Migration of Photoinduced Oxidative Damage in Models for DNA

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Laser flash photolysis with 193 nm light of single stranded oligodeoxynucleotides in aqueous solution indicates that the photoinduced radical cation of adenine or cytosine migrates to guanine, whereas the oxidative damage is not transferred from adenine to either cytosine or guanine *via* a cytosine linker.

Radical cations of the nucleic acid bases are major, primary species produced by the action of ionising radiation on DNA. Migration of this oxidative damage to the most easily oxidised base, guanine, is suggested for frozen samples.[1] The route of migration, predicted from their oxidation potentials, is T → C → A → G.[2] Indeed a study of electron transfer between monomeric nucleic acid bases and dinucleotides is consistent with this prediction.[3] Although DNA acts as a good matrix for photoinduced electron transfer between intercalated donor and acceptor molecules,[4] definitive evidence for migration of the radical cations of nucleic acid bases in aqueous solutions of DNA or model systems (*i.e.* oligodeoxynucleotides) has not been reported. Evidence for photoionisation of aqueous solutions of DNA and its components, at 293 K, with 193 nm light, has been obtained from laser flash photolysis studies.[5,6] Spectral identification of the resulting radical cations has been reported for the nucleic acid base species and various polynucleotides;[3,5] these absorption spectra compare remarkably well with those obtained by pulse radiolysis. There is some indirect evidence for migration of oxidative damage to guanine for DNA samples with differing GC:AT ratios after their photoionisation with 193 nm light.[6] This evidence is only tentative and a more systematic study is required.

Transient absorption spectra, obtained by laser flash photolysis by 193 nm light, of a series of single stranded oligonucleotides, were assessed for the migration of oxidative damage. The oligodeoxynucleotides (**1–5**, Table 1) were synthesised as well as three nonamers containing either guanine (**G9**), adenine (**A9**) or cytosine (**C9**). The initial distributions of photoinduced radical cations formed at each of the nucleic acid base species can be estimated from the extinction coefficients (193 nm) and the quantum yields of photoionisation of the monomeric nucleic acid bases[5] and are shown in Table 1. Optical absorption spectra obtained 5 μs after photolysis with 193 nm light of single stranded oligodeoxynucleotides and the nonamers in oxygenated, aqueous solution (293 K, pH = 7 ± 0.5) are displayed in Figs. 1–3. The extinction coefficients shown were determined by reference to the absorbance of the hydrated electron at 650 nm (ε_{650} = 1580 m^2 mol^{-1}) for identical solutions of these oligodeoxynucleotides, but saturated with argon.[5] The quantum yield for photoionisation of the oligodeoxynucleotides studied are in the range 0.043–0.110. Evidence for migration is obtained by comparison of the transient optical spectra obtained for **1–5** with that obtained for the nonamer containing the most easily oxidised base.

The first group of oligodeoxynucleotides (**1,2,3**) contains two nucleobases, whereby the less easily oxidised base is in excess.

Table 1 Calculated mole fraction of radical cations formed by photoionisation

Oligodeoxynucleotide	G	A	C
AGAAGAAGA **1**	0.68	0.32	—
CGCCGCCGC **2**	0.74	—	0.26
CACCACCAC **3**	—	0.40	0.60
CAACGCAACGCAAC **4**	0.44	0.32	0.22
CAACCGCCAAC **5**	0.33	0.32	0.35

The absorption spectra for **1** and **2**, following photolysis, were remarkably similar to that obtained for **G9** (Fig. 1). It is estimated that migration of the radical cation from either adenine or cytosine to guanