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BIOCHEMICAL ANALYSIS OF DAMAGE IN PLASMID pBR 322 DNA PRODUCED BY THE REACTION WITH RADIATION-INDUCED ORGANIC RADICALS

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It is considered that radiation-induced organic radicals, which are produced within the cell by irradiation with ionizing radiation, react with cellular DNA and inactivate it, because many organic molecules that can form organic radicals exist in the vicinity of DNA molecules. As an *in vitro* model system, we have investigated what types of DNA damage are induced by organic radicals when the aqueous solution of plasmid DNA pBR 322 is irradiated in the presence of organic compounds. Nitrous oxide-saturated aqueous solution of DNA was irradiated with X-rays in the presence of an excess concentration of organic compounds such as formate, histidine and glycerol to make the radiation-induced organic radicals react exclusively with DNA molecules. The frequency of DNA strand breaks was measured using agarose gel electrophoresis. Our first observation was that organic radicals induced a certain number of DNA strand breaks. The second observation was focused on detecting other kinds of DNA damage which were not strand breaks but specific sites which were recognized and converted to strand breaks by the use of endonucleases. When the irradiated DNA sample was treated with endonuclease extracted from *Micrococcus luteus*, which recognized various kinds of base damage, the frequency of DNA strand breaks was 4.85 times greater than the non-treated sample, whereas when the DNA sample was treated with Sl-nuclease, which recognized light structural disintegration, no additive strand breaks were observed. It was concluded that in addition to the strand breaks, organic radicals also produced DNA damage, which was base alteration without any accompanying structural disintegration.