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Kr-86 Ion-Beam Irradiation of Hydrated DNA: Free Radical and Unaltered Base Yields

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Abstract

This work reports an ESR and product analysis investigation of Kr-86 ion-beam irradiation of hydrated DNA at 77 K. The irradiation results in the formation and trapping of both base radicals and sugar phosphate radicals (DNA backbone radicals). The absolute yields (G , $\mu\text{mol/J}$) of the base radicals are smaller than the yields found in similarly prepared γ -irradiated DNA samples, and the relative yields of backbone radicals relative to base radicals are much higher than that found in γ -irradiated samples. From these results, we have elaborated our radiation chemical model of the track structure for ion-beam irradiated DNA as it applies to krypton ion-beams. The base radicals, which are trapped as ion radicals or reversibly protonated or deprotonated ion radicals, are formed almost entirely in the track penumbra, a region in which radiation chemical effects are similar to those found in γ -irradiated samples. By comparing the yields of base radicals in ion-beam samples to the yields of the same radicals in γ -irradiated samples, the partition of energy between the low-LET region (penumbra) and the core is experimentally determined. The neutral sugar and other backbone radicals, which are not as susceptible to recombination as are ion radicals, are formed largely in the track core. The backbone radicals show a linear dose response up to very high doses. Unaltered base release yields in Kr-86 irradiated hydrated DNA are equal to sugar radical yields within experimental error limits, consistent with radiation-chemical processes in which all base release originates with sugar radicals. Two phosphorus-centered radicals from fragmentation of the DNA backbone are found in low yields.

INTRODUCTION

Cucinotta and coworkers recently pointed out that a detailed understanding of the biological effects of heavy nuclei radiation is needed for space radiation protection as well as for cancer therapy (1). The initial biophysical events that ion radiation causes in effected biomolecules, such as DNA, are thus “key” to understanding the risk from heavy ions in space (2) or their potential for treatment of cancer. The fact that heavy ions have very different radiation effects from low-LET radiations such as γ and X rays has been well documented and these differences are currently a focus of much research. For example, with a therapeutically relevant 2 Gy dose, activation of DNA damage signaling pathways does not occur in adenocarcinoma A549 cells using γ irradiation, but has been found to occur with an oxygen ion beam (3). It is known that heavy-ion radiation tracks result in clusters of radiation damage in DNA in which the damage is often far more complex than caused by lower LET radiation (4) and that these damage clusters are more resistant to repair than the more isolated damage that results from lower LET radiation (5). For example, there is enhanced formation of increased chromosomal aberrations in carbon and lithium ion-beam irradiated CHO cells (6). With respect to cancer therapy, in a recent review, Noda and

Kamada point out the great success of carbon ion-beam therapy for treating tumors in Japan and Germany and remark on the importance of basic research in the future of these therapies (7).

In response, there has been a steadily increasing interest in understanding the radiation effects of high-energy ions on DNA. It is pertinent that to understand the biological effect of heavy-ion radiation, the manner in which the track structure of the ion affects the irradiated genes must be considered (4).

The overall physical track structure that results from heavy-ion irradiation is, in broad stroke, well understood, both from early experiments (8, 9) as well as computer modeling that includes the physics of energy deposition (10, 11), presumed damage to DNA (11–14) and broader biological effects (15). All physical models share the concept that a heavy-ion track consists of a relatively small cylindrical-tapered region with a high density of energy deposition (the core), caused by the ion itself interacting with the medium with small impact parameters, and a much larger region in which energy deposition is caused by electrons that are ejected from the core (δ rays). This latter region experiences radiation effects similar to those caused by low-LET radiation.

However, with regard to the direct-type effects for DNA, the chemical effects of track structure are only beginning to be understood. The chemical processes that occur after heavy-ion radiation depends not only on the amount of energy that is deposited in the DNA (the dose), but also on the spatial details of the energy deposition. To fully understand and model heavy-ion effects, the radiation chemistry track structure (as opposed to the physical track structure), which describes the very different chemical processes that occur in different regions of the ion track subsequent to ion radiation, must also be understood.

From previous work using oxygen and argon ion beams, we have developed a model for the chemical track structure based on the populations of trapped radicals observed at 77 K after irradiation (16, 17). In this model, in the high-energy density core, ion radical recombinations are rapid, so few ion radicals survive at 77 K. The relatively high numbers of neutral sugar radicals found in ion-beam-irradiated samples are thought to originate largely from core processes (13, 14). With Argon-40 ions of energy 100 MeV/u, the core radius is about 6.8 nm (18). In the low-LET region, called the penumbra by Chatterjee (11), the radiation chemistry is similar to that found from γ irradiation. Most of the base radicals that are trapped at 77 K in ion-irradiated samples are formed in this region.

In this work, the radiation chemistry in Kr ion tracks, as it pertains to free radical formation from direct-type effects, and the likely subsequent formation of DNA strand breaks, is explored. The energy partition between the core and the penumbra is determined, and a new as yet unidentified phosphorus-centered core radical is found. Studies of prompt base release from Kr ion beam irradiated samples show that the unaltered base release observed when samples are warmed to 280 K are equal to the trapped sugar radicals observed at 77 K.

MATERIALS AND METHODS

Origin of Compounds

Salmon testes DNA (Type III, sodium salt, 57.3% AT and 42.7% GC) were purchased from Sigma Chemical Company (St. Louis, MO). For HPLC standards, all bases were purchased from Sigma. All of the compounds were used without further purification.

