Electron addition to DNA–thymine *vs* cytosine radical anion?

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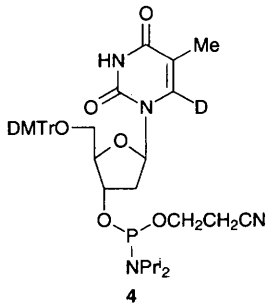
Comparison of the EPR spectra of a self-complementary oligonucleotide (24-mer) containing either thymidine or [6-$^2H_1$]thymidine residues following irradiation in frozen aqueous LiCl matrices allows the unambiguous analysis of the radical anion population in model DNA systems; these studies show that $C^{\cdot-}$ (1):$T^{\cdot-}$ (2) is 85:15.

Ionising radiation leads to damage to DNA by two distinct pathways, one involving the radiolysis products of water, particularly hydroxyl radicals (the indirect mechanism), and the other involving the direct ionisation of DNA (the direct mechanism).[1,2] In contrast to the indirect pathway the direct mechanism is less well understood despite the belief that this pathway may be of significance *in vivo*.[3] EPR spectroscopic studies of irradiated frozen aqueous solutions of DNA have established that although the initial sites of ionisation may be essentially random, hole migration must occur to account for the radical cation being localised on the purine bases and selective trapping of the electron must occur for the radical anion to be formed predominantly at the pyrimidine bases.[4,5] There is general agreement that the radical cation formed in DNA is predominantly the guanine radical cation, however, the nature of the radical anion has proved more controversial because of the close similarity of the EPR spectra of the cytosine or thymine radical anions.[6–9] The unambiguous assignment of the DNA radical anion signal is a longstanding major problem. Furthermore, it has important ramifications for the source of the well characterised radical TH· **3** which must either arise directly from $T^{\cdot-}$ by protonation on C-6 or indirectly from $C^{\cdot-}$ by electron transfer to T followed by protonation. The latter pathway would require electron addition to be initially reversible, Scheme 1. Ultimately a knowledge of the nature of the primary radicals is crucial to an understanding of the distribution of DNA damage and its relevance to subsequent chemical and biological events involving cellular DNA. Here we report a definitive approach to the problem of identifying the primary sites of electron capture and discuss the implication for reversibility of the electron addition. We describe the preparation of an oligonucleotide containing [6-$^2H_1$]thymidine at each of the T sites and its use to determine the extent of electron capture at C and T upon irradiation in frozen LiCl matrices.

The one-electron-reduced products derived from cytosine- and thymine-containing nucleosides and nucleotides have been well characterised by EPR spectroscopy. In DNA and duplex forming oligonucleotides both bases give rise to very similar doublet EPR spectra arising from a single large hyperfine coupling to H-6, Fig. 1, and this close similarity has lead to difficulties in assigning the anion component in irradiated DNA samples. The significant yields of TH·, the 5,6-dihydrothymin-5-yl radical, seen in the spectra of irradiated frozen aqueous



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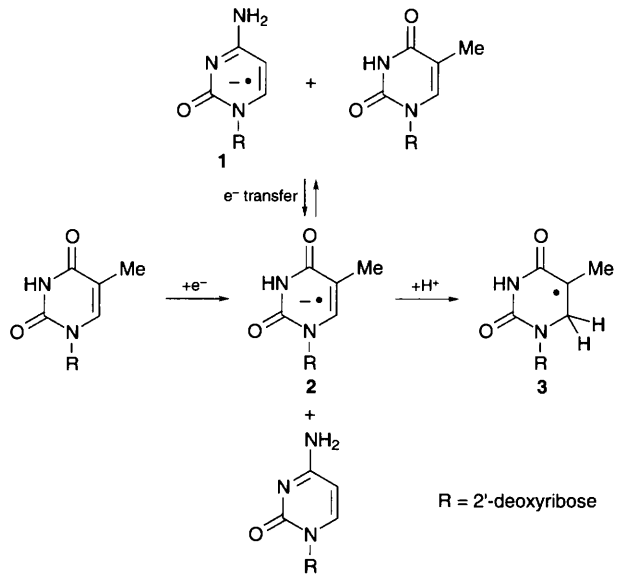

