The Electron Spin Resonance Spectrum of γ-Irradiated Deoxyribonucleic Acid: a Novel Interpretation based on Molecular Orbital Calculations and Computer Simulations

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A novel interpretation of the e.s.r. spectrum of irradiated deoxyribonucleic acid is put forward on the basis of the linear combination of three hyperfine components: the previously known thymine octet [7—15% radical (T_1)], a doublet of *ca.* 20 G (10—20%), a singlet (70—80%). Tentative proposals for the assignment of the doublet have been made involving radicals arising from the pyrimidine and purine bases and from the ribose and protein moiety. No definite interpretation of the singlet could be attained because of the lack of resolution; however, it is worth pointing out that this signal has the characteristics expected, under the experimental conditions employed, from the hydrogen atom adducts at O(2) and O(4) of thymine and cytosine and at O(6) of adenine, whose formation is likely to be favoured by the hydrogen-bond system.

When subjected to the effect of ionizing radiations at room temperature, 'dry' deoxyribonucleic acid (DNA) affords a complex spectrum where a typical eight-line pattern of *ca.* 20 G peak-to-peak average, due to the thymine radical (T_1), is clearly detectable.[1—4] This component, however, accounts for only a minority of the radical population; most radicals, in fact, contribute to the central part of the spectrum which, because of the lack of resolution, has not yet been conclusively interpreted. Our approach to the unfolding of this problem relies on the adoption of different experimental conditions with the aim of altering the relative abundance of the radicals and, consequently, the e.s.r. signal shapes. It was thought that the simulation by computer of various e.s.r. spectra thus obtained could lead to the identification of individual hyperfine components.

Experimental and Calculations

Pure DNA was extracted from calf thymus according to the usual procedure[5] and purified from residual proteins, down < 0.4%, by digestion with a proteolytic enzyme (pronasis); final purification was achieved by dialysis using triply distilled water followed by repeated precipitation with ethanol; 'dry' DNA samples were obtained by lyophilization at −10 °C. For the e.s.r. measurements, DNA samples were sealed under vacuum in quartz tubes and irradiated at room temperature in a ^{60}Co source at a dose rate of 0.12 Mrad h^{-1} with total doses of 10—130 Mrad. The e.s.r. spectra were recorded on a Varian *X*-band 4500 spectrometer.

Computer simulation and MO calculations were carried out on Honeywell DPS8 and HP 9835 A computers. The INDO method[6] was employed to obtain predicted hyperfine splittings for hypothetical radicals arising from purine and pyrimidine bases; *ca.* 50 different species have been investigated (the computed results are available on request) formally arising from electron capture, electron loss, homolysis of exocyclic C–H, C–C, and C–N bonds and H addition at C=C, C=O, and C=N double bonds. Radical geometries were based on *X*-ray diffraction data on model compounds of purine and pyrimidine bases.[7]

