

# Dose and LET dependence on the formation of free radicals and products after irradiation of dry DNA

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The main aspect of this report deals with the correlation of dose, LET and the formation of free radicals and products after irradiation of dry DNA. The methods applied were EPR spectroscopy for the distribution of free DNA radicals and RP-HPLC for the quantitative analysis of the stable products generated.

X-irradiation and bombardment with heavy ions (Ti, Ni, Xe, Au, Bi (all 11.4 MeV/u); Zn (5.0 MeV/u)) were carried out with different doses varying from 16 kGy to 680 kGy. The influence of dose on the generation of free DNA radicals established earlier in our group (the oxidised guanine ( $G^{+\bullet}$ ), the reduced thymine and cytosine (two protonation states;  $T^{\bullet-}/C^{\bullet-}$ ), cytosine as aminoprotonated form ( $C(N4H)^{\bullet}$ ), two deoxyribose radicals ( $C1^{\bullet}$  and  $C3^{\bullet}$ ), the thymine allyl radical ( $TCH2^{\bullet}$ ) and the 5-thymyl radical ( $TH^{\bullet}$ )) was evaluated [1], [2]. Comparing X-rays (fig.1B) with Ti (fig.1A) for small doses between 20 and 40 kGy we found significant differences for the  $T^{\bullet-}$  (reduction of 20 %) and  $C1^{\bullet}$  (increase of 21 %).  $C(N4H)^{\bullet}$ ,  $TH^{\bullet}$  and  $C3^{\bullet}$  radicals show only a small rise (2-3 %) after Ti-bombardment, whereas  $G^{+\bullet}$  slightly decreases (6 %) in comparison to X-irradiation. The verification of  $C^{\bullet-}$  was only possible in the case of X-irradiated DNA. Considering the whole dose effect curve reveals an analogous evolution for X-rays and Ti ions: the intense reduction of  $G^{+\bullet}$  (about 9-13 %) and  $T^{\bullet-}$  (20 %) as well as the increase of  $C3^{\bullet}$  and  $TH^{\bullet}$  (4-5 %). The  $C1^{\bullet}$  deoxyribose radical contribution appears more distinct in the case of X-irradiation (30 %; Ti about 20 %). In order to compare all heavy ion experiments and to analyse the effects of LET we studied the behaviour of X-rays and various heavy ions (Ti, Ni, Xe, Au, Bi and Zn) at three different doses (50 kGy, 160 kGy (data not shown) and 400 kGy (fig.2)). Independent from dose we found the same LET correlation of all DNA radicals contributions. For  $G^{+\bullet}$  a total decrease of about 13 % was observed with higher LET. In the case of  $C(N4H)^{\bullet}$  and  $C3^{\bullet}$  a slight rise of about 8 % and 4 %, respectively, was detected, whereas the analysis of  $C1^{\bullet}$  and  $T^{\bullet-}$  yields a contrary step-like behaviour.  $T^{\bullet-}$  first decreases by about 15 %, then remains constant over a wide range of LET and increases finally with higher LET values.  $C1^{\bullet}$  shows the same three steps but in reversed order.

In a complementary HPLC study the LET dependence of the yield of released bases was reported [3]. Additionally we examined the correlation between dose and product yield for Ni ions (8.6 MeV/u) and X-rays (fig.3). For doses up to 500 kGy a linear correlation is found, whereas for DNA bombarded with high doses (2000-4000 kGy) of accelerated Ni ions an incipient saturation of the base yield is emerging. Comparison of the single DNA bases formed after irradiation with Ni or X-rays shows smaller concentrations for Ni, depending on DNA base species: for purines a smaller difference (1.5 fold) was detected than for pyrimidines (about 2.4 fold).

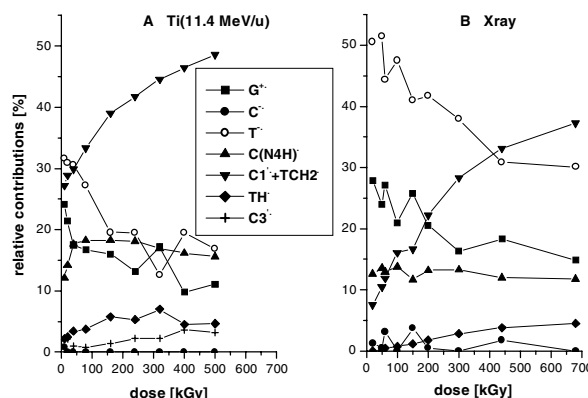


Fig.1 Dose dependence of the free radical contribution

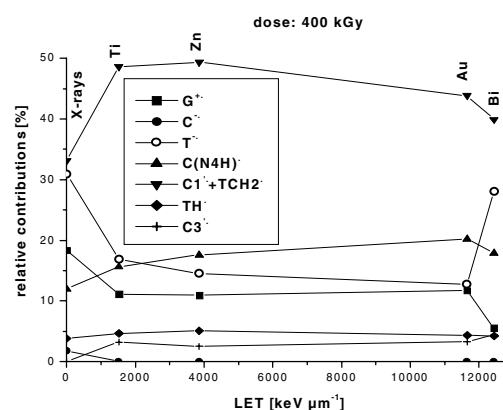


Fig.2 Correlation between LET and radical contribution

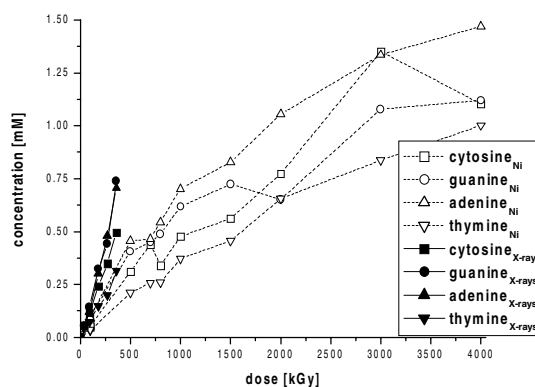


Fig.3 Influence of dose on the release of free bases

## References

- [1] B. Weiland and J. Hüttermann, *Int. J. Radiat. Biol* 1998, 74, 341-358
- [2] B. Weiland and J. Hüttermann, *Int. J. Radiat. Biol* 1999, 75, 1169-1175
- [3] H.M. Luxenburger et al., *GSI scientific report* 2001, 159