Sample Preparation

Hydrated ($\Gamma = 12 \pm 2$ D₂O/nucleotide) salmon testes DNA samples (19) are pressed into a rectangular parallelepiped shape (4 mm $\times$ 10 mm $\times$ ca. 1.2 mm) under an N₂ atmosphere and immediately frozen in liquid N₂. Samples are handled in a glove bag with D₂O vapor present so that the hydration level does not significantly change during processing. Seven samples are lined up in a sample packet for irradiation (Scheme 1). The dose and LET of each small sample is dependent on its position in the sample packet. Because of variability in sample depth, the LET for each sample at each position in the packet will change from sample packet to sample packet. For example, for position 4 (Scheme 1), the calculated LETs varied from 1813 keV/ μ m to 2183 keV/ μ m in the five sample packets used. The average LETs ($\pm$ one standard deviation) for (coincidentally) five samples at each LET, calculated at each position using the SRIM code and sample depth, are: position 1, 1080 ± 5 keV/ μ m; position 2, 1175 ± 20 keV/ μ m; position 3, 1460 ± 35 keV/ μ m; position 4, 2000 ± 150 keV/ μ m; position 5, 4000 ± 400 keV/ μ m; and positions 6 and 7, unirradiated.

The accuracy of the calculated LET depends on the accuracy of the measurement of the depth of each small sample, which may have an irregular depth, as well as the accuracy of the SRIM program. Because of these uncertainties, we estimate absolute LETs to have an uncertainty of approximately $\pm 20\%$.

Heavy Ion and γ Irradiation

Kr irradiation samples were irradiated at 77 K at the National Superconducting Cyclotron Laboratory at Michigan State University. A Kr-86 beam with a specific energy of ~ 135 MeV/u was used. The color development (gray in Scheme 1) and radical formation in the samples were reasonably consistent with the range (6.13 mm) given by the SRIM program (20). The dose rate for ion-beam irradiated samples was typically between 40–210 kGy/min. The γ irradiation was carried out with a J. L. Shepherd & Associates Model 109-GR9 Co-60 irradiator. The γ -irradiation dose rate was approximately 600 Gy/h.

Ion-Beam Dosimetry

Dosimetry is approximate. The dose is calculated using cyclotron parameters, SRIM program energy deposition values, beam current, beam area, sample area irradiated and the duration of irradiation (17; see supplementary material: <http://dx.doi.org/10.1667/RR3066.1.S1>). Samples are positioned so that the ion-beam path is perpendicular to the long axis of a packet of seven samples stacked to give a approximately 8.4 mm long packet. Dose and LET vary from sample to sample and are calculated for each small sample in a packet. We estimate uncertainties in dose to be $\pm 15\%$.

Electron Spin Resonance

Spectra are generally recorded at 77 K at 41 dB (16 μ W) or at 24 dB (0.80 mW) for phosphorus-centered radicals. Fremy's salt, with $A_N = 1.309$ mT and $g = 2.0056$, is used for field calibration and hyperfine splitting measurements. Spectral analysis is carried out using programs developed in our laboratory (ESRADSUB, ESRPLAY).

Computer Analysis of DNA Spectra

The low temperature ESR spectra of irradiated samples results from the overlap of the spectra from a number of free radicals. Benchmark ESR spectra (Fig. 1; see supplementary material: <http://dx.doi.org/10.1667/RR3066.1.S1>) are used for deconvolution of these composite spectra. The four benchmark spectra represent: (1) the reversibly deprotonated guanine cation-radical, $G(-H)^{\bullet}$, (2) the reversibly protonated cytosine anion radical, $C(N3)H^{\bullet}$, (3) the thymine anion radical, $T^{\bullet-}$, and (4) a mixture of sugar radicals, $dR^{\bullet}$. This

last benchmark, $dR^\bullet$, is a composite of the spectra of a variety of sugar radicals. We have tentatively identified line components from $C1'^\bullet$, $C3'^\bullet$, $C5'^\bullet$ and $C3'^\bullet_{\text{dephos}}$ in this spectrum, but have not yet been able to develop a satisfactory quantitative model that accounts for all of the underlying spectra. Scheme 2 gives structures for these radicals. The goodness of fit of the reconstructed spectrum to the experimental spectrum is indicated by the sum of the squared residuals, R^2 . In all cases, the goodness of fit was also evaluated visually to assure the accuracy of deconvolutions.

Because of the presence of a large amount of the complex ESR spectrum of $dR^\bullet$, it is our judgment that the apportionment of concentrations of $C(N3)H^\bullet$ vs. $T^{\bullet-}$ in our deconvolutions is only approximate. The percentage of each pyrimidine radical shifts by a few percent depending on the specific benchmark spectrum used. However, the percentage of radicals represented by the sum of $C(N3)H^\bullet + T^{\bullet-}$ was very stable in all of our deconvolutions and, at times, we use this sum as a measure of radical concentrations rather than the percentages of the individual pyrimidine radicals.

Calculation of G and k Values

Using the deconvolution results, it is possible to obtain doseresponse relationships for each of the base radicals and for the $dR^\bullet$ benchmark. The yields are fit to relationship (1), yielding G and k . In relationship (1) D is the dose in Gray,

$$\text{Yield} = \left(\frac{G}{k}\right)(1 - e^{-kD}) \quad (1)$$

G is the traditional G value in $\mu\text{mol/J}$, and k (Gy^{-1}) is a constant that expresses the sensitivity of preexisting trapped radicals to destruction by additional radiation: a larger k implies a higher sensitivity to such destruction. Specifically, k is 1 divided by the dose required to destroy $1/e$ of previously trapped radicals. In the range of doses used in this work, the yield of the cohort of free radicals represented by $dR^\bullet$ is found to be nearly linear to the maximum dose used and the yield is fit to the simple linear relationship: $\text{yield} = G \times D$. G values are calculated using the hydrated mass of DNA. We estimate that the relative error in ESR determined radical G values is approximately $\pm 15\%$ and the absolute error is approximately $\pm 25\%$.

