10 HEAVY ION ACTION ON SINGLE CELLS: CELLULAR INACTIVATION CAPABILITY OF SINGLE ACCELERATED HEAVY IONS

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Introduction

Heavy ions (HZE-particles) constitute an important part of radiation in space. Although their number is small the high amount of energy transferred by individual particles may cause severe biological effects. Their investigation requires special techniques which were tested by experiments performed at the UNILAC at the GSI (Darmstadt). Diploid yeast was used which is a suitable eucaryotic test system because of its resistance to extreme conditions like dryness and vacuum. Cells were placed on nuclear track detector foils and exposed to ions of different atomic number and energy. To assess the action of one single ion on an individual cell, track parameters and the respective colony forming abilities (CFA) were determined with the help of computer aided image analysis. There is mounting evidence that not only the amount of energy deposited along the particle path, commonly given by the LET, is of importance but also the spatial problem of energy deposition at a submicroscopical scale. It is virtually impossible to investigate track structure effects in detail with whole cell populations and (globally applied) high particle fluences. It is, therefore, necessary to detect the action of simple ions in individual cells. The results show that the biological action depends on atomic number and specific energy of the impinging ions, which can be compared with model calculations of recent track structure models.

Techniques and Methods

Diploid wildtype yeast cells, *Saccharomyces cerevisiae*, are plated as monolayers with a cell density of $3*10^6$ / cm^2, embedded in a thin layer of nonnutrient agarose gel completed by D-Trehalose, on the surface of the detector foil. As track detectors 200 μm polycarbonate "LEXAN" and 100 μm CN-foil were used. Their advantages are good mechanical rigidity and easy handling. Irradiation was performed with about 10^6 particles/cm^2, the X-ray dose used in combination experiments was 360 Gy. In order to simulate the effect of a mixed radition field, as can be found in space in a rather complex composition, combined irradiations with X-rays and Oxygen-ions and α-particles, respectively, were performed, using the methods mentioned above. Preirradiation took place about 1 h before particle irradiation, the resulting radial inactivation dependencies are shown in the figures 2 and 3 below. The method of analysis based on a computer-aided image analysis equipment, is depicted in figure 1.

The biological samples, consisting of a track detector with a biological layer, were irradiated, then an additional layer of nutrient agar was moved on the area of interest, providing on the one hand a suitable growth substrate, on the other hand the quality of microscopic images could be improved. Colony forming ability (CFA) was tested by incubating under growth conditions for 6 hours, based on the assumption, that after a lag time of about 2 hours, the cells can divide two times to form a microcolony. Colonies with at least four cells after the above mentioned time were accepted as survivors. After removal of the biological layer , track etching took place. Microscopical pictures of specially

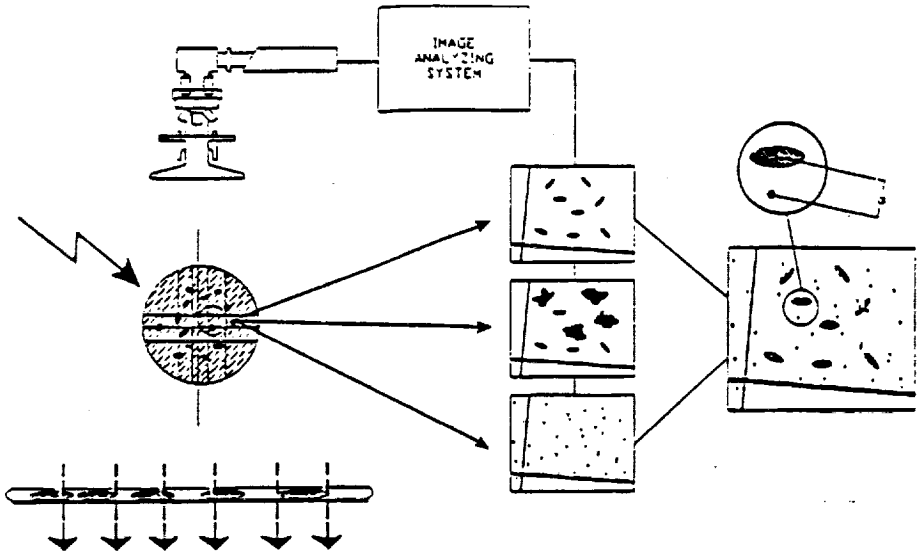

Figure 1: The principle of the Experimental Method

marked areas were taken by the computer-coupled Video-camera at three stages: 'Single cells', 'colonies' and 'etch-pits'. The superposition of the three pictures on sreen provided information on the CFA of specified single cells as well as on the impact parameter of ion tracks in the vincinity of individual cells with an accuracy of about 0.5 μm (see figure 1).

