The Site of Electron Capture in Irradiated Deoxyribonucleic Acid: Cytosine *vs.* Thymine

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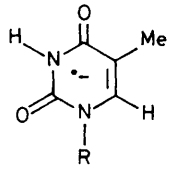
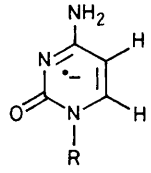
Evidence from e.s.r. spectroscopy for the facile *N*-protonation of the radical anion of cytosine generated in an aqueous glass, even at 4 K, supports the conclusions that electrons are captured predominantly at thymine when DNA is exposed to ionising radiation.

Electron capture is thought to be a key stage in the radiolysis of DNA.[1—3] On the basis of a number of e.s.r. studies it is widely accepted that the major electron gain (and loss) occurs at the heterocyclic bases rather than the sugar or phosphate moieties. Although there is evidence that the electron becomes localised on pyrimidine rather than purine bases, there is considerable controversy regarding the precise sites of trapping, despite the fact that this is a key step in the direct damage pathway.[4] The problem arises because it has been well established by single crystal e.s.r. and ENDOR studies at low temperature using thymine (T) and cytosine (C) derivatives, that the e.s.r. spectra for the electron-capture centres in both cases are characterised by an anisotropic doublet.[5,6] The direction cosines for this show clearly that it is due to coupling to the C^6-H proton, (**1**) and (**2**). These studies failed to show any other coupled nuclei. The result for $T^{\bullet -}$ is nicely confirmed by liquid-phase studies which give an isotropic coupling equal to the average of the x, y, and z components for C^6-H (Table 1), all other hyperfine couplings being small.[7] Unfortunately, attempts to prepare $C^{\bullet -}$ anions using similar methods have so far failed.[8]

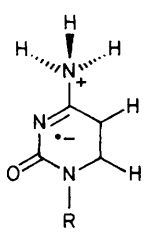
The spectra for cytosine and thymine derivatives in aqueous glasses rather than crystals are also reported to be closely



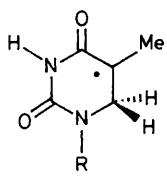
(**1**)



(**2**)



(**4**)



(**3**)

Table 1. E.s.r. hyperfine parameters for thymine ($T^{\bullet -}$) and cytosine ($C^{\bullet -}$) radical anions and for a protonated form of the cytosine radical anion ($CH^{\bullet}$).

Source	Medium	T/K	^{1}H hyperfine coupling/G[a]			
			x	*y*	*z*	*iso*
Thymidine[b]	Single crystal	4	19.8	10.7	4.6	11.7
Cytidine 3′-monophosphate[b]	Single crystal	4	21.2	12.1	5.25	12.8
Thymine[c]	H_2O	278	—	—	—	11.8
Thymidine	10 M LiCl/H_2O	77	21	11	5	12
Cytidine	10 M LiCl/D_2O	77	21	12	5	13
Cytidine	10 M LiCl/H_2O	77	21 —	12 —	5 —	13 11

[a] G = 10^{-4} T. [b] Ref. 6. [c] Ref. 7 (A_{Me} = 0.9 G; A_{N1} = 1.35 G; A_{N2} = 0.9 G; $A_{(2H)}$ = 0.2 G).

similar. Since most of the studies on DNA are conducted in aqueous media because of the relevance to the situation *in vivo*, the results from the aqueous glasses may be more comparable with the situation in DNA itself. Using aqueous D_2O glasses, which normally give better resolution that H_2O glasses, well defined doublets are indeed obtained for both C and T derivatives (Figure 1).[2,9] These glasses are specially selected because it has been well established that electron-capture by suitable solutes is far more efficient than electron-loss,[10] so there is no ambiguity in assignment of the doublets to electron-capture centres.

For frozen aqueous DNA, one component of the low temperature e.s.r. spectra is also a doublet, almost indistinguishable from those described above. The nature of the controversy concerns whether the radical anions formed in DNA and detected by low temperature e.s.r. spectroscopy are primarily $T^{\bullet -}$ or whether $C^{\bullet -}$ makes a significant or even major contribution. Given that the e.s.r. spectra for these two species are so similar there is clearly a problem of assignment.

In pursuing this important point we have returned to studies on model mononucleosides and nucleotides in water and aqueous glasses. The latter systems have been shown to be particularly good for forming electron-capture centres.[10] We have confirmed that in all systems T gives a well defined doublet closely similar to the DNA doublet. However, although C gives an *apparently* identical doublet in D_2O glasses, it gives a well defined *triplet* when H_2O glasses are used. The radical anion formed from C in the crystal is a doublet. The extra splitting that arises in H_2O glasses is clearly due to a proton derived from the solvent, which means that the species in aqueous glasses is *not* $C^{\bullet -}$ but $CH^{\bullet}$. The C^6-H coupling in $CH^{\bullet}$ is almost identical to $C^{\bullet -}$ (Table 1), which rules our protonation on carbon as occurs with $T^{\bullet -}$ to give $TH^{\bullet}$ (**3**). The most reasonable proposal is that the NH_2 group has become protonated rather than the ring nitrogen. Provided rotation of the resulting $-NH_3^+$ group is prevented by hydrogen bonds, one large coupling could result, (**4**).