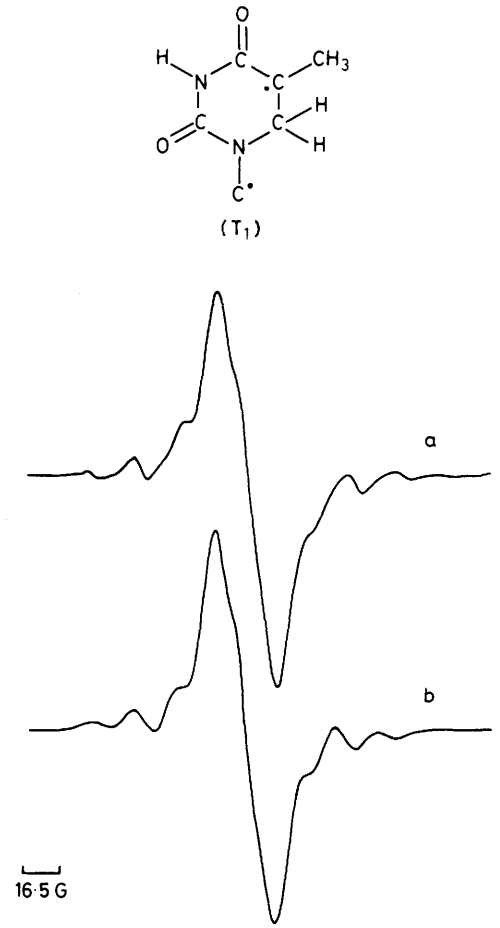


Figure 1. a, Experimental e.s.r. spectrum of DNA irradiated with a total dose of 9.2 Mrad at room temperature; b, computer simulation performed with the hyperfine components shown in Figure 4 (singlet 72%, doublet 13%, octet 15%)

Results and Discussion

The spectra shown in Figures 1—4 were obtained by adopting the following experimental conditions. (a) Irradiation took place at room temperature with different total γ-doses (9.2 Mrad, Figure 1a; 136 Mrad, Figure 2a). (b) Prior to irradiation at room temperature, the samples were kept 24 h at 50 °C under continuous evacuation at 10^{-5} Torr (Figure 3a); this procedure,

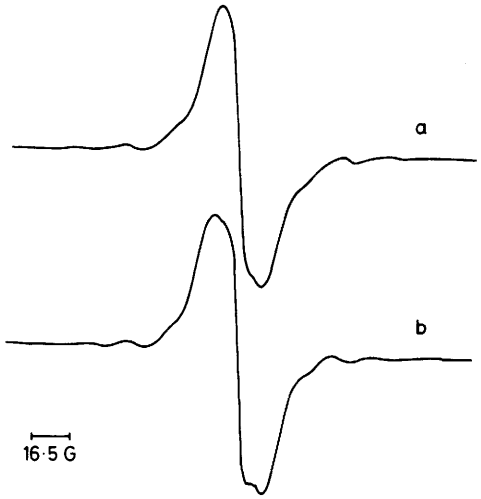


Figure 2. a, Experimental e.s.r. spectrum of DNA irradiated with a total dose of 126 Mrad at room temperature; b, computer simulation with 84.5% singlet, 99% doublet, 6.5% octet

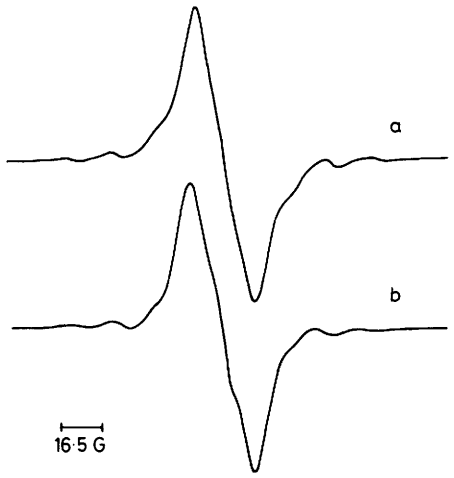


Figure 3. a, Experimental e.s.r. spectrum of DNA γ-irradiated at room temperature with a total dose of 9.2 Mrad; the DNA sample was kept 24 h under vacuum (10^{-5} Torr) at 50 °C prior to irradiation; b, computer simulation based on 72% singlet, 20% doublet, 8% octet

by monitoring the sample weight loss on a thermobalance, was seen to cause the exhaustive dehydration of DNA. (c) The samples were submitted to prolonged thermal annealing between 40—80 °C, following the irradiation at room temperature with the highest dose (Figure 4a). The adoption of the most drastic procedure (c) leads to a 'singlet' (peak-to-peak width 15.5 G and traces of hyperfine structure at the limit of resolution), which decays slowly at 80 °C without further appreciable change of shape (Figure 4a). This singlet is present in all the e.s.r. spectra recorded and its subtraction from the spectrum of Figure 3a reveals the existence of a doublet of *ca.* 20 G superimposed on the thymine octet. In agreement with these observations, satisfactory computer simulations of the spectra in Figures 1a—3a were performed by linear combination of the 'singlet' of Figure 4a with a doublet (17.9 G splitting, line widths 13 G, Gaussian shape), and an octet (simulated with $3a^H$ 19 G, $2a^H$ 38 G, line width 15 G, Gaussian shape). The changes induced by the different experimental conditions are thus interpreted in terms of changes in the relative abundance of the three pattern as shown in the Table; the singlet is enhanced

