COMMENTS

Comment on Electron Transfer vs Differential Decay in Irradiated DNA

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A recent report by Pal and Hüttermann[1] proposes that radical fading explains the results we find in our series of papers on excess electron transfer in DNA.[2−8] In this comment, we point out that the substantial signal loss with time (fading) observed by Pal and Hüttermann did not occur in our work and therefore cannot explain our results.[2−8] We show in Figure 1 the total double integrated total radical intensity vs time series for two samples reported in our first publication.[2] These samples contain DNA intercalated with mitoxantrone (MX) at ca. 1 MX per 23, and 46 base pairs (bp) (equivalent to 1/46 and1/92 nucleotides) in 7 M LiBr. These samples were irradiated for 20 min, and the first spectrum was taken ca. 10 min after irradiation. Pal and Hüttermann reported their spectra were taken 30 min after the completion of irradiation.[1] Figure 1 shows no substantial loss in total radical signal intensity even out to long times as we reported.[2] We note that Pal and Hüttermann report a 50% to 60% drop in total radical intensity over this time period. Further, they report the most substantial loss in signal intensity during the first ca. 1500 min. The insert in Figure 1 for the 1/23 loading shows that during the first 1500 min our signal intensity is constant. In our work, an absolute increase in MX radical intensity with the loss of DNA anion radicals is observed, which is strong evidence for electron transfer from DNA to MX. In the supplement, we show scanned images of chart recordings showing actual ESR scans of the 1/23 sample during the first 1500 min. These spectra show no decrease in signal intensity during the same time period that Pal and Hüttermann report a dramatic change in signal intensity. They further show an increase in the signal of one electron reduced MX (from 57.1 to 62.6%) with the loss of DNA anion radical (from 42.9 to 37.4%). Note that the peak height of the central peak from MX electron adduct increases while the outer peaks from the DNA anion radical decrease.

Given these results, it is clear that γ-irradiated DNA samples in our work behave profoundly differently from the X-irradiated samples used in the work of Pal and Hüttermann. Several differences in the procedures used by Pal and Hüttermann may explain the different results found in their work. There are a number of factors that must be met to properly duplicate our work that include low dose, appropriate concentration of scavengers, uniform dose distribution, and the absence of light.

1. Low doses of γ-irradiation (ca. 700 Gy) should be employed to keep the hole concentration low. The holes produced by ionization result in the $Br_2^{\bullet-}$ radical which has a broad ESR signal which does not significantly interfere in the region of interest.[9]

2. Sufficient scavenger concentration to compete with the matrix hole for excess electrons must be present. We note that

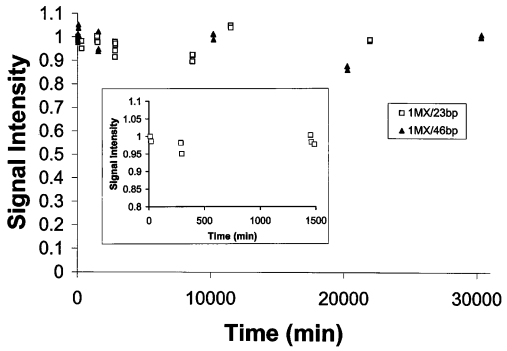

Figure 1. Total radical signal intensity with time in two samples of DNA intercalated with mitroxantrone (MX) in 7 M LiCl at 77 K at two ratios of MX to DNA base pairs, 1/23 and 1/46. These samples contained 20 and 10 mg/mL DNA respectively.

our analyses precisely measure the relative amounts of MX and DNA electron adducts. The absolute intensities have greater variation and were not used although we found no substantial decrease for loadings from 1MX/20bp to 1MX/50bp (see Figure 1). At loadings of 1MX/100 base pairs and above we do see some loss in signal (ca. 10%) with time as reported earlier.[2] By varying the loading of scavenger, we gauge the effect of this competitive scavenging by the $Br_2^{\bullet-}$. For variations in the loading from 1MX /200 bp to 1MX /20bp, we found only a small change in our values of β which are slightly (ca. 0.1−0.2) lower at 1/200 than at 1/50,[1,5] and we find no change in the electron-transfer distance reported in bps at 1 min, which are consistent at loadings from 1/200 to 1/20.[1,5] The β values from higher loadings are considered the most reliable and yield consistent values of β for loadings of 1/20 to1/50.

We also note that in our work in solid-state hydrated DNA lower loadings of intercalator need to be employed because the dominant pathway is interduplex transfer.[5−7] In effect, the transfer is in 3 dimensions and provides multiple pathways from the electron to intercalator.[5,7]

3. A third condition is that the dose distribution must be uniform. Co-60 γ-irradiation, by its nature, provides an equal probability of ionization throughout the 4 mm samples used in our work. While the first two conditions we believe were met by Pal and Hüttermann, this third condition may not have been met by the use of 95 kV X-rays; these sources have a significant soft X-ray component that if unfiltered would give a nonhomogeneous dose distribution. Such a distribution would produce high concentrations of radicals near the surface and fading by recombination would be likely.

4. All of our work, including the taking of the ESR spectra was done with samples kept in the dark. As we reported in our initial work,[2] this is necessary because the DNA anion radicals in glassy systems absorb visible light and photoeject the electron. Samples exposed to light will undergo fading of the signal. Although Pal and Hüttermann state their samples where stored in the dark, they do not mention whether they shielded their samples during measurement.

Another difference between our two works is that our samples were formed in 4 mm quartz tubes whereas Pal and Hüttermann

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used small glass beads prepared by dropping the solution from a thin head Pasteur pipet into liquid nitrogen. These beads have a much higher surface area which might increase the effect of both soft X-rays and light.

Finally, we note that to test electron transfer within DNA the scavenger must be intercalated within DNA. This is checked by the shift in the visible absorption of the scavenger on intercalation.[2,4] No such check was reported by Pal and Hüttermann.

We are confident that researchers will find the results we report[2−8] if the above procedures are followed. We welcome the interest in our work and encourage others interested in excess electron transfer in DNA to apply these techniques.

Supporting Information Available: Scanned images of chart recordings showing actual ESR scans of the 1/23 sample during the first 1500 min. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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