Molecular mechanisms of heavy-ion induced radiation damage:

Free radicals and products from DNA and chromatin

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Free radical formation in dry DNA and chromatin

A recent EPR analysis of spectra from X-irradiated freeze-dried DNA (77 K to 300°K, 10 kGy to 480 kGy) yielded <u>ten</u> assigned radicals: the oxidized guanine, the reduced thymine and cytosine (two protonation states), the thymine allyl radical, two deoxyribose radicals (C1' and C3'), the 5-thymyl radical, the deprotonated guanine cation and a radical at N7 of a purine [1]. Bombardment with heavy ions (^{50}Ti (11.4 MeV/u), ^{68}Zn (5 MeV/u), ^{197}Au (11.4 MeV/u) and ^{209}Bi (11.4 MeV/u)) at about 100 K confirmed the presence of most primary radicals. The spectra at 9.5 GHz were nearly identical to those after X-irradiation. Dose response curves gave G-values and saturation concentrations in the same order of magnitude [2]. At identical doses, a significant increase of the thymine allyl and the C1' radicals was detected after for heavy ions in aggreement with reports for oxygen impingement [3]. Radical formation in dry chromatin (calf thymus) gave again strong similarities in the EPR spectra after irradiation with either X-rays or heavy ions. Quantitative measurements of pure DNA and of chromatin after X-irradiation together with spectral analysis point at a spin transfer from protein to DNA as was suggested earlier in the literature but was proven only now [4]. The effect of the Bragg-peak was probed specifically by stacking pellets of dry DNA. Xe (11.4 MeV/u) and Ni (6.0 and 11.4 MeV/u) ions were used. For Ni and Xe at 11.4 MeV/u the fourth pellet contains the Bragg-maximum, with Ni at 6.0 MeV/u it is located in the second disk. Fig. 1 shows, that the LET has no effect on the total radical yield in each disk before and in the Bragg-maximum but the amount of thymine allyl radicals as well as C1'- and C3'-deoxyribose radicals increases with LET and dose. The sugar radicals are potential precursors of strand breaks, which in turn are connected with abasic sites.

Product formation in dry DNA

Solid DNA and DNA-nucleotides were used to study the products formed from direct radiation action at 300 K (X-rays and heavy ions in the beam vacuum, respectively). Polycrystalline pyrimidine nucleotides showed the release of unaltered bases as investigated by HPLC and NMR. [5] Further heavy ion experiments with pyrimidine as well as purine nucleotides showed for all these DNA model compounds the formation of the free bases [6]. Recently we found the release of the bases adenine, cytosine and thymine also for dry DNA after heavy ion bombardment. The modified base 8-hydroxy-adenine was identified as another product. X-irradiation and bombardment with Ti (11.4 MeV/u) led to a similar release of unaltered and modified bases, whereas bombardment with Bi (11.4 MeV/u) resulted in a decreasing release of bases. In contrast to the low-LET-irradiation, bombardment with heavy ions effected an increased formation of formate (Fig. 2). This is connected with oxidative sugar dammage and thus is a probe for strandbreaks. The release of bases is also connected with strand breaks in DNA.. If the induced single strand breaks (ssb) appear within few base pairs, a double strand break (dsb) is fromed. The maximum dsb/ssb ratios were calculated for neon and titanium ions. [7] With the assumption that the release of bases and the formation of formate can be connected with a ssb of DNA, we determined a maximum for titanium ions

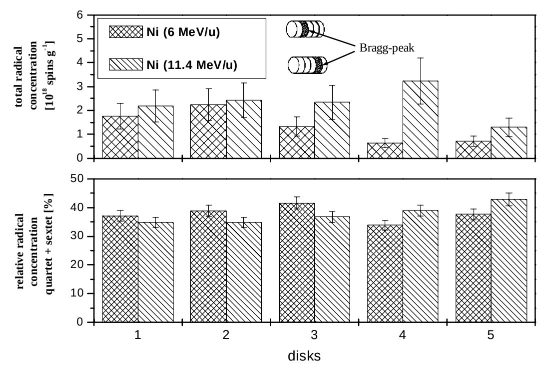

Fig.1 Total and relative radical yield vs. sample thickness

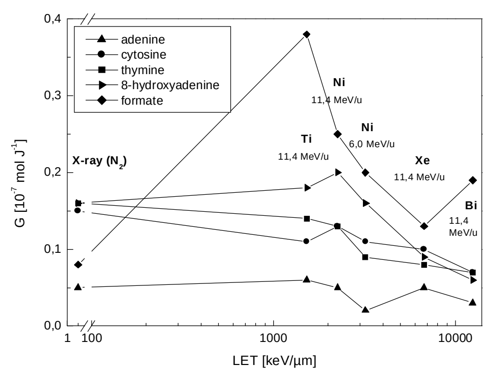

Fig.2 LET-dependence of product formation from DNA

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