10 mM the signals of {**1**} and {**3**} disappeared and only the broad singlet resonance was left.

Acknowledgement

Skilful technical assistance by Mr. H. Niehaus with the ESR measurements is gratefully acknowledged.

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Accepted by Prof. H. Sies