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FREE RADICALS FORMED BY ELECTRON GAIN IN OLIGOMERS OF DNA

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A direct effect of ionizing radiation is to produce geminate ion pairs in DNA. At temperatures of 77 K and below, there is evidence that the radical anion is selectively trapped at T, giving $T^{\cdot -}$, and the radical cation at G, giving $G^{\cdot +}$.[1,2] If this is the case, then the hole must be mobile, trapping out at G, and the electron must either hop between bases trapping out at T or react selectively with T. It has been proposed that geminate pairs, consisting of $T^{\cdot -}$ and $G^{\cdot +}$ and separated by 10–30 Å, set the stage for double strand breaks.[3] Of the various aspects of the $T^{\cdot -}/G^{\cdot +}$ model, one aspect is well substantiated; electrons are trapped preferentially by the pyrimidines. We are trying to determine whether $[T^{\cdot -}] > > [C^{\cdot}]$, $[T^{\cdot}] \cong [C^{\cdot}]$, or $[T^{\cdot}] < [C^{\cdot -}]$ in irradiated duplex DNA at 78 K and below. Also, we are trying to learn more about what factors govern electron transfer in DNA, and thereby the final distribution of electron adducts.

The approach is to study one-electron reduced bases in oligomers using ESR at Q- and X-band microwave frequencies. The trapping matrix is 12 M LiCl with oligomer concentrations of ~ 0.3 mM. An 8 μl aliquot, contained in a thin wall quartz tube, is x-irradiated and observed at 4 K. Most of the data is taken at Q-band for reasons of sensitivity and improved information content. The ESR spectra are being analyzed using a non-linear least squares computer program recently developed for this purpose. The spectra can be accurately simulated but a unique set of simulation parameters cannot be determined.

One-electron reduced thymine has been studied in a variety of thymine derivatives and matrices. Although a unique set of hyperfine and g-tensors could not be determined, it has been possible to establish boundaries for these interactions and propose a parameter set that is likely and reasonable for $T^{\cdot -}$ trapped in a DNA duplex. Also, variations in the interaction parameters were measured as a function of oligomer chain length. The changes are not large enough to account for the differences between hfc values measured from DNA fiber data[1,2] versus hfc values mesured from monomer systems. The analysis also indicates that $T^{\cdot}$ does not protonate at 04 between 4 K and 77 K at neutral pH.

The analysis of the other three one-electron reduced bases ($C^{\cdot -}$, $A^{\cdot -}$, $G^{\cdot}$) is in progress. The ESR spectrum of $C^{\cdot}$ changes from a broad doublet for CdR in $LiCl/H_2O$ to a sharper doublet for d(pCpGpCpG) in $LiCl/H_2O$. The spectrum of the latter was used to obtain a bench mark simulation of $C^{\cdot}$. Using an analogous approach, the bench mark spectra of $A^{\cdot}$ and $G^{\cdot -}$ were obtained. The spectrum of $A^{\cdot -}$ is a broad singlet, with a slight indication of hyperfine structure, and the spectrum of $G^{\cdot -}$ is a narrow singlet. The spectra of $C^{\cdot}$ and $T^{\cdot -}$, trapped in oligomers, are very similar.

The four bench mark spectra, of $T^{\cdot -}$, $C^{\cdot -}$, $A^{\cdot}$ and $G^{\cdot -}$, are used as a basis set for simulating spectra believed to be a composite of one or more of these radicals. Using this basis set, the spectrum of one-electron reduced d(pApGpCpT) in $LiCl/H_2O$ was simulated. This self associating oligomer, which we expect forms duplex DNA, is simulated by the weighted sum of $0.18[T^{\cdot -}] + 0.78[C^{\cdot -}] + 0.04[A^{\cdot -}] + 0.00[G^{\cdot -}]$. These results indicate that, in the LiCl glasses at temperatures below 78 K, electron attachment decreases in the following sequence: $C^{\cdot -} > T^{\cdot -} > A^{\cdot -} > G^{\cdot -}$. Surprisingly, cytosine is a significantly better trap than thymine.

In conclusion, it seems quite probable that in DNA the cytosine base is the preferred site of electron attachment. This opens the possibility that thermally assisted electron transfer between stacked bases may be very limited or even nonexistent at temperatures below 78 K.

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