Scheme 1

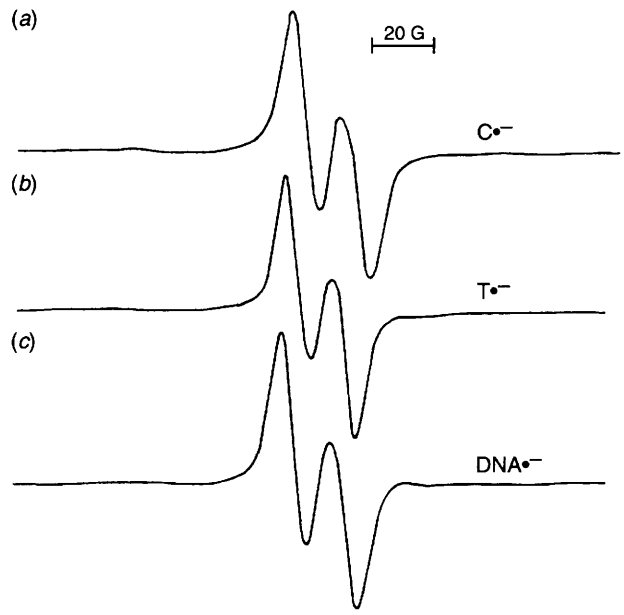

Fig. 1 X-Band EPR spectra of DNA and the pyrimidine radical anions generated by γ-irradiation in 10 mol dm^{-3} LiCl glasses illustrating the similarity of the $C^{\cdot-}$ and $T^{\cdot-}$ spectra and the difficulty in deconvoluting these signals in DNA. (*a*) Poly[dA-dT]•Poly[dA-dT], $T^{\cdot-}$; (*b*) Poly[dG-dC]•Poly[dG-dC], $C^{\cdot-}$; (*c*) $DNA^{\cdot-}$. All spectra were recorded at 77 K and are shown with the solvent signal substracted.

DNA samples on annealing led to the early assumption that the principal site of electron addition in DNA was the thymine base. More recent work based on deconvolution of composite EPR spectra of irradiated DNA in frozen matrices has suggested that the major electron addition site is in fact the cytosine base,[6,7] but these analyses are dependent on the quality and validity of the bench-mark spectra, the way in which slight differences in g-shift are handled *etc*; and these issues become critical if one is to deconvolute very similar spectra such as the $C^{\cdot -}$ and $T^{\cdot -}$ doublets.

We have shown that the selective deuteriation[10] of the 6-position of thymidine leads to the expected collapse of the doublet EPR spectrum resulting from irradiation in a LiCl matrix at 77 K, Fig 2. Incorporation of this [6-$^2H_1$]thymidine (>95% deuteriated at C-6 as judged by ^1H NMR) into a self-complementary oligonucleotide (5'-TCAGCATG-CATGCATGCATGCTGA) was accomplished from the synthesised [6-$^2H_1$]thymidine phosphoramidite monomer[11] **4** by automated oligonucleotide synthesis (Midland Reagents Ltd, Texas). The deuteriation of all of the thymidine residues means that the thymine anions and cytosine anions are now easily distinguished, the former give rise to singlets while the latter remain as doublets; analysis is therefore straightforward. The undeuteriated and deuteriated sequences were prepared and purified under identical conditions and irradiation of samples of each in 10 mol dm^{-3} LiCl glasses gave rise to the spectra in Fig. 3. The spectrum of the oligonucleotide containing [6-$^2H_1$]thymine bases shows a marked reduction in the resolution in the central region, Fig 3(*b*), consistent with a part of the 'doublet' seen for the undeuteriated oligonucleotide, Fig. 3(*a*), collapsing to a singlet. Successful simulation of the spectrum of the deuteriated oligonucleotide is achieved with 15% of the $T^{\cdot -}$ and 85% of $C^{\cdot -}$, Fig. 3(*c*) using the radical anion spectra of [6-$^2H_1$]thymidine, Fig. 2, and (Poly[dG-dC]•Poly[dG-dC]) as benchmarks. These values are remarkably close to those obtained from the previous deconvolution/simulation of EPR spectra of frozen aqueous DNA solution and oligonucleotides irradiated in LiCl matrices.[6]