Results and Conclusions

Single ion experiments

The inactivation range of α-particles is much larger than the calculated penumbra-radii and the inactivation probability of the α-particles (fig. 2) is lower compared to accelerated oxygen ions (fig. 3). Even direct hits into the cell nucleus show an inactivation probability of less than 100%. For the oxygen ions the calculated penumbra radii are similar to the experimental data.

Combination experiments with X-preirradiation

For both ion types it can be clearly seen that, except for small impact parameters, that preirradiation with α-particles causes a significant expansion of the effect towards higher impact parameters. This might be understood as an additional inactivation of sublethally damaged cells in this area, whereas for small impact parameters, the large amounts of energy transferred by the particle exert an overriding influence. In conclusion it can be stated that these data can complete the hitherto existing results with respect to the understanding of the biological effect of heavy ions on cellular systems.

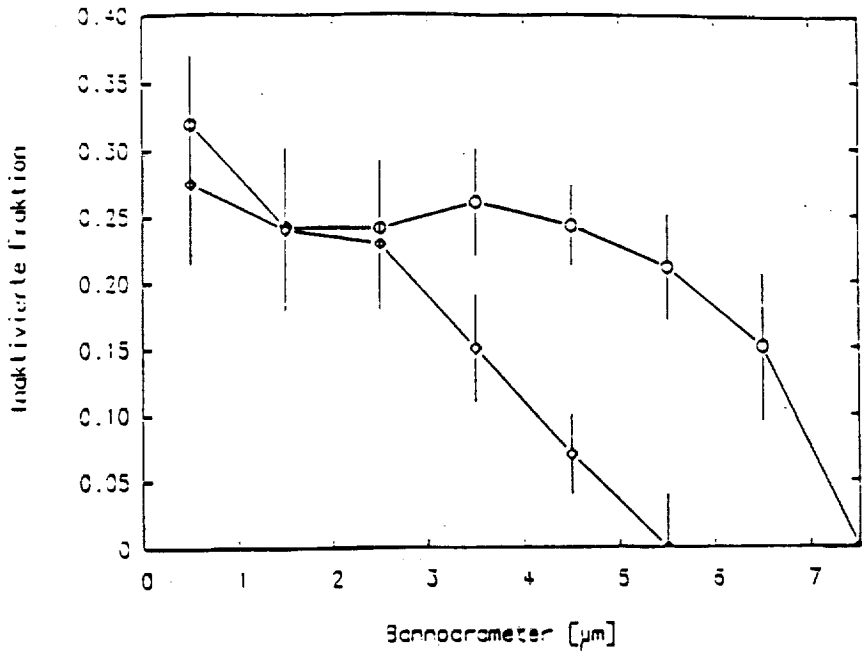

Figure 2: Normalized inactivated fraction of yeast cells for individual impact parameters after irradiation with 1.13 MeV/u α-particles (diamonds) and additionally preirradiated with 360 Gy 80 keV X-Rays (circles).

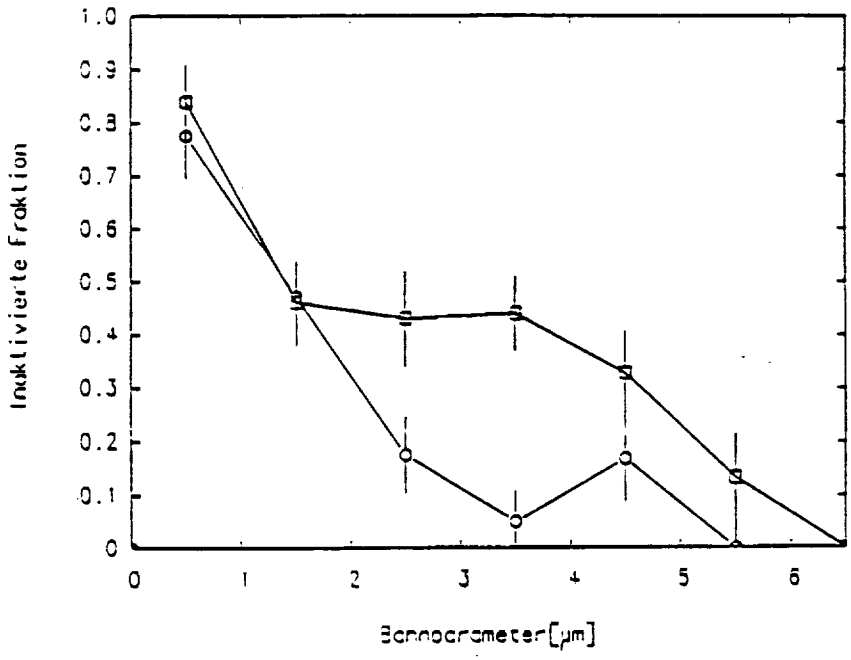

Figure 3: Normalized inactivated fraction of yeast cells for individual impact parameters after irradiation with 11.4 MeV/u oxygen-ions (circles) and additional preirradiation with 360 Gy 80 keV X-Rays (squares).