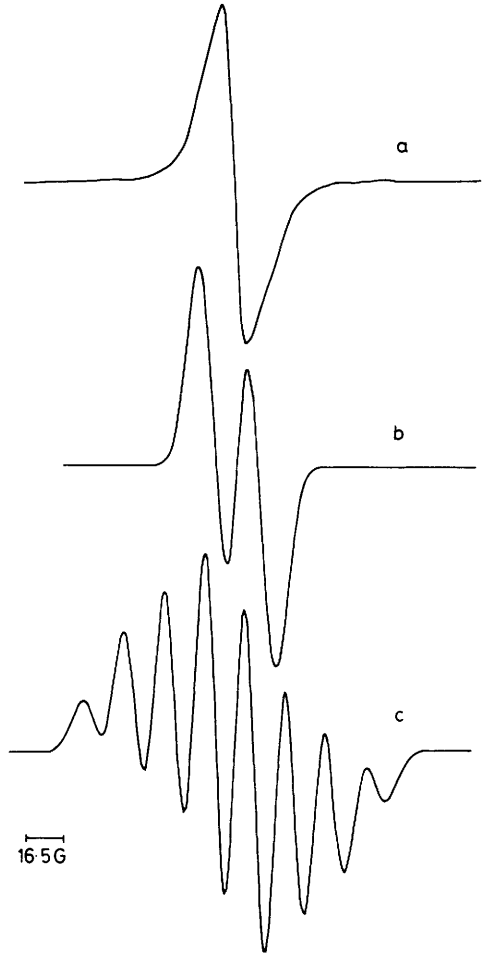


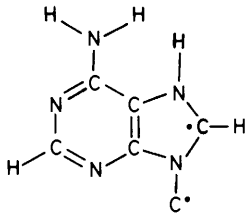
Figure 4. Hyperfine components adopted for the computer simulation of the spectra in Figures 1a—3a. a, Experimental e.s.r. signal obtained by submitting the DNA sample to thermal treatment under vacuum after the irradiation (15 min at 40 °C, 22 h at 60 °C, 15 min at 80 °C); b, doublet simulated with a^H 17.9 G, line width 13 G, Gaussian shape; c, the thymine octet simulated with $3a^H$ 19 G, $2a^H$ 19 G, $2a^H$ 38 G, line width G, Gaussian shape

Table. Computer simulation of the spectra of Figures 1a—3a: relative intensities (% of total area) of the hyperfine components

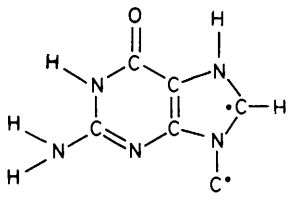
	Hyperfine components (%)		
Spectrum	Singlet	Doublet	Octet
Figure 1a	72	13	15
Figure 2a	84.5	9	6.5
Figure 3a	72	20	8

either by increasing the radiation dose or by post-irradiation thermal annealing; the decrease of the water content seems to have the effect of decreasing the intensity of the octet and of enhancing the intensity of the doublet.

