Ne-22 Ion-Beam Radiation Damage to DNA: From Initial Free Radical Formation to Resulting DNA-Base Damage

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Introduction

Ionization of cellular DNA leads to a variety of electron and hole transfer processes that result in strand breaks, damaged bases or, in the case of the C5'-radical (C5'•), cyclization to form 8,5'-cyclopurine-2'-deoxynucleosides which can be difficult to repair. As the linear energy transfer (LET) of the radiation changes, so too do the radicals formed, subsequent chemistry and consequent final damage products and the difficulty of repair. According to track structure models, in the high LET core of an ion-beam, ionization events are so densely clustered that charged radicals recombine at high rates driven by coulombic interactions even at 77 K while neutral radicals are more likely to escape recombination. Hence, in DNA, along the core of the ion-beam, neutral sugar radicals continue to accrue with more radiation while the yields of base radicals, which are either charged or come from charged precursors, quickly taper off. In the low LET penumbra, however, ionization events are sparsely distributed and both charged and neutral radicals can be found at 77 K. Neutral sugar radicals are the precursors to strand breaks in DNA which lead to ionizing radiation-induced cellular death, mutations and aging. The extent to which strand breakage and other base and sugar damage has occurred can be monitored via quantification of the final products. This work tests the hypothesis that LET, and, therefore, recombination at the Bragg peak is so high that maximum damage due to heavy ion-beam irradiation actually occurs just before the Bragg peak by quantifying radicals trapped at 77 K with electron spin resonance (ESR) spectroscopy and quantifying final base damage products with liquid and gas chromatography mass spectrometry (LC-MS/MS and GC-MS/MS) along the path of the beam. (Ref: *ACS Omega* **2021**, *6*, 16600 – 16611.)

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Significance

This work is the first to use the combination of ESR spectroscopy, LC-MS/MS and GC-MS/MS, and to report the formation of DNA products that had not been reported previously in DNA ion-beam irradiated at 77 K. Our results show that both DNA-radicals and DNA-base damage products formed via both oxidative and reductive pathways (e.g., Figure 10) increase along the track until just before the Bragg peak is reached; in addition, the DNA-radical and product yields are reduced at the Bragg Peak. This work thus enables a better understanding of the mechanisms of radiation damage to DNA along the ion-beam track in terms of the formation of DNA-radicals, the stable products that are formed from the radicals, and the location of products in the ion track.

Methodology

DNA Sample Preparation: Salmon testes DNA was used as received from Sigma-Aldrich. The DNA is hydrated to Γ = 12±3 H_2O/nucleotide by equilibration over a saturated NaCl/H_2O or NaCl/D_2O solution for two weeks under a N_2 atmosphere. 50 mg to 75 mg of the hydrated DNA is pressed into rectangular parallelepiped blocks using an aluminum dye and press. Samples are then rehydrated for a few weeks. Approximately 7 to 9 individual parallelepiped samples are then assembled into a sample packet as shown in Figure 1 and placed in a plexiglass sample packet holder for irradiation.

Rehydration is done once more to ensure that these samples stay hydrated at Γ = 12±3 H_2O/nucleotide. Before irradiation, the plexiglass sample holders containing the DNA samples are rapidly plunged into liquid N_2 (77 K) for transportation to the National Superconducting Cyclotron Laboratory (NSCL) at Michigan State University, East Lansing. After Ne-22 ion-beam irradiation and ESR analysis at 77 K, the samples are warmed to room temperature and sliced by hand with a razor blade for product analysis.

Ne-22 ion-beam and γ-irradiation: DNA samples were irradiated at 77 K at the NSCL using the Coupled Cyclotron Facility. The Ne-22 beam had an energy of 1.514 GeV at the exit of the fragment separator, which was used for the energy selection of the degraded primary beam. After passing through a 75 µm zirconium window, 433 mm of air and 28 mm of Styrofoam, the nominal energy of the beam at the front of and before it enters a sample packet was calculated by LISE++ to be 1.363 GeV. However, the depth of formation of color centers in the plexiglass sample holders and the measured yields of products in the sample packets themselves both indicate that the actual energy at the sample packet entrance is ca. 1.14±0.05 GeV.

ESR Spectroscopy: A Varian Century Series X-band (9.3 GHz) ESR spectrometer with an E-4531 dual cavity, 22.9 cm magnet, and 200 mW Klystron was used, and Fremy's salt [g = 2.0056, A_N = 13.09 G] was employed for the field calibration. All ESR spectra were recorded at 77 K and at 41 dB (16 µW). Spectral recording is done at 77 K.

Measurements of DNA Lesions: See *ACS Omega* **2021**, *6*, 16600 – 16611.

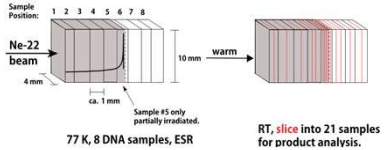

Fig. 1

ESR Measurement of DNA Radicals

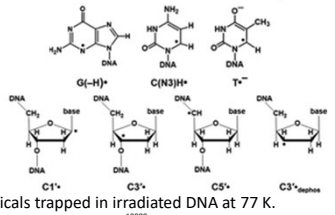

Fig. 2 Radicals trapped in irradiated DNA at 77 K.

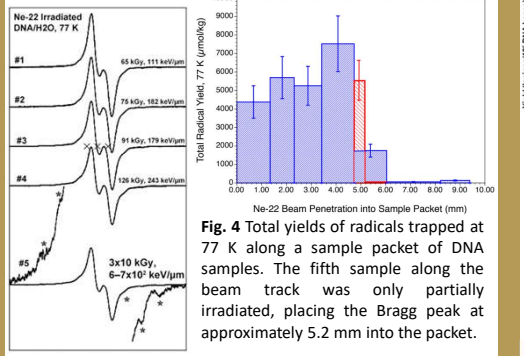

Fig. 3 ESR spectra obtained from Ne-22 irradiated hydrated DNA at 77 K containing the cohort of radicals in Figure 2 at various doses and LETs. Spectral decomposition was performed using an in-house generated program to quantify the base radicals and sugar radicals separately. The wings of the sample that received the highest dose have been expanded to show line components assigned to the neutral sugar radicals. The three X's represent Fremy's salt resonances.

Fig. 4 Total yields of radicals trapped at 77 K along a sample packet of DNA samples. The fifth sample along the beam track was only partially irradiated, placing the Bragg peak at approximately 5.2 mm into the packet.

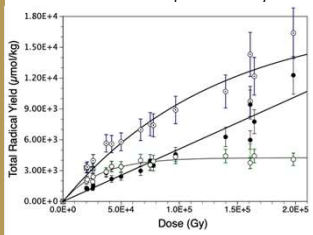

Fig. 5 Dose response of trapped radicals. The upper curve (dotted circles) represents total radical yield, black circles indicate sugar radical yields, and open circles show the sum of base radicals. Uncertainties are estimates based on typical spin-counting variabilities.

Measurement of DNA Base Damage Products

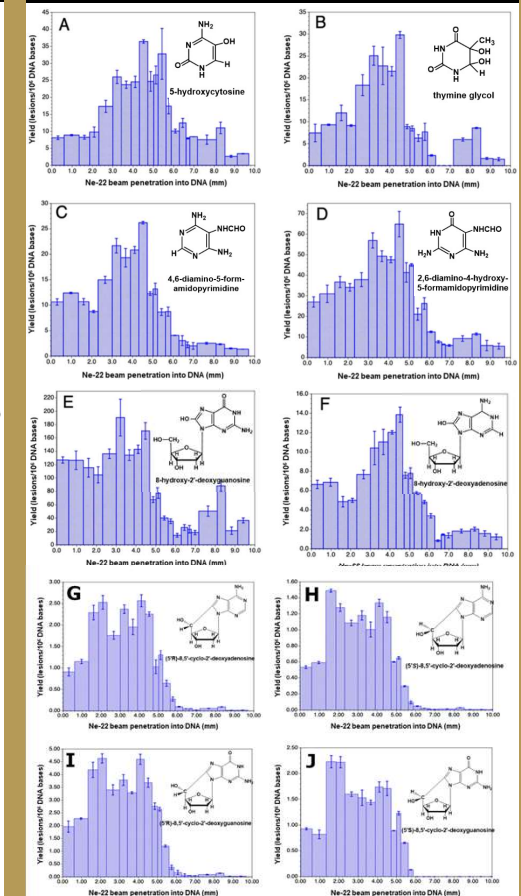

Fig. 6 DNA damaged base products along the beam track. For yields of dihydrouracil and cytosine see *ACS Omega* **2021**, *6*, 16600 – 16611