

DNA FRAGMENTATION AND REJOINING IN HUMAN CELLS EXPOSED TO γ -RAYS AND CHARGED PARTICLES

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INTRODUCTION

The biological consequences of protons and HZE particles (the components of space radiation of major concern) are expected to be related to DNA lesions. Since DNA damage produced by charged particles occurs in a correlated manner along the particle track, the current working hypothesis is that spatial correlation of damage, that depends on the particle type and energy, affects its reparability. Several characteristic distances for damage correlation can be considered, related to various levels of chromatin organization. We have investigated the DSB induction and rejoining in AG1522 human fibroblasts through the analysis of the DNA fragmentation patterns in the size range 23 kbp - 5.7 Mbp, i.e., dimensions related to the loop structure of chromatin, using calibrated Pulsed Field Gel electrophoresis (PFGE). We have compared γ -rays and various charged particles i.e., low-energy protons of 0.88 MeV (representative of stopping protons at the Bragg peak) and Fe ions of 1050 and 414 MeV/u.

DNA FRAGMENT INDUCTION AND DISTRIBUTION

The number of DNA DSB induced in the size range studied, at doses between 40 and 200 Gy, was evaluated using the fragment counting method. The general trend observed is a linear, or almost linear, increase of DSB with the dose for all the radiation types. 1050 MeV/u Fe ions are the most effective particles in terms of either unit dose or unit fluence.

The frequency distributions of fragments induced by the charged particles are shifted towards smaller sizes with respect to those induced by comparable doses of γ -rays. The cumulative distributions suggest that at doses of the order of 100 Gy most of the fragments induced by γ -rays or protons have size larger than 23 kbp, while for Fe ions it is expected a significant amount of fragments smaller than that size. In effect, a significant amount of fragments induced by 1050 MeV/u Fe-ions has been detected by “ad hoc” measurements of DNA fragments in the size range 2 – 23 kbp.

DNA FRAGMENTATION SIMULATIONS

Simulation of DNA fragment distributions was performed using a generalized broken-stick (GBS) model, in which the radiation-induced DSB are considered randomly placed, but the non-random fragmentation of the control sample is taken into account and considered as the initial fragment distribution. The agreement between these simulations and the experimental distributions is much better after gamma or proton irradiations than after Fe-ions irradiations. The deviation from randomness observed for these ions are related to an enhanced induction of small fragments.

REJOINING OF DNA FRAGMENTS

Rejoining of radiation-induced DNA fragments was studied by comparing the fragmentation distributions after 30 and 120 min repair following exposure with 160 Gy of γ -rays or protons. The fraction of residual DSB is larger after proton irradiation, especially at 30 min rejoining. Also, the distribution of residual fragments appear different, in that cells exposed to protons show a less extent of rejoining with respect to γ -rays in the entire size range analyzed.

CONCLUSIONS

For AG1522 cells, the DSB yields evaluated by PFGE in the range 23-5700 kbp are in the following decreasing order: Fe (1050 MeV/u), Fe (414 MeV/u), p (0.84 MeV/u), γ -rays. The higher effectiveness of Fe-ions compared to protons seems to be related to the occurrence of fragments smaller than 23 kbp. In general, the fraction of short fragments induced by Fe-ions is higher than that predicted by a random fragmentation (GBS) model. The distributions after rejoining show that fragments induced by protons are rejoined slower with respect to γ -rays.

REFERENCES

[1] Belli M. et al (2001) *Adv Space Res* 27, 393-399 [2] Belli M. et al (2002) *Int J Radiat Biol* 78, 475-482.

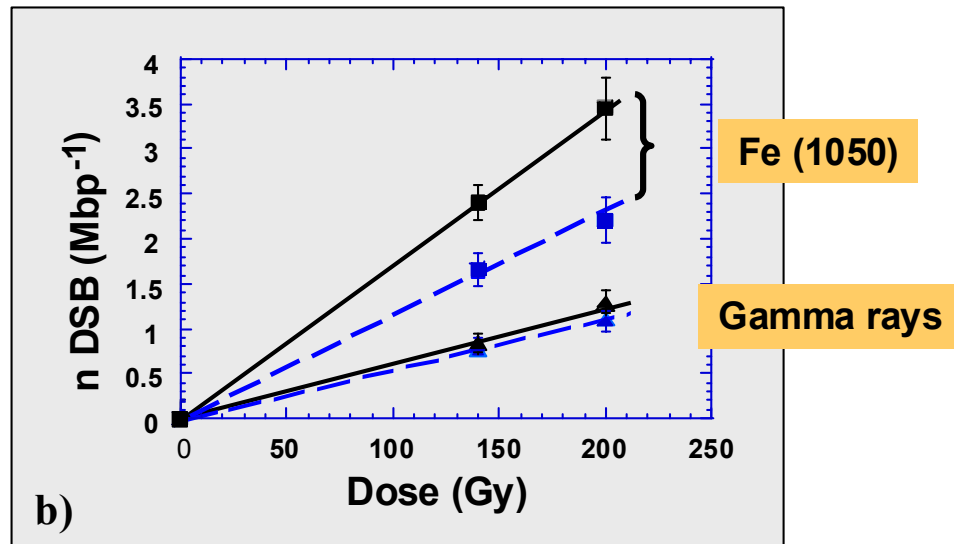
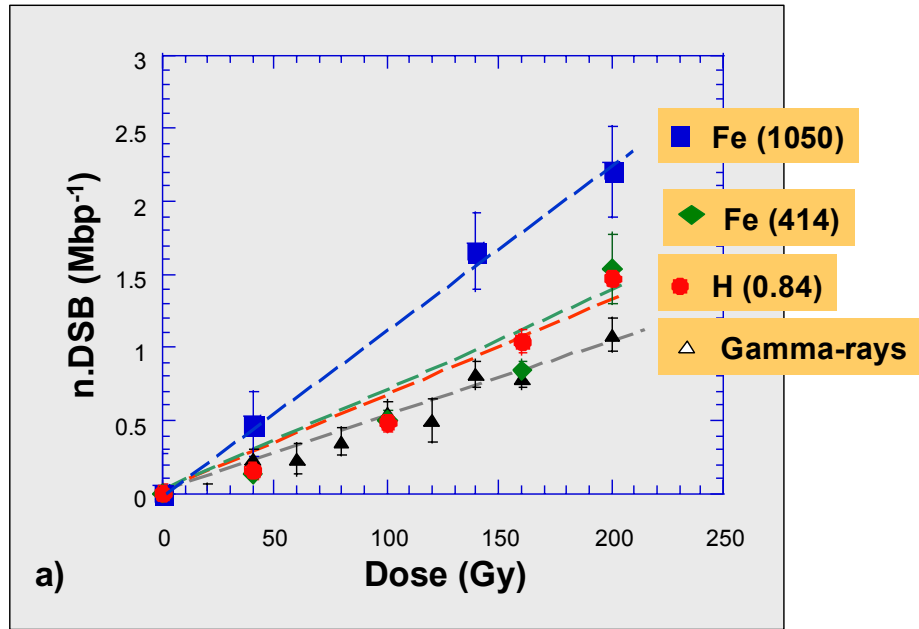