Preparation for Base Release Analysis

All solutions were prepared using Baker HPLC water. After the sample irradiation process, the ESR spectra of these samples were recorded at 77 K. Samples were then stored for approximately 2 years in the dark in liquid N_2 until HPLC investigations could be performed. After storage, the ESR spectrum of the sugar radicals was identical to that taken immediately after irradiation. Each sample was weighed and dissolved in N_2 saturated 10 mM triethylammonium acetate (TEAA) buffer with pH 7 in the ratio of 2 mL buffer per 20 mg sample. Following the earlier protocol of Swarts *et al.* (19), samples were then stored in a refrigerator for 3 days to allow the DNA to completely dissolve. In doing so, a viscous solution was formed. Absolute (99.99%) ethanol (10 mL per 20 mg sample) was then added to precipitate the DNA and the mixture was stored in a refrigerator for 2 days. The vial holding the mixture was gently mixed a few times during this period. A 1.5 mL aliquot of the resulting liquid was placed in an Eppendorf vial and was subsequently centrifuged in a Baxter Scientific Product Biofuge A centrifuge at 13,000 rpm for 70 min. One milliliter of the supernatant was then transferred into a round bottom flask and was flash-dried on a Buchi rotary evaporator at 37°C leaving a film on the bottom of the flask. One milliliter of the TEAA buffer was then added to the flask and the flask was vortexed for 2 min to thoroughly dissolve the bases. The resulting solution was used for analysis.

Because samples were kept refrigerated during the workup and analysis, only prompt base release was determined. However, Swarts *et al.* showed that heat labile base release is a minor effect in γ -irradiated hydrated DNA, increasing the total yield of base release by approximately 18%, even after heating for 24 h at 37°C (19). Interestingly, no increase in purine base release was found with the pyrimidines accounting for all of the change. We also note that in several studies on γ - and ion-beams (He^{2+} , C^{5+6+} , Ne^{8+10+}) irradiated (at 5.6°C) hydrated pUC18 DNA, Yokoya *et al.* report that no increase in single-strand breaks are found after postirradiation heat treatment to 37°C for 30 min over that found at 5.6°C (21–23). Both of these results suggest that there would be only a small increase in base release from heat labile base release in our Kr-irradiated samples.

We note that in previous work, hydrated DNA samples that were γ irradiated at room temperature under an N_2 atmosphere and in 100% O_2 show no difference in base release yields (19). We therefore expect that Kr ion-beam-irradiated samples would behave similarly.

Analysis of the Released Bases

Released unaltered base amounts were determined using reverse phase HPLC with a Perkin Elmer Binary Pump LC 250 with a six-port valve and an LC 235 diode array detector. A 250 mm $\times$ 4.6 mm i.d. Adsorbosphere C18 5 μ column from Grace Davison Discovery Sciences was used. In the primary method used, the aqueous mobile phase is 10 mM TEAA and the organic mobile phase is 100% acetonitrile. The primary elution gradient was 3% acetonitrile/97% TEAA for 10 min followed by a column washing using 30% acetonitrile/70% TEAA mixture. The flow rate was 1 mL/min. Eluted bases were monitored at 260 nm and were quantified by comparison with known standard solutions that were prepared with authentic compounds. This resulted in excellent separation of the four bases in approximately 11 min of elution. A secondary gradient described in the supplementary information (<http://dx.doi.org/10.1667/RR3066.1.S1>) was occasionally used to confirm guanine and cytosine. Data were analyzed using the Perkin Elmer TotalChrom software. We estimate that the relative error in base release G values is approximately $\pm 5\%$ and the absolute error is $\pm 25\%$.

RESULTS

In Fig. 1 we show experimental Electron Spin Resonance (ESR) spectra obtained for DNA that was hydrated to $\Gamma = 12 \pm 2$ D_2O /nucleotide at 77 K. These spectra are similar to those obtained from other heavy-ion-irradiated hydrated DNA samples (16, 17). It is notable that small line components in the wings of the spectra become more visible with higher dose and that these originate with neutral sugar radicals (Scheme 2). The higher concentration of sugar radicals also causes slight changes in line shapes at higher doses.

Figure 2 shows the yields of all radicals at each of the LETs used. As expected, there is a monotonic increase in radical yield with dose. The yields do not show a plateau because of the large relative number of neutral sugar radicals that are formed with increasing dose. These radicals are resistant to destruction by additional radiation once they are trapped at 77 K, and thereby show a near linear dose response to the highest doses used. This behavior prevents the total yield curve from reaching a plateau in the dose range used.

Four benchmark spectra (see Materials and Methods; see supplementary material: <http://dx.doi.org/10.1667/RR3066.1.S1>) were used to analyze all of the spectra presented in this work, with the exception of the phosphorus radical spectra, which lie in a different magnetic field range with line components up-field and down-field by approximately 300 G.

In Fig. 3, we present a set of typical dose responses for the four different benchmark spectra, in this instance were obtained by the analysis of spectra for samples with LET = 4,000 keV/ μm . From the dose response, the yields, as G values, and destruction constants, as k values can be determined. The pattern seen for the three base radicals is very similar to that observed using γ irradiation, that is, the yield of radicals tends to reach a plateau as the dose increases. This occurs when, after an ionization event, a preexisting trapped radical combines with a new radiation-produced ion radical, likely of opposite charge. Thus, for each new radical formed, a pre-existing radical is destroyed and a plateau in radical yield occurs. Coulomb attractions largely drive this process. As found in our previous studies (16, 17), there is no significant observable tendency at the doses used for the yield of sugar radicals ($\text{dR}^\bullet$) to plateau as do the base radical yields (Fig. 3). Because of the absence of the Coulomb force, neutral sugar radicals are less susceptible than are ion radicals to the “killing” process described earlier, and a linear dose response to higher doses is observed for them.

Table 1 presents the G values obtained for each of the radicals at the five LETs used. γ irradiation results are included for comparison.

