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# DNA DAMAGE IN FROZEN AQUEOUS SOLUTION: SEQUENCE DEPENDENCE AND END GROUPS

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Strand breaks arising in deoxyribonucleic acids and oligonucleotides following irradiation under conditions of *direct* damage have been shown to occur and every site with no apparent sequence specificity and the end-groups arising at the new 5'- and 3'-termini have been identified as phosphate monoesters.

The cytotoxic, mutagenic and carcinogenic effects of ionising radiation are well documented and are usually assumed to be associated with damage to the nuclear deoxyribonucleic acid (DNA).[1] The chemical lesions induced by exposure to ionising radiation that have been well characterised include single and double strand breaks, base release sites, and sugar and base modifications.[2] Some of the radical precursors to each of these types of DNA damage have been identified or inferred.[2-4] The majority of the *in vitro* model studies have been conducted in dilute aqueous solution where the damage to DNA is dominated by the reactions of hydroxyl radicals. We and others have argued that within the cell the nuclear DNA is in a compact state that is not well modelled by a very dilute solution. In addition, hydroxyl radicals formed at a distance from DNA within the cell will not contribute to DNA damage because these will be scavenged by other biomolecules (GSH, lipids, proteins etc.), hence the direct damage pathway may be more important *in vivo*. In the light of this we have been studying the direct effects of ionising radiation by a combination of electron spin resonance (e.s.r.) spectroscopy and biochemical analyses.[5] E.s.r. spectroscopy on frozen aqueous solutions of DNA have been interpreted in terms of two primary radicals, the radical anion of the thymine ($T^{-\cdot}$) and the radical cation of guanine ($G^{+\cdot}$), although there are some suggestions that there may be more than one component contributing to the radical anion.[6] The detection of significant levels of TH· arising from protonation of some or all of the anion population on annealing is not in doubt, and we have detected this radical species on irradiation of chromatin and cell nuclei.[5g] We have previously suggested that these base radicals must initiate reactions leading to damage such as single and double strand breaks. In pursuing these studies further we have now looked at the sites and distribution of single strand breaks within a known sequence of DNA and have chemically identified both the 3'- and 5'-end groups.

The distribution of strand breaks within a known DNA sequence was investigated by irradiation of the 3'-$^{31}$P-end-labelled restriction fragment from pBR 322 (Bam H1/Hind III; 346 bp). Irradiations were carried out under the following conditions (i) as a dilute solution at room temperature, (ii) as a frozen aqueous solution at 77 K, (iii) as a "dry" film at room temperature. Analysis on polyacrylamide sequencing gels revealed that under each of the above conditions fragments arise from cleavage at every nucleotide site, with no detectable sequence specificitity. Single bands produced at each of the radiation induced cleavage sites comigrate with the products of the Gilbert-Maxam sequencing reaction which identifies the 5'-end groups as simple

