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STUDIES OF RADIATION DAMAGE USING DNA OLIGOMERS

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Radiation-induced damage has been studied in the synthetic DNA oligomers d(TpA),[1,2] d(ApT),[2] d(CpG),[3] d(GpC)[3] and d(TpApCpG).[4] There are two main advantages of exploring DNA damage at the oligomer level. Firstly, the damage can be analyzed in intact molecules, thus avoiding the uncertainties that attend the degradation of damaged DNA to the monomer level. The principal experimental method for identifying the lesion borne by the modified oligomer has been proton nuclear magnetic resonance spectroscopy, often with corroboration by mass spectrometry using ionization by fast atom bombardment. Secondly, oligomers bearing specific lesions can be a valuable resource for identifying and quantitating that same lesion in polymer DNA.

Currently there is considerable interest in DNA damage incurred by the cell under prooxidant stress.[5] Prooxidant stress may be induced by tumor promoters, ionizing radiation, metabolic oxidation-reduction couples or such other processes as generate superoxide anion, hydrogen peroxide or hydroxyl radicals. The thesis that tumor promoters act by a genetic mechanism, as opposed to an epigenetic mechanism, is provocative by unproven at this time. Although superoxide anion and hydrogen peroxide are clearly progenitors of oxidative DNA damage, a growing concensus imputes hydroxyl radical as the ultimate agent. Molecular oxygen may participate in the oxidative damage process in two distinct roles. In mechanisms involving superoxide, the presence of oxygen is obviously required for the formation of the molecular anion. Molecular oxygen may also enter the process after the initial OH attack produces a free radical species which can add O_2 to forma peroxyl radical. Thus oxygen in this role determines the final form of DNA damage.

In studies of damage to dinucleoside monophosphates conducted in this laboratory, the product profiles were substantially the same whether the damage process was initiated by ionizing radiation or by an autoxidation process initiated by ascorbic acid provided oxygen was present.[6] In the case of dinucleoside monophosphate containing thymidine the principal modification is one in which the base moiety is degraded to formamide. In the case of a dinucleoside monophosphate containing deoxycytosine two major products are stereoisomers of an imidazolidine modification of the cytosine base, namely 1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine.[7] Both lesions are of interest in connection with oxidative DNA damage but the formamido lesion takes on added importance because it can be derived from damaged bases other than thymine. In this report evidence that the formamido lesion is produced as consequence of radiation-induced degradation of the cytosine base is presented.

Figures 1a, 1b and 1c show the HPLC profiles of products obtained from d(TpA), d(ApT) and d(TpApCpG) X-irradiated in oxygenated solutions. In each case the damaged oligomer produced in largest yield, based on absorption at 254 nm, is one bearing a formamido modification. The significance of the formamido lesion is enhanced by the fact that it is generated by more than one mechanism as demon-