The radiation chemical model that we have developed for ion-beam-irradiated DNA (17) explicates the trends observed in Table 1. The greatly decreased yield of base radicals in Kr-86 irradiated samples relative to γ irradiation is explained by the details of the track structure of the Kr-86 ion-beam: a partition of energy between the core and the penumbra of the track occurs. Because of high ion recombination in the core, virtually all of the base ion radicals (momentarily) formed recombine, leaving few trapped base radicals from the core. Thus, the base radicals trapped at 77 K are nearly exclusively formed in the more sparsely ionized penumbra, which receives only a fraction of the total energy of the ion. Because a smaller fraction of the total energy of the ion beam relative to γ irradiation is effective in producing base radicals, a lower yield of base radicals relative to γ -irradiated samples, results. Conversely, the neutral sugar radicals formed do not as readily recombine as do the charged base radicals, and they survive to be trapped at 77 K. In fact, in ion-beam-irradiated DNA, these neutral sugar radicals, $\text{dR}^\bullet = (\text{C1}'^\bullet + \text{C3}'^\bullet + \text{C5}'^\bullet + \text{C3}'^\bullet_{\text{dephos}})$, are formed mostly in the track core, with approximately 25% formed in the low-LET penumbra. The formation of radicals in the penumbra is similar to the radical formation found in γ -irradiated samples (24, 25), that is, similar to γ irradiation, the ion-beam penumbra is a region of low-LET energy deposition.

We also note that the higher dose rate attained in the ion-beam penumbra relative to that attained with γ irradiation may be responsible for some of the decrease in base release found. This possibility will be investigated in future studies.

Table 2 presents the k values for each of the radicals observed using Kr-86 ion-beam irradiation. In general, the k values are smaller for Kr-86 irradiation than for γ irradiation and they generally get smaller as LET increases. This is expected from the model we develop in the discussion.

Table 3 presents the theoretical yield of base radicals at infinite dose, Y_∞ , for each of the LETs used as well as for γ -irradiated samples: using Eq. (1), $Y_\infty = G/k$. This is not necessarily the actual plateau yield that might be observed at high doses because of changes in the irradiated medium that might occur as the dose gets very high, and also due to secondary and tertiary processes in radical formation.

The average value of Y_∞ for Kr-86 ion-beam irradiated samples is the same as that found for γ -irradiated samples, suggesting that the maximum base (“ion radical”) yield is a

property of the DNA sample and not of the ionization source, and that similar processes occur in the γ -like penumbra as in γ -irradiated samples.

Consideration of the relative concentrations of the base radicals found in Kr-86 irradiated samples relative to that found in γ -irradiated samples also lends support to our radiation chemical track structure model, which posits that the base radicals found in Kr-86 irradiated samples are formed mostly in a γ -like track penumbra. Table 4 shows the percentage of base radicals in the pertinent samples, using the sum of the G values of the bases only as the basis for the calculation, i.e., the sugar radicals are not included.

As indicated earlier, we consider the sum of the G values of $C(N3)H^{\bullet} + T^{\bullet-}$ to be more reliable than the individual yields of these two radicals, so this sum is used in Table 4. As can be seen, the distribution of radicals in Kr-86 irradiated samples is reasonably close to that found in γ -irradiated samples, again supporting the supposition that the base radicals are formed in a γ -like region of the track.

Phosphorus-Centered Radicals

The ESR spectrum that results from a 1000 G scan of a hydrated ($\Gamma = 12 \pm 2$ D₂O/nucleotide) DNA sample is shown in Fig. 4A (solid line). This spectrum was taken at 24 dB (0.80 mW) and at a modulation amplitude of 8 G, and it is greatly increased in intensity relative to those shown in Fig. 3, as can be seen from the large off-scale central portion of the spectrum that results from line components such as those shown in Fig. 1. The two line components on the far outside wings of the spectrum are characteristic of a radical with a large spin density on phosphorus and, in fact, have been assigned to the immediate strand break phosphorus-centered radical $ROPO_2^{\bullet-}$ (Scheme 2) in oxygen ion-beam and argon ion-beam-irradiated hydrated DNA (16, 17). This radical likely forms through dissociative electron attachment from low-energy electrons (LEEs) (see Discussion). Figure 4B shows a simulated spectrum for this radical using the parameters given in Table 5.

Components from a second phosphorus-centered radical were also present in Kr-86-irradiated hydrated DNA samples, as evidenced by the two inner line components (asterisks) with smaller separation than the line components from $ROPO_2^{\bullet-}$ (Fig. 4). A simulation of the spectrum from this as yet unidentified radical using the parameters in Table 5 resulted in the spectrum shown in Fig. 4C. In the simulation, the unknown radical has approximately 20% the intensity of $ROPO_2^{\bullet-}$. Summing the spectra given in Fig. 4B and C gives the spectrum shown in Fig. 4A (dotted line). The sum is an excellent match to the experimental spectrum shown in Fig. 4A (solid line).

For most of the spectra recorded, it was possible to determine the integrated area of $ROPO_2^{\bullet-}$ and thereby calculate the absolute concentration of $ROPO_2^{\bullet-}$. For four samples in which baseline drift and/or noise made it impossible to determine the integrated area (one sample at LET = 1175 keV/ μ m and all four samples at LET = 4000 keV/ μ m), the height of the spectrum and a well-determined area-to-height ratio (from the 11 other samples) was used to estimate an integrated area. The resulting dose-response curves are shown in Fig. 5.

The G values for formation of $ROPO_2^{\bullet-}$ obtained as the slopes of the lines shown in Fig. 5 are quite small, ranging from a maximum of 1.8×10^{-4} μ mol/J to 4.5×10^{-5} μ mol/J (Fig. 6). In these samples, it is a minor radical.

Base Release in Kr-86 Irradiated Samples by HPLC

The study of prompt base release in Kr-86 irradiated samples is motivated by the desire to explore the relationship between sugar radical formation and base release using the base release as a surrogate for strand breaks. High-LET irradiated samples have relatively large

yields of sugar radicals (*vide supra*) and it is a relatively straightforward matter to determine the G values for these radicals without undue interference from the accompanying yields of base radicals. Base release studies were carried out on the same samples that were investigated using ESR spectroscopy, and therefore the yields of sugar radicals (Table 1) and base release could be directly compared (26).