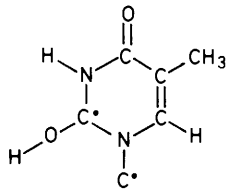
Origin of the Doublet.—Electron capture and loss are dominant radiolytic processes leading to the formation of radical anions and cations;[8] the anions of thymine and cytosine are reported to give doublets,[9–11] of splitting not far from those of the DNA doublets, which arise from the dominant interaction of the single proton at C(5) at the end of the allyl



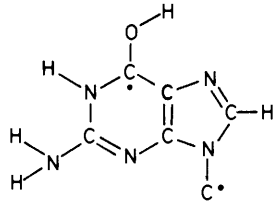
(A_1)
a_8^H - 16·3 G
a^N < 3 G
a^H < 6 G



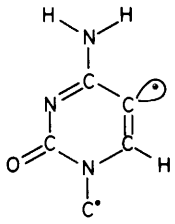
(G_1)
a_8^H -16·5 G
a^N < 4 G
a^H < 6 G



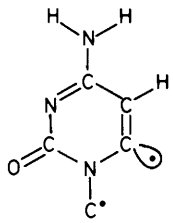
(T_2)
a^H < 4 G
a^N < 2 G



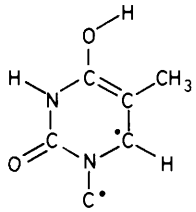
(G_2)
a^H < 3 G
a^N < 2 G



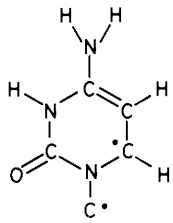
(C_1)
a_6^H 16·4 G
a^H < 1 G
a^N < 4 G



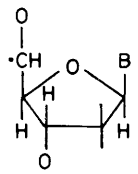
(C_2)
a_5^H 12·7 G
a^H < 4 G
a^N < 3 G



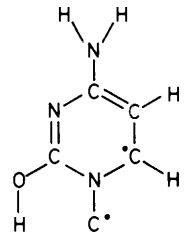
(T_3)
a_6^H - 6·7 G (ref. 16)
a^H - 9·2 G
a^H < 7 G
a^N < 2 G



(C_3)
a_6^H - 11 G other $a^H \leq 5$ G
$a^N \leq 5$ G



(R_1)
$a_\alpha^H \sim$ 15-20 G
$a_\beta^H \sim$ 5-7 G



(C_4)
a_6^H - 8·5 G
$a_6^H \sim$ 10 G (ref. 13) other $a^H \leq 4$ G
$a^N \leq 5$ G

triad. This assignment, however, conflicts with the recognized instability of these species at room temperature; furthermore they are bleached by u.v. light whilst the DNA doublet is not. Hydrogen-atom addition at the double bonds is another favoured radiolytic process, leading to thermally and u.v.-stable neutral radicals; many hydrogen-atom adducts of purine and pyrimidine derivatives have indeed been isolated, such as the hydrogen-atom adducts at C(5) and C(6) of thymine and cytosine, at C(8) of guanine and adenine,[12-14] at O(2) of cytosine and O(4) of thymine.[13-16] Among these species the only acceptable candidate seems to be the O(2) and O(4) adducts, since their hyperfine structures are characterized by the dominant interaction of a single proton at C(6); however, the reported splitting (s_{iso}^H *ca.* 7 G) is too small to be resolved in the polycrystalline matrix; in consequence they are more likely to contribute to the singlet rather than to the doublet. Two not yet experimentally detected adducts, which are predicted by the INDO method to yield doublets of the expected splittings, are the radicals (A_1) and (G_1) resulting from hydrogen (or the other radical precursors) addition at N(7) of adenine and guanine. N(7) is the favoured site of protonation for both adenine and guanine;[17] therefore the hypothesis of the existence of radicals (A_1) and (G_1) appears to be consistent with the widespread opinion that neutral radicals in irradiated DNA are mainly formed by protonation of radical anions precursors.[2-4,11]

Water, as a proton donor, does not seem to be a likely candidate since the intensity of the doublet is not depressed by a decrease in the water content; more likely proton donation to guanine and adenine radical anions might take place from the >N–H and $-NH_2$ groups of nearby thymine and cytosine units which have been seen by inspection of non-bonded interatomic distances in DNA to be at a favourable reaction distance ($\leq$ 5 Å).[18]

