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Effects of Gamma Radiation on the pBs-KS DNA plasmid

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DNA is considered to be the most important and critical target in a cell from the point of view of radiation damage. It is responsible for conservation and transmission of all the cell genetic information. It is constantly submitted to different kind of damages, where repair could or couldn't take place. The energy transferred by ionizing radiation to the DNA strands can induce mutation, carcinogenic process and cell death. The DNA damage involves nucleotide base alterations, single (SSB) and double (DSB) strand breaks and also chromatin rupture. The DNA strand break (mainly DSB) is the most critical damage induced by ionizing radiation. Specifically, gamma radiation is used in a large number of illness treatment including cancer diagnosis, treatment and cure. In fact, this radiation is the base of conventional radiotherapy using 60 Co and 137 Cs sources. Beside, this radiation is also present in treatment which involves neutrons and others types of particles, such as protons, alphas and heavy ions (e.g. Ne. Ar). The 250 MeV proton beam therapy, largely used in recent years as an effective non invasive cancer treatment. produces gamma rays as a secondary radiation which can generate radiobiological effects on healthy tissue. This kind of radiation with low linear energy transfer (LET) interacts with the tissue producing secondary electrons. which directly produce excited and ionized states into the DNA strands and inducing the formation of energetic free radicals in the aqueous solution containing the DNA molecule. The DNA strand damages produced by these interactions must be well known in order to prevent the radiobiological effects on human being. The investigation of DNA radiation effects has been very intensive in the last years^[1-3], and the present study is a contribution to better understand the mechanism of single (SSB) and double (DSB) strand breaks of pBs KS (+) plasmid DNA. with 1 MeV gamma radiation. About 10 ml of DNA was irradiated at a concentration of 15 ng/ml in a cylindrical plastic tube (eppendorf) in the 60Co Gamma Cell at IPEN (Instituto de Pesquisas Energéticas e Nucleares, São Paulo) facility. The dose rate was 5 kGy/h, and the biological material was submitted to total doses ranging from 0 to 200 Gy. Supercoiled (FI), Circular (FII) and Linear (FIII) forms of the plasmid were separated by agarose gel electrophoresis at 10 volts overnight with 1% agarose. The results exhibit a decreasing of the FI plasmid fraction and an increasing number of the FII and FIII fractions with the dose, suggesting the presence of SSBs and DSBs in the irradiated DNA plasmid. The detectable molecule fractions of each form of plasmid were analyzed by means of a statistical treatment^[4,5] allowing the

calculation of the average number of SSB and DSB per plasmid for each interaction dose. These two quantities are shown in the figure. The curve is only to guide the eyes(preliminary data).

In this analysis it was assumed that the strand break distribution obeys Poisson' laws. These calculations were compared with the corresponding experimental data. It was found that the statistical results describe satisfactory the data for gamma radiation.

REFERENCES

- [1] Chatterjee A., Radiological Effects of High-Let particles: DNA strand breaks. Nucl. Instr. & Meth. In Phys. Res. 1989, A280:439-448.
 - [2] Lewin B., in Genes V, chapter IV, Oxford Univ. Press, NY, 1994.
- [3] Cook V. E. and Mortiner R. K., A Quantitative Model of the DNA Fragments Generated by Ionizing Radiation and Possible Experimental Application. Radiation Research 1991, 125:102-106.
- [4] Cowan R., Collis C.M., Grigg G.W. Breakage of double -stranded DNA due to single-stranded nicking. Journal of Theoretical Biology, 1987, 127: 229-245.
- [5] Hempel K. Mildenberger E. Determination of g- values for singles and double strand breaks induction in plasmid DNA using agarose gel electrophoresis and a curve- fitting procedure. International Journal of Radiation Biology, 1987, 52: 125-138.