Figure 7 shows a typical HPLC chromatogram for Kr-86 irradiated hydrated ($\Gamma = 12 \text{ H}_2\text{O}/\text{nucleotide}$) DNA. In Fig. 8, the dose response for base release for each of the four bases for samples with $\text{LET} = 1080 \text{ keV}/\mu\text{m}$ is shown. To prepare these graphs, raw base release versus dose graphs were prepared and a linear fit was plotted. The y-intercept (base release at zero dose) obtained was assumed to be an artifact introduced by the sample work-ups and/or contaminants in the original DNA, and was subtracted from the each of the yields of irradiated samples to obtain corrected yields. These artifactual yields at zero dose were typically in the range $50\text{--}100 \mu\text{mol/kg}$ for the individual bases. The yields for base release in Fig. 8 were linear to the highest dose used.

Table 6 gives the G values for base release for each of the bases at the five LETs used, and that for γ irradiation for comparison (26). The G values for base release with γ irradiation are in excellent agreement with those obtained earlier in Swarts *et al.* (19). With regard to krypton irradiated samples, because the relative internal error in doses can be as high as approximately 25%, the results show no statistically valid trend in base release as LET increases from $1080\text{--}2000 \text{ keV}/\mu\text{m}$ for any of the bases. The total yield of bases is remarkably constant for these LETs. Although an accurate dose is more difficult to determine for the samples at $\text{LET} = 4000 \text{ keV}/\mu\text{m}$, the last sample in a sample packet to be irradiated, than it is for the four other samples in the packet, it is quite clear that base release is lower at $4000 \text{ keV}/\mu\text{m}$ than in the range of $1080\text{--}2000 \text{ keV}/\mu\text{m}$. This is ascribed to the track structure of the ion and the partition of energy between the core and penumbra of the track (see Discussion).

From the yields given in Table 6, the A/T ratio of base release for all five LETs is 0.97 ± 0.06 , and that for the G/C ratio is 1.13 ± 0.08 . These ratios are both close to 1.0 as they should be if base release were completely random. In addition, the average percentage base release of GC is $46.4 \pm 1.3\%$, this is close to the actual 42.7% GC found in salmon sperm DNA (see supplementary data: <http://dx.doi.org/10.1667/RR3066.1.S1>). These observations lead to the conclusion that, in Kr-86-irradiated hydrated ($\Gamma = 12 \pm 2 \text{ H}_2\text{O}/\text{nucleotide}$) DNA, base release is random. This is expected if the ionization event is followed by rapid deprotonation to form the sugar radical. Even though approximately half of the holes on the sugars do transfer to the bases (19), there is no evidence of preferential hole migration to any specific base.

DISCUSSION

The data presented gives firm support for our radiation chemical track structure model (16, 17), in which trapped neutral sugar radicals are formed mostly in the densely ionized ion-beam track core, and trapped base radicals are formed largely in the sparsely ionized track penumbra. The terminology and concepts used here borrow from that of the Chatterjee group, which developed a physical model for energy deposition (11). Our model is a radiation chemical model, in which the yields of trapped radicals at 77 K are related to the track structure. The model is schematically shown in Scheme 3.

From this model, it is possible to calculate the partitioning of energy between the track core and the track penumbra based on the yield of trapped radicals. To simplify this calculation, we assume that all, rather than most, of the trapped base radicals are formed in the

penumbra. Knowing that the yield of trapped base radicals for γ -irradiated identical samples is $0.19 \mu\text{mol/J}$ (Table 1), the fraction of energy in the penumbra for any given Kr-86-irradiated sample is:

$$\text{Fraction energy in penumbra} = \frac{G_{\text{base}}(^{86}\text{Kr})}{0.19 \frac{\mu\text{mol}}{\text{J}}} \quad (2)$$

Table 7 shows the results of this calculation.

G values as a function of LET for DNA hydrated to $\Gamma = 12 \text{ D}_2\text{O/nucleotide}$ were determined earlier for argon-ion-beam irradiated samples (17), and the percentage energy deposited in the core for argon-ion-beam irradiated samples can be calculated using Eq. (2) (see supplementary data: <http://dx.doi.org/10.1667/RR3066.1.S1>).

Figure 9 shows the percentage energy in the core as a function of $\log(\text{LET})$ for both argon-ion-beam irradiated and krypton-ion-beam irradiated DNA at $\Gamma = 12 \pm 2 \text{ D}_2\text{O/nucleotide}$, with LET expressed in $\text{keV}/\mu\text{m}$. Chatterjee *et al.* suggest that the radial energy deposition for high-energy ions is universal (27), depending primarily on the LET of the ion rather than its identity. Therefore, it can be concluded that the energy partition between the core and penumbra is also a universal function of the LET (28). The results for Ar and Kr ion-beam irradiation, as determined by the experiment, shown in Fig. 9, agrees with this suggestion.

The ratio of the yield of trapped sugar radicals (dR^*) to trapped base radical are higher in high-LET irradiated samples when compared to the same ratio in low-LET irradiated DNA. However, when the yields of sugar radicals in the core are calculated based on the actual energy deposited in the core for LET in the range $1080\text{--}2000 \text{ keV}/\mu\text{m}$, they are very close to the yields found using γ irradiation. Table 8 presents these yields, calculated from their overall yield (Table 1) and the percentage of energy deposited in the core (Table 7), but corrected for the expected yield of dR^* in the penumbra. This is assumed to be 15% of the yield of base radicals, as found for γ -irradiated samples (24). The agreement between these indicates that sugar radical formation processes in the core are much the same as in low-LET spurs. Since the core may be thought of as overlapping spurs, this seems reasonable. However, since the high-energy density core is expected to be the home of multiple processes such as knock ons, multiple ionizations, Coulomb explosions, ion recombinations and associated excited state processes, it is possible that there are unique radical formation and radical destruction processes in the core that coincidentally balance to give the same yield of dR^* as is found in γ -irradiated samples.