Among the hydrogen-abstraction radicals arising from purine and pyrimidine derivatives, the only experimentally detected ones are those formed by the loss of hydrogen at N(1) of thymine, cytosine, and uracil derivatives[16,19a] and from the methyl group of thymine;[19b] both types of species [the former could be formed in DNA through the less likely rupture of the C(1′)–N(1) bond] would not yield doublets because of the large $A_{||}$ nitrogen coupling and because of the number and magnitude of α-allyl protons (*ca.* 15—20 G). Inspection by the INDO method (which proved to be capable of correctly predicting the isotropic splittings of the methyl protons in the thymine hydrogen-abstraction radical) has led to the identification of two other possible candidates (C_1) and (C_2) for the doublet (INDO major splittings). Another hypothesis for the

doublet assignment may be based on sugar–phosphate backbone radicals, such as those arising for the hydrogen abstraction at C(5′) and C(11) and from the sugar-phosphate bond scission, which were observed and characterized by e.s.r. for a model compound.[20] The C(5′) hydrogen-abstraction radical, as obtained from deoxyadenosine[20a,b] and 3′-cytidylic acid, is reported to have one α-hydrogen with a 15—20 G coupling and one β-hydrogen with a smaller coupling of 5—7 G which probably would not be resolved; therefore this species might be expected to yield a doublet when obtained from DNA in our experimental conditions.

All the other species are reported to have at least two major interacting protons and should therefore be disregarded.

Ultimately the possibility cannot be ruled out that the doublet be generated by protein radicals $-\dot{C}H-\underset{|}{N}-$ (P_1) from the residues of the protein coat surviving the attack by proteolytic enzymes; however, the low concentration of these protein impurities (0.4%) and the occurrence of energy transfer and spin migration phenomena toward DNA,[21] which would cause further lowering of the protein radical signal, make this hypothesis less probable.

Origin of the Singlet.—The singlet is by far the major component of the spectrum, therefore its assignment is of decisive importance for the elucidation of the major features of the solid-state radiation chemistry of DNA; unfortunately the absence of resolution makes it impossible to formulate any conclusive hypothesis about its origin so that only a tentative guess can be made, based on stable species which can be predicted not to afford resolved splittings in the experimental conditions employed. A radical-forming process which seems to be suitable on this respect is the hydrogen (or other radical precursors) addition at O(2) of thymine and O(6) of guanine yielding (T_2) and (G_2) (INDO splittings). To these, also the adducts at O(4) of thymine and at O(2) and N(3) of cytosine should be added since the major splittings due to protons at C(6) are likely not to be resolved. These hypotheses are tempting since hydrogen addition at oxygen can take place by protonation through the hydrogen-bond system of the base pairs which is favoured by quantomechanical tunnelling. There are, however, other possibilities, including for instance hydrogen (or radical) addition at N(3) of guanine and adenine and N(1)–H bond rupture of guanine.

Conclusions.—A novel interpretation of the e.s.r. spectrum of irradiated DNA is put forward based on the linear combination of three hyperfine components: the previously known thymine octet [7—15% radical (T_1)], a doublet of *ca.* 20 G (10—20%), and a singlet (70—80%). Tentative proposals for the assignment of the doublet have been made involving radicals arising from pyrimidine and purine bases [hydrogen-addition radicals (G_1), (A_1); hydrogen-abstraction radicals (C_1), (C_2)], and from the ribose and protein moiety [hydrogen-abstraction radicals (R_1), (P_1)]. No definite assignment of the singlet could be attained because of the lack of resolution; however, it is worth pointing out that this signal has the characteristics expected, under the experimental conditions employed, for hydrogen adducts at O(2), O(4) of thymine and cytosine, and at O(6) of adenine, whose formation is likely to be favoured by the hydrogen-bond system.

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