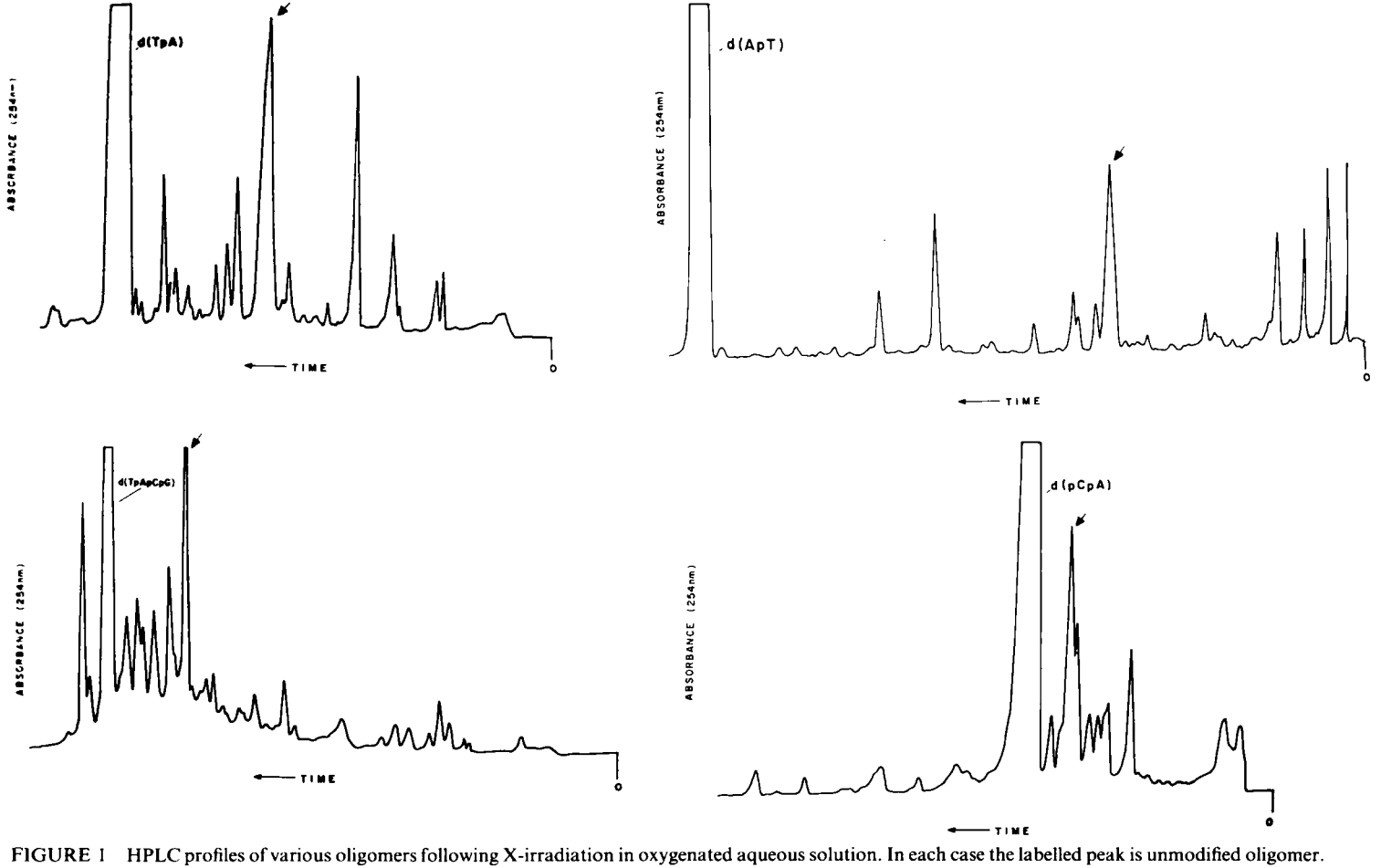

FIGURE 1 HPLC profiles of various oligomers following X-irradiation in oxygenated aqueous solution. In each case the labelled peak is unmodified oligomer. The peak indicated by an arrow is the modified oligomer which bears the formamido lesion. Chromatography utilized C18 reverse columns and an acetonitrile graidient. Details of the chromatography of irradiated d(TpA),[1,2] d(ApT)[2] and D(TpApCpG) are given in the original papers. Elution of d(pCpA) was by a 0–10% acetonitrile gradient in 0.1 M ammonium acetate.

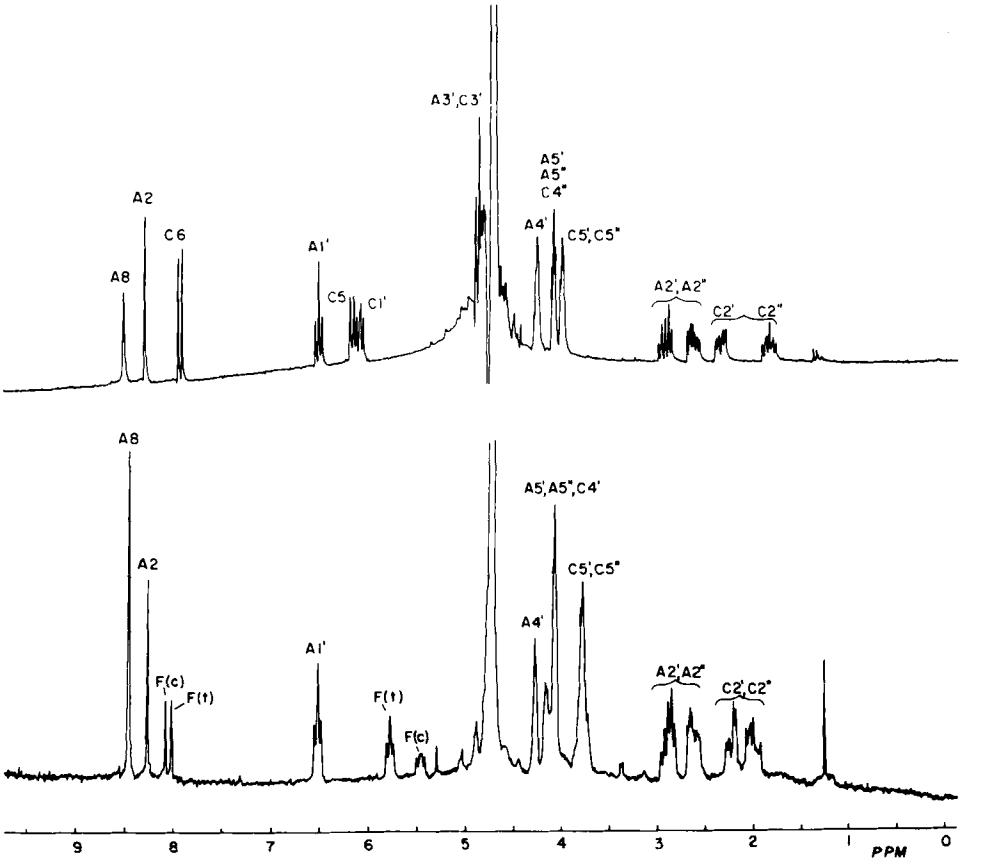

FIGURE 2 The proton NMR spectra of d(pCpA) (upper) and of the major product (see Figure 1d) of d(pCpA) following X-irradiation in oxygenated aqueous solution (lower).

strated in Figure 1d and Figure 2. Irradiation of the dinucleotide d(pCpA) in oxygenated solution yields a product which also bears a formamido modification. The proton NMR spectrum of this product and unmodified d(pCpA) in D_2O are compared in Figure 2. The chemical shifts of the resonances associated with the deoxyadenosine moiety of the product molecule are essentially unperturbed when compared with d(pCpA). However, the H5 and H6 doublets characteristic of cytosine base are absent. The H1′ resonance of the damaged nucleotide occurs at two distinct frequencies, the major component at 5.8 ppm and the minor component at 5.5 ppm. Corresponding to the major and minor components there are low field resonances at 8.0 ppm and 8.1 ppm which are due to the non-exchangeable formamido proton. Split resonances are observed due to restricted rotation about the N–C bond.[8]

In summary, the formamido lesion is consistently found to be the most prominent lesion produced in DNA oligomers irradiated in oxygenated aqueous solutions. Moreover at least two routes of formation are available for the production of this lesion.

During the course of this work an abstract was published which indicates a formamido modification is obtained as a radiation product of the 2′-deoxycytidine nucleoside.[9] This work was supported by grants CA46838 and CA44808 from the National Cancer Institute.

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