Fig.1. DSB induction as a function of dose for various radiation types, evaluated by fragment counting in the ranges 23 kbp – 5.7 Mbp (panel a) and 2 kbp - 5.7 Mbp (panel b). In panel b) dotted lines represent the results shown in panel a) for comparison.

SIMULATED FRAGMENT DISTRIBUTIONS

D = 200 Gy

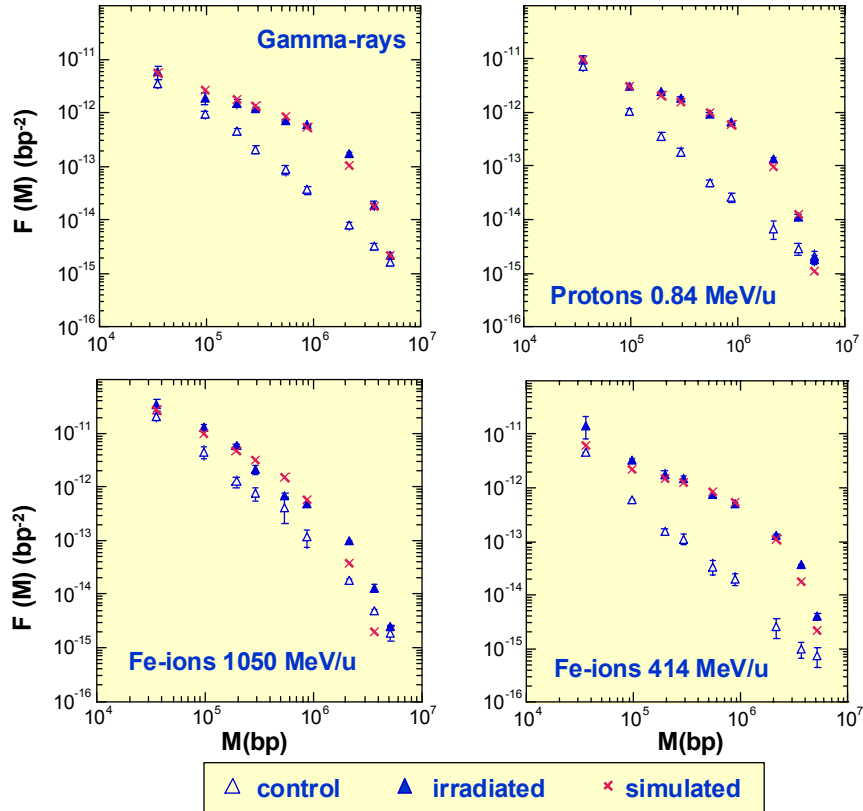


Fig.2. Comparison between experimental (triangles) and simulated (crosses) fragment distributions for various radiation qualities.

FREQUENCY DISTRIBUTION OF RESIDUAL DSB

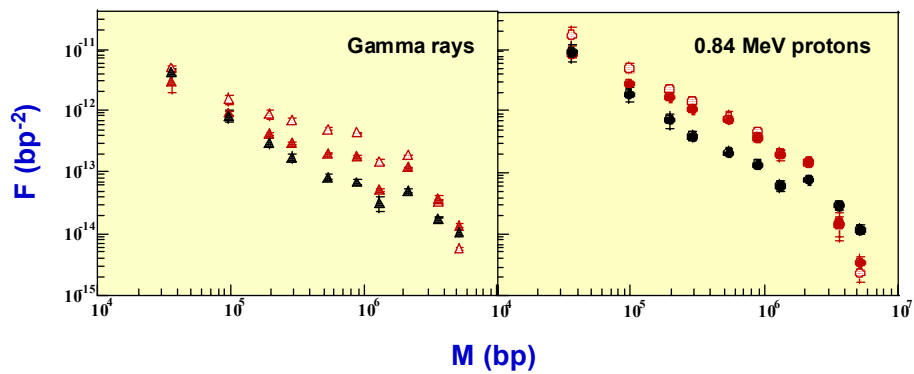


Fig.3. Experimental frequency distribution of DNA immediately after 160 Gy irradiation (open symbols) and after 30 and 120 min rejoining (closed symbols).