The situation in the $4000 \text{ keV}/\mu\text{m}$ samples is unique; here we believe that 93% of the beam energy is deposited in a very small core, and that early radical recombinations are dominant because of the proximity of early ion radicals. This suppresses formation of all radicals, including sugar radicals.

Base Release

Table 9 presents a comparison of the G values for sugar radical formation at each LET and the G value for base release from the same samples. For LET from $1080\text{--}2000 \text{ keV}/\mu\text{m}$, the G for sugar radical formation and base release are, essentially, the same. At $4000 \text{ keV}/\mu\text{m}$, the divergence appears larger than that at lower LETs, but it is within our error limits. The base release yield is also low, as is expected from the far lower yield of sugar radicals.

For the four lower LETs, the agreement between the $G(\text{dR}^\bullet)$ and $G(\text{base release})$ is entirely consistent with a mechanism in which base release originates only with sugar radicals (29, 30).

$\text{dR}^\bullet \rightarrow \text{strand break} \rightarrow \text{base release}$

It is well established that $\text{C3}'^\bullet$, $\text{C4}'^\bullet$ and $\text{C5}'^\bullet$ lead to prompt strand breaks and a concomitant base release. $\text{C1}'^\bullet$ does not lead to a significant yield of prompt single-strand breaks (31), but it does result in unaltered base release. In our ESR analyses of irradiated hydrated DNA, $\text{C1}'^\bullet$ is found to be a minor radical (ca. 10% of sugar radicals). We note here that evidence suggests that direct irradiation of DNA systems also does not form significant amounts of $\text{C4}'^\bullet$ (32,33). Therefore, it is typically assumed that the yield of strand breaks is roughly equal to the yield of base release (34), and that the G values for base release found in this work gives a correlation between the trapped sugar radicals, such as $\text{C3}'^\bullet$, $\text{C5}'^\bullet$ and $\text{C3}'^\bullet_{\text{dephos}}$, and strand breaks.

The agreement between $G(\text{dR}^\bullet)$ and $G(\text{base release})$ in Kr-86 irradiated samples is different from the situation found in γ -irradiated DNA, where $G(\text{base release})$ is significantly larger than the $G(\text{dR}^\bullet)$. For example, for hydrated ($\Gamma = 12 \text{ D}_2\text{O}/\text{nucleotide}$) DNA, $G(\text{dR}^\bullet) = 0.030 \mu\text{mol/J}$ (Table 1) and $G(\text{base release}) = 0.048 \mu\text{mol/J}$ (Table 6). An excess of base release over that of sugar radicals in X-irradiated hydrated pUC18 DNA films has also been reported by Bernhard and coworkers (34, 35). They suggested that the excess base release in γ -irradiated samples originates with a double oxidation through which G^{+} oxidizes the chemically reducing radical $\text{dR}^\bullet$ in a spur. This results in loss of $\text{dR}^\bullet$ before it is trapped at low temperature and eventual base release on warming, causing $G(\text{dR}^\bullet)$ to be lower than $G(\text{base release})$. One would expect substantial early formation of G^{+} proximate to $\text{dR}^\bullet$ in the core of a high-LET ion in which there is a high ionization density. This mechanism is shown to be feasible by recent DFT calculations, which confirm that oxidation of $\text{dR}^\bullet$ by G^{+} is energetically downhill for all sugar radicals except $\text{C2}'^\bullet$ (36). However, if this process were occurring in our Kr-irradiated samples, then we would expect excess base release: i.e., $G(\text{base release}) > G(\text{dR}^\bullet)$. This is not observed for $\text{LET} = 1080 \text{ keV}/\mu\text{m}$ to $\text{LET} = 2000 \text{ keV}/\mu\text{m}$, although it may occur at the very highest LET of $4000 \text{ keV}/\mu\text{m}$.

Formation of sugar radicals by excited base cation radicals may also be expected to contribute to base release in ion-beam irradiated DNA and this mechanism has been established experimentally and theoretically in our laboratory (30, 31). However, events such as double oxidation or sugar radical formation by excited base cation radicals would suggest that ion-beam irradiated DNA should produce more base release than γ -irradiated DNA, but the reverse is actually observed (Table 6). This is an unexpected result and is under further study.

Phosphorus Radicals

The larger line components from a phosphorus-centered radical have previously been assigned to the phosphoryl radical, $\text{ROPO}_2^{\bullet-}$ (16, 17). This radical arises from low-energy electrons (LEE) and can be formed by P–O bond cleavage at either $\text{C5}'$ or $\text{C3}'$. Scheme 4 shows the proposed reaction pathway for bond cleavage at $\text{C3}'$. In pathway B, $\text{ROPO}_2^{\bullet-}$ forms directly as one of the products of a strand break, hence its designation as a “strand-break radical.” The radical $\text{C3}'^\bullet_{\text{dephos}}$ is also found in Kr-86 irradiated hydrated DNA, and arises from pathway A in Scheme 4. It also is an immediate strand-break radical. Estimates of radical concentrations indicate that path A accounts for approximately 97% of the reaction of the transient excited state anion (in brackets), and pathway B approximately 3%.

The unknown phosphorus radical ($P_x^\bullet$) observed is not yet identified. However, possible structures include $(RO)_2PO^\bullet$, which would be produced by loss of O^- from the phosphate moiety, and has been suggested by Sanche and coworkers from LEE-induced damage to DNA via dissociative electron attachment (37).

Summary

The radiation chemistry in Kr-86-irradiated samples is highly dependent on the track structure of the ion. A summary of this model is shown in Scheme 5.

From our finding of the sugar radical yield in the core, many strand breaks are expected in the very small volume of the core, suggesting substantial amounts of clustered damage from high-LET radiation. Computer codes that model DNA damage must certainly account for the different radiation chemistries in different parts of the track if they are to correctly predict the damage that occurs from an ion beam. Knowledge of the spatial location of damage and the specific chemical nature of the damage will certainly become more important to the targeting of ion beams for effective therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Experimental spectra of DNA hydrated to $\Gamma = 12 \pm 2$ D₂O/nucleotide, at LET = 4000 ± 400 keV/ μ m, at three different doses. The samples used were all in position 5 in the sample packets. All ESR spectra were taken at 77 K.

