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FREE RADICAL AND RADIATION-INDUCED DNA DAMAGE TO OLIGONUCLEOTIDES

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The chemical nature of many DNA lesions induced by the action of gamma rays is known very precisely and many radiation products derived from nucleosides have been synthesized. A variety of DNA defects are produced non-specifically along the DNA chain: base and sugar modifications, abasic sites, cross-linkings, single and double strand breaks.[1-4]

Direct chain ruptures are produced during radiolysis. They result mainly from the attack of OH radicals at the sugar moiety.[5] Additional chain ruptures are observed when irradiated DNA fragments are heated in the presence of piperidine. They are due to the decomposition of radiation-modified base and sugar residues which are alkali labile. The way the base radiation products are formed by the attack of radicals provided by water radiolysis will be discussed. The alkaline medium catalyses base ring openings and rearrangements to give rise to N-glycosylic bond rupture and then to oligonucleotide chain breakage. The occurrence of damage along the DNA chain is not homogeneous.[6]

The phenomenon has been investigated with synthetic oligonucleotides ^{32}P labeled at one end. The fragments resulting from gamma irradiation of these oligonucleotides were heated with piperidine and separated by polyacrylamide sequencing gel electrophoresis according to their chain lengths. After autoradiography the electrophoresis bands were counted but quantitative interpretation is not simple because the same oligonucleotide molecule can be ruptured at more than one site. The experimental values of the band intensities must be corrected. mean relative radiosensitivity values for the four natural nucleotides corresponding to alkali-induced chain rupture can be calculated by computer iteration.[7] From these constants the bands intensities can be predicted for every level of oligonucleotide degradation. The ratio of experimental to theoretical values gives information on the local reactivity of radicals for a nucleotide in a well defined position. Reactivity depends on the oligonucleotide conformation. The influence of the vicinal surrounding bases is not well understood. When a single stranded oligonucleotide which is not self complementary is irradiated, the calculated values of the electrophoresis band intensities are very close to the experimental ones. In the hairpins, large differences between experimental and predicted band intensities are observed. Thus the reactivity is related to the nature of the base and to the local distortion and bending of the oligonucleotide chain. It should be noted that the preferential degradation due to the position of a given nucleotide is rather insensitive to the delivered radiation dose. The same features are exhibited by different curves when less than 80 per cent of the oligonucleotide molecule is cut.

The relative radiosensitivity of a base DNA defect in the structural environment of the biopolymer chain can be measured precisely by computer simulation. Synthetic oligonucleotides bearing a modified nucleotide were prepared by a new method involving labile amino protecting groups.[8] They were irradiated and the gel electro-

phoresis band intensities predicted. The radiation product of adenine residues, 7,8-dihydro-8-oxoadenine was inserted by chemical synthesis in an oligonucleotide 39 units long in place of adenine. Its radiation-induced degradation was compared to that of the normal nucleotide. Chain ruptures induced by gamma irradiation and piperidine heating are three times more frequent with 7,8-dihydro-8-oxoadenine.

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