In these model studies the protonation of $C^{\bullet -}$ in aqueous glasses is an extremely facile process, and occurs extensively *even at* 4 K. Given that protonation of $T^{\bullet -}$ formed in DNA to give $TH^{\bullet}$ has been unambiguously demonstrated, we believe that the protonation of $C^{\bullet -}$, if formed in DNA, might reasonably be expected to occur readily. On the basis of these present results we would expect therefore to detect a triplet in frozen aqueous DNA systems in H_2O if there was a significant $C^{\bullet -}$ population initially. However, we detect only the doublet, which is replaced by the characteristic eight-line spectrum of $TH^{\bullet}$ on annealing. It is possible that there may be special reasons why protonation of $C^{\bullet -}$ might be prevented in duplex DNA but given the ease of this process for C and the behaviour of $T^{\bullet -}$ outlined above this seems unlikely.

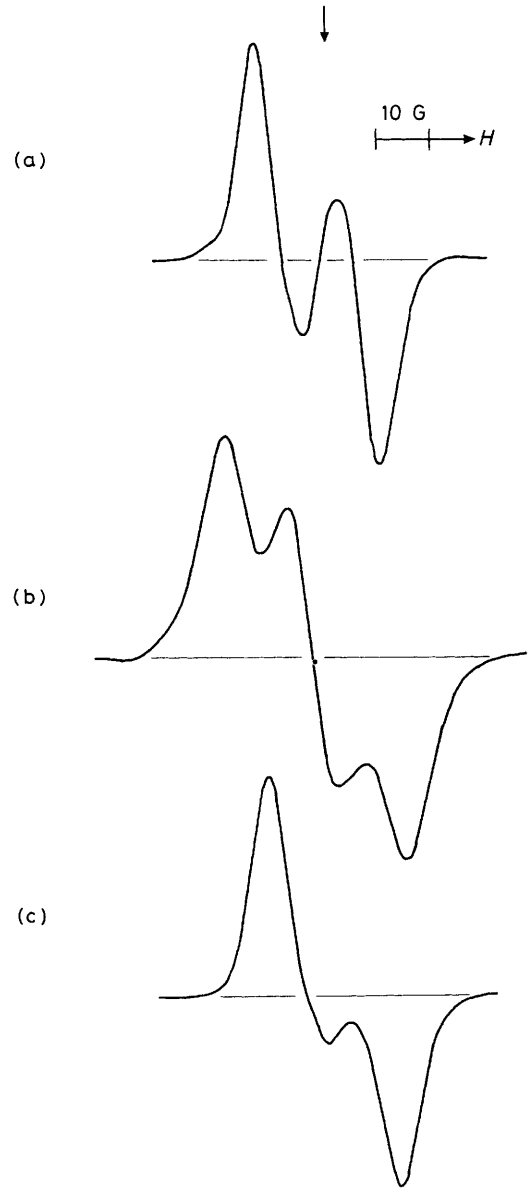


Figure 1. First derivative *X*-band e.s.r. spectra for (a) a solution of thymidine in H_2O/LiCl glass after exposure to ^{60}Co γ-rays at 77 K, showing the doublet assigned to $T^{\bullet -}$; (b) a solution of cytidine in H_2O/LiCl glass showing the triplet assigned to $CH^{\bullet}$, and (c) as in (b) using D_2O, showing the doublet previously assigned to $C^{\bullet -}$.

Finally, we call attention to a much cited study in which equal concentrations of T and C in water were frozen to 77 K, irradiated, and annealed so that any $T^{\bullet -}$ formed would be converted to $TH^{\bullet}$.[11] We have repeated these studies and find that $T^{\bullet -}$ and $CH^{\bullet}$ are formed in about equal yields at 77 K, the former being fully converted into $TH^{\bullet}$ on annealing. In the light of our present observations it is clear that this does *not* mean that C and T have equal electron affinities. Given that the initial electron capture is not selective, as expected, then it is subsequent electron-transfer such as in equation (1) that would establish which centre had the higher electron affinity. Provided that conversion into $CH^{\bullet -}$ is fast, then reaction (1) will be blocked, even if the equilibrium of this reaction lies far to the right.

$$C^{\bullet -} + T \rightarrow C + T^{\bullet -} \quad (1)$$

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