FIG. 2.

Total yield of radicals at each LET used. Panel A: All 5 samples in position 1 (see Materials and Methods). Panel B: All 5 samples in position 2. Panel C: All 5 samples in position 3. Panel D: All 5 samples in position 4. Panel E: All 5 samples in position 5. In all cases the yield (y axis) is expressed in micromoles per kilogram and the dose (x axis) is expressed in grays. The total hydrated sample mass is used for calculating the yields and doses. Note that the maximum dose on the x axis varies. Lines are drawn for ease of visual presentation only.

FIG. 3.

Dose response at 77 K of Kr-86 irradiated DNA, hydrated to $\Gamma = 12 \pm 2$ D₂O/nucleotide, at LET = $4,000 \pm 400$ keV/μm. Symbols used: dR• (◇); G(-H)• (Δ); C(N3)H• (○); T•- (□)

FIG. 4.

ESR spectra of hydrated ($\Gamma = 12 \pm 2$ D₂O/nucleotide) salmon sperm DNA irradiated with a Kr-86 ion beam to a dose of 47.9 Mrad with LET = 2183 keV/ μ m. Panel A: Solid line, represents experimental spectrum; dotted line, represents simulated spectrum from summation of spectra B and C. The intensity of spectrum C is 20% that of spectrum B. Panel B: Simulated spectrum of ROPO₂•⁻ using the parameters given in Table 2. Panel C: Simulated spectrum of unknown phosphorus centered radical using the parameters given in Table 5. The large off-scale central portion of the experimental spectrum arises from the relatively large amounts of base and sugar radicals whose spectra of which are shown in Fig. 1. The centrally located “X” is at approximately $g = 2.00$. All spectra are taken at 77 K.

FIG. 5.

Dose response for ROPO_2^{*-} . The yield of ROPO_2^{*-} as a function of dose is shown at different LETs. The response is nicely linear at the four lowest LETs. At $\text{LET} = 4000 \text{ keV}/\mu\text{m}$, there is a larger degree of uncertainty in the determination of both dose and yield, and the data are more scattered, but it is clear that a significantly lower yield is found at 4,000 $\text{keV}/\mu\text{m}$ than is found for the lower LET spectra.

FIG. 6.

G values for $\text{ROPO}_2^{\bullet-}$ as a function of $\log(\text{LET})$, LET in $\text{keV}/\mu\text{m}$. $\text{ROPO}_2^{\bullet-}$ likely forms in the core as $\pm$ irradiation does not result in significant amounts of this radical. The increase in G as LET increases from 1080 $\text{keV}/\mu\text{m}$ to 2000 $\text{keV}/\mu\text{m}$ suggests more core reactions occur as LET increases. At the highest LET of 4000 $\text{keV}/\mu\text{m}$, the energy density is very high and the concentration of all of the radicals observed (including $\text{ROPO}_2^{\bullet-}$) decreases. The error bars reflect an estimate of the relative uncertainty of the values. The absolute uncertainties are larger, approximately $\pm 30\%$.

FIG. 7.

HPLC chromatogram of DNA hydrated to $\Gamma = 12 \pm 2$ H₂O/nucleotide, Kr-86 ion-beam irradiated at 77 K to a dose = of 233 kGy at LET = 1080 keV/ μ m. Unaltered free bases were detected by absorbance at 260 nm after passing through a reverse-phase column. Each base was quantified against a standard for that base.

FIG. 8.

Dose response for base release for each of the four bases in hydrated DNA for samples irradiated with Kr-86 at LET = 1460 keV/ μ m. In all cases, the dose (x axis) is in units of gray and the yield (y axis) in micromole/kilogram. The dose response is linear to the highest dose used.

FIG. 9.

Percentage energy in core as a function of $\log(\text{LET})$ (LET in $\text{keV}/\mu\text{m}$) for argon-ion-beam radiation (triangles) and Kr-ion-beam radiation (squares). The least squares drawn line has $R^2 = 0.829$.

SCHEME 1.

This scheme shows the DNA sample configuration for one sample packet. There are five such packets in this work. The Kr-86 beam penetrated partially into sample 5, and samples 6 and 7 were unirradiated. Sample 5 contains the Bragg peak.

SCHEME 2.
Structures of radicals discussed.

SCHEME 3.

In the track core, Coulomb forces drive rapid recombination of proximate ion radicals, therefore very few trapped base radicals originate in this region. In the track penumbra, secondary electrons that escape the core form spurs, long tracks and blobs, much as are formed from secondary electrons produced by γ irradiation. It is in the penumbra in which essentially all of the trapped base radicals are found. [Used with permission from ref. (26).]

SCHEME 4.

Formation of C3'•_{dephos} and ROPO₂•⁻ from low-energy electrons. After formation of an excited state transient radical anion, two alternate reaction paths may occur. Path A results in formation of C3'•_{dephos} and a diamagnetic anion, whereas path B results in formation of ROPO₂•⁻ and also a diamagnetic anion. Both paths cleave a bond in the sugar-phosphate backbone and thereby cause an immediate strand break.

SCHEME 5.

Summary of radiation chemistry track structure model.

TABLE 1

G Values for Radicals in Kr-86 Ion-Irradiated-Hydrated DNA ^{a,b,c}

LET (keV/μm)	G(-H) ^e	T ⁺ ₂ ^d	C(N3)H ^d	(Base) ^e	(dR) ^e
γ Irradiation	0.095 ^e	0.045 ^e	0.050 ^e	0.19 ^e	0.030 ^e
1080	0.033	0.0097	0.013	0.056	0.027
1175	0.043	0.0099	0.017	0.070	0.027
1460	0.031	0.0081	0.011	0.051	0.027
2000	0.022	0.0058	0.010	0.038	0.025
4000	0.0069	0.0031	0.0031	0.013	0.0075

^a *G* in μmol/J.