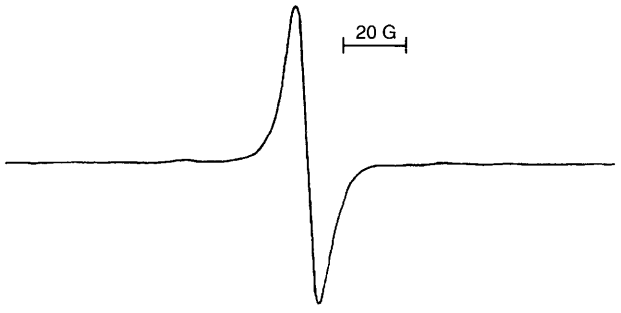

Fig. 2 X-Band EPR spectrum of the radical anion spectrum of [6-$^2H_1$]thymidine on γ-irradiation in 10 mol dm^{-3} LiCl glasses showing the collapse of the doublet signal into the singlet. The spectrum was recorded at 77 K and is shown with the solvent signal subtracted.

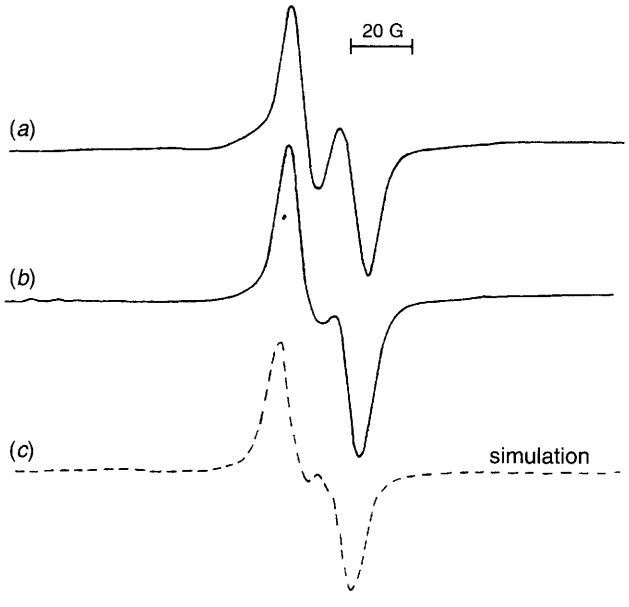

Fig. 3 X-Band EPR spectra of the radical anion of (*a*) the undeuteriated and (*b*) the [6-$^2H_1$]thymine deuteriated oligonucleotide generated by γ-irradiation in 10 mol dm^{-3} LiCl glasses. Simulation of the partially collapsed doublet with 85% $C^{\cdot -}$ doublet and 15% $T^{\cdot -}$ singlet using the benchmark spectra in Figs. 1(*b*) and 2 respectively. All spectra were recorded at 77 K and are shown with the solvent signal subtracted.

The implications of these results for the situation in frozen aqueous solution is dependent on the effect of the LiCl matrix on the relative electron affinities, potential electron transfer reactions or protonation/deprotonation equilibria. If $T^{\cdot -}$ does indeed represent 15% of the total anion population in frozen aqueous solutions there are important implications. We have shown that the yield of TH· on annealing irradiated frozen aqueous DNA solutions to 200 K represents *ca.* 18% of the initial total radical population (anion and cation).[9] Assuming that the anion/cation ratio is 50:50 then 36% of the anion component would have to have been $T^{\cdot -}$ if the only source of TH· was directly from $T^{\cdot -}$. If the ratio of anion to cation in frozen aqueous DNA solutions is closer to 70:30 seen in the nucleosides thymidine and deoxycytidine then 24% of the initial anion must be capable of forming TH·. Both of these values are significantly larger than the percentage of $T^{\cdot -}$ seen experimentally in this study. This must mean either that the ratio of $T^{\cdot -}$/$C^{\cdot -}$ is larger in frozen aqueous solution or that the irreversible C-6 protonation of $T^{\cdot -}$ giving rise to TH· on annealing may be able to draw over the equilibrium between $C^{\cdot -}$ and $T^{\cdot -}$, Scheme 1. Attempts to probe this possibility in the LiCl matrices are problematic. Only low yields of TH· are seen on annealing such samples (5-10%) but this is probably due to competing electron transfer reactions such as shown in eqn. (1).

$$DNA^{\cdot -} + Cl_2^{\cdot -} \rightarrow DNA + 2Cl^- \quad (1)$$

This definitive demonstration of preferential electron capture at cytosine is consistent with suggestion that the distribution of radical anions reflects the relative electron affinities of the various trapping sites.

Footnote

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Received, 23rd January 1996; Com. 6/00679E