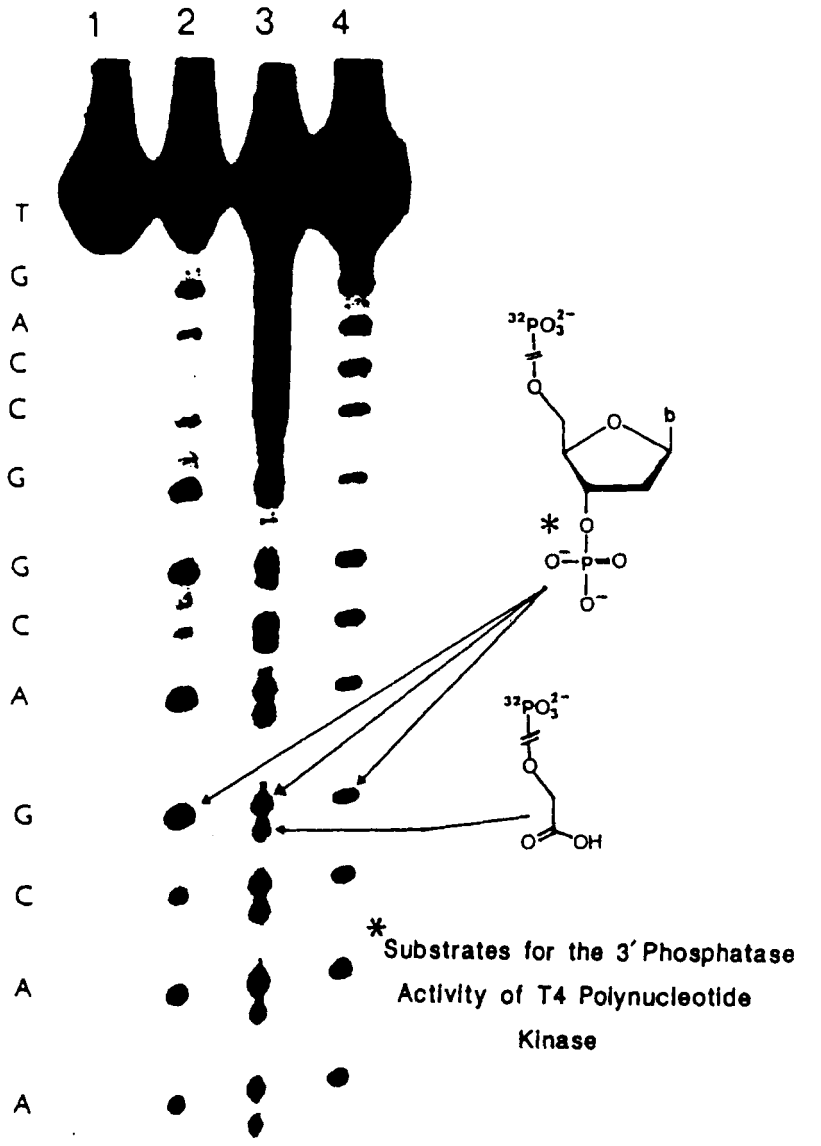


FIGURE 1 Electrophoresis of a 5'-end labelled oligonucleotide. Track 1: the unirradiated control; track 2: G & A Maxam-Gilbert sequencing reaction; track 3: irradiation with 0.6 Mrad in aqueous solution ($100_5$g ml$^{-1}$) at ambient temperature; track 4: irradiation of the frozen aqueous solution at 77 K with 6 Mrad.

phosphates as deduced by Henner *et al.*[7] for hydroxyl radical induced cleavage. Thus the direct and indirect pathways produce the same 5'-end groups.

The nature of the 3'-end group was probed using 5'-$^{32}$P-end labelled restriction fragments andoligonucleotides irradiated as in (i), (ii) and (iii) above. Under dilute

aqueous conditions at room temperatures cleavage occurred to give two bands at every nucleotide site in agreement with the results of Henner.[7] These bands have previously been identified as the 3'-phosphate and the 3'-phosphoglycollate. In contrast, under conditions of direct damage, (ii) and (iii), we detect only a single band at each site. Figure 1. The single bands were identified as fragments terminating in a 3'-phosphate by demonstrating that it was a substrate for the phosphatase activity for T 4 polynucleotide kinase. Thus we have found a significant chemical difference in the end groups produced under direct and indirect conditions. This difference does not arise as a result of differences in the irradiation temperature since the "dry" film was irradiated at room temperature and this showed the same result as the irradiation of the frozen aqueous solutions at 77 K.

The development of strand breaks involves hydrogen atom abstraction from a sugar moiety, which in the case of hydroxyl radicals occurs from C-4'. Under direct damage conditions the hydrogen atom abstraction from the sugar is presumably by the base radicals in an intramolecular reaction. The site of the hydrogen atom abstraction has not yet been identified however, in the B-conformation the nearest hydrogen atoms to the TH· radical are those attached to C-2' and C-1' of the neighbouring sugar residue 5' to the base radical, Figure 2. Although the lack of local

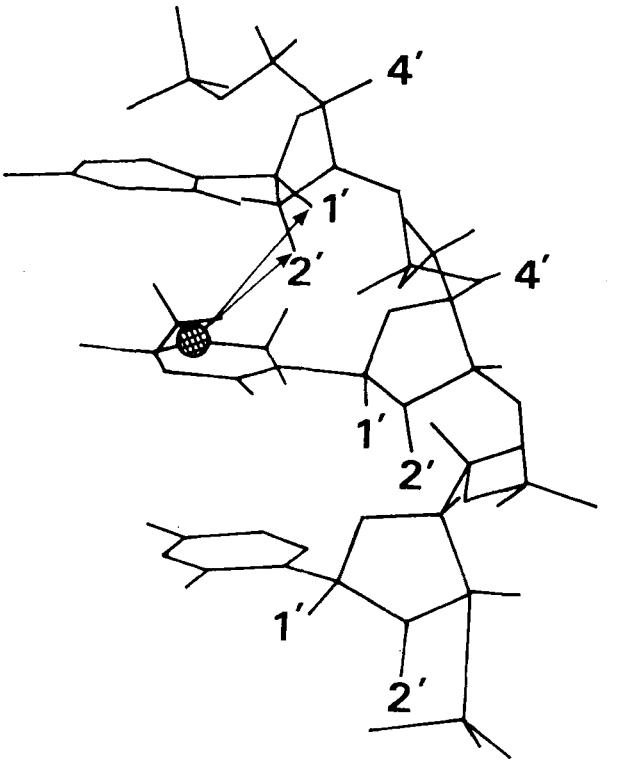


FIGURE 2 Structure of the trinucleotide fragment CTC taken from a section of B-DNA. TH· is the central nucleotide with the radical centre shaded. The distances to the nearest hydrogen atoms that are candidates for hydrogen atom abstraction by the base radical are indicated by the arrows.

sequence specificity in strand breaks produced under direct conditions may appear to conflict with the initial base specificity detected by e.s.r. spectroscopy, if $C^{-\cdot}$ does contribute to the radical anion signal[6] then, for the sequences thus far studied, each of the nucleotide sites have either a flanking T, C or G.

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