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PULSE RADIOLYSIS OF DNA — EFFECTS OF ENVIRONMENT

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The mutagenic and lethal effects of ionising radiation in biological systems are thought to be as a result of chemical modifications induced within essential biomolecules such as DNA. In order to study radiation induced changes in solid DNA, studies have generally concentrated on ESR techniques using low temperatures. Up to date, time-resolved studies of chemical modifications induced within DNA and model systems at room temperature have concentrated on the interactions of water radicals generated in aqueous solution. The few, time-resolved studies on solid DNA have concentrated on radiation-induced electrical conductivity[1] or luminescence.[2]

Since conventional pulse radiolysis with optical detection is generally used to investigate transparent samples, we have utilised the luminescence technique[2] and the recently developed time-resolved technique based upon diffuse reflectance[3] to study radiation induced changes in opaque samples of DNA upon pulse irradiation. The aim of these studies is to gain an insight into the chemical events occurring upon energy deposition within DNA. We have initially concentrated upon radiation-induced energy migration within DNA and the role of hydration in modifying the properties of DNA radicals.

Radiation-induced luminescence from dry DNA produced upon irradiation with 30 ns electron pulses of energy < 260 keV has been investigated in vacuo or in the presence of O_2 at 77 and 293 K. One atmosphere of O_2 partially quenches the radiation-induced luminescence from DNA. The effect of doping DNA with an electron-trap (misonidazole) upon the luminescence intensity was investigated in vacuo. The 'in-pulse' luminescence intensity is critically dependent upon the misonidazole content of the DNA (3:1 to 333:1 DNA base pairs: misonidazole molecule). The luminescence intensity decreases with increasing content of misonidazole within DNA whereby the amount of misonidazole to afford a 50% change in the overall effect is equivalent to one misonidazole molecule per 25 base pairs. At the highest concentration of misonidazole used of 3:1 (base pairs: misonidazole molecule) the luminescence intensity is reduced to $\sim$ 50% of that observed in the absence of misonidazole. It should be noted that at these concentrations of misonidazole $\sim$ 90% of the total energy is deposited in the DNA.

It is concluded[4] that radiation-induced energy migration may occur within DNA. Therefore, in the presence of an appropriate trap, radiation-induced DNA damage may be formed at significant distances from the site of the original energy deposition. In order to complement these radiative processes, preliminary studies have been initiated into the formation of radiation-induced free radicals within dry/hydrated DNA at room temperature using diffuse reflectance.[5] The optical absorption spectrum of pulse irradiated 'Dry' DNA (A-form) is weak with broad bands of centre at 580–600 nm and a shoulder at 400–420 nm. Upon hydration of DNA (1:1 w/w) the optical spectrum increases in intensity with two peaks centered at $\sim$ 380 nm and $\sim$ 470 nm. This amount of water is sufficient to fully hydrate the first solvent shell and

convert the DNA predominently into the B-form.[6] Further hydration of DNA up to 3:1 (w/w) results in additional changes of the post-pulse spectrum. Hydration of DNA result in a significant proportion of the transient absorption especially at $\lambda > 450$ nm decaying within a few milleseconds ($t_{1/2} \sim 0.6$ ms) compared to the longer lived transients observed in the absence of added water. Both kinetic and spectral features of these transient species are not appreciably affected by oxygenation. In these studies whereby energy partition with DNA relative to water molecules varies from $\sim$ 100 to 20 per cent, it is inferred that water radicals and/or water dependent conformational changes of DNA as a result of the primary hydration shell play a role in modifying the DNA species produced.

Such studies on energy deposition within DNA and the effects of environmental changes (e.g. hydration, additives) are particularly relevant to the understanding of molecular radiobiology. Indeed radiation-induced energy migration within DNA adds further support to a potential mechanism of radiosensitization based upon the hypothesis of Adams and Cooke[7] whereby the sensitizer acts as an electron sink thereby preventing ion recombination.

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