^b $\Gamma = 12 \pm 2$ D₂O/nucleotide.

^c Estimated relative error in *G* values is approximately ±15%, and the absolute error ±25%.

^d These are approximate; see Materials and Methods.

^e See supplementary material (<http://dx.doi.org/10.1667/RR3066.1.S1>).

TABLE 2

k Values for Radicals in Kr-86 Ion-Irradiated-Hydrated DNA^{a,b}

LET ^c	G(-H)•	T•-	C(N3)H•
γ Irradiation	3.2×10^{-5}	6.4×10^{-5}	1.7×10^{-5}
1080	1.3×10^{-5}	9.3×10^{-6}	9.9×10^{-6}
1175	1.9×10^{-5}	8.5×10^{-6}	1.6×10^{-5}
1460	1.2×10^{-5}	5.7×10^{-6}	8.9×10^{-6}
2000	8.8×10^{-6}	4.1×10^{-6}	9.9×10^{-6}
4000	2.3×10^{-6}	2.6×10^{-6}	2.8×10^{-6}

^a *k* in Gy⁻¹.^b Γ = 12 ± 2 D₂O/nucleotide.^c keV/μm.

TABLE 3Calculated High-Dose Yields of Base Radicals in Kr-86 Ion-Irradiated-Hydrated DNA^a

LET (keV/μm)	Y _∞ (μmol/J)
γ Irradiation	5278 ^b
1080	4895
1175	4490
1460	5240
2000	4925
4000	5299
Average (Kr-86)	4970 ± 324

^aThese are not necessarily the actual high-dose yields. See narrative.

^bCalculated using $G = 0.19 \mu\text{mol/J}$ (Table 1) and $k = 3.6 \times 10^{-5} \text{ Gy}^{-1}$ (25).

TABLE 4Calculated Percentages of Base Radicals in Kr-86 Ion-Irradiated-Hydrated and in γ -Irradiated DNA^a

LET (keV/ μ m)	%G(-H) $\cdot$	%(C(N3)H $\cdot$ + T $\cdot^-$)
γ Irradiation ^b	50	50
1080	59	41
1175	61	39
1460	61	39
2000	58	42
4000	53	47
Average (Kr-86)	58	42

^aPercentages are calculated using G values. Relative errors in these values are estimated at $\pm 10\%$.^bFrom reference (24), which gives $G(\text{G}(-\text{H})\cdot) = 0.11 \mu\text{mol/J}$, $G(\text{C}(\text{N3})\text{H}\cdot + G(\text{T}\cdot^-)) = 0.11 \mu\text{mol/J}$ at $\Gamma = 14 \pm 2 \text{ H}_2\text{O/nucleotide}$.

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TABLE 5

Parameters used in Simulations of Phosphorus-Centered Radicals

Radical	$A_{\parallel}^a$	$A_{\perp}^a$	$g_{\parallel}^b$	$g_{\perp}^b$	Linewidth ^{a,c}
ROPO ₂ ^{•-}	793	631	ca. 2.0028	ca. 2.0060	35
Unknown Phosphorus-centered	546 ^d	435	ca. 2.0020	ca. 2.0020	30

^aGauss.

^bThese are very approximate and are presented solely to show relative *g* values suitable for a simulation.

^cLorentzian lineshapes used throughout.

^dThe line components for $A_{\parallel}$ were obscured by the ROPO₂^{•-} spectrum. It was assumed that the ratio of $A_{\perp} / A_{\parallel}$ for the unknown radical is the same as that for ROPO₂^{•-}. This gave a satisfactory simulated ESR spectrum, but other assumptions regarding $A_{\parallel}$ will also do so.

TABLE 6

G Values for Base Release in Kr-86 Irradiated-Hydrated DNA^a

LET (keV/μm)	<i>G</i> Value (μmol/J)			
	C	G	T	Total
γ Irradiation ^b	0.014	0.0076	0.011	0.015
γ Irradiation ^c	0.013	0.0080	0.013	0.014
1080	0.0068	0.0065	0.0070	0.0077
1175	0.0060	0.0055	0.0067	0.0078
1460	0.0058	0.0055	0.0065	0.0067
2000	0.0058	0.0055	0.0059	0.0075
4000	0.0027	0.0026	0.0029	0.0032

^aEstimated relative error in *G* values is *ca.* ±15%, absolute error *ca.* ±25%.

^bThis work.

^cRef. (19).

TABLE 7

Energy Partition in Kr-86 Ion-Irradiated-Hydrated DNA

LET (keV/ μ m)	G(base ^a) μ mol/J	Fraction energy in penumbra ^a	Fraction energy in core ^a
1080	0.056	0.31	0.69
1175	0.070	0.37	0.67
1460	0.051	0.27	0.73
2000	0.038	0.20	0.80
4000	0.013	0.068	0.93

^aEstimated relative error approximately $\pm 15\%$, and the absolute error $\pm 25\%$.

TABLE 8Core Yields of $\text{dR}^\bullet$ Based on Actual Energy Deposited in Core

LET (keV/ μm)	$G(\text{dR}^\bullet)^a$ $\mu\text{mol/J}$	$G_{\text{core}}(\text{dR}^\bullet)^b$ $\mu\text{mol/J}$
1080	0.027	0.026
1175	0.027	0.024
1460	0.027	0.026
2000	0.025	0.024
4000	0.0069	0.0059

^aTable 1.^b $G_{\text{core}}(\text{dR}^\bullet) = [G(\text{dR}^\bullet) - 0.15G_{\text{base}}]/(\text{fraction energy in core})$ (Table 7).

TABLE 9*G* Values (μmol/J) for Sugar Radical Formation and Base Release

LET (keV/μm)	<i>G</i> (dR [•])	<i>G</i> (base release) ^a
1080	0.027	0.028
1175	0.027	0.026
1460	0.027	0.024
2000	0.025	0.025
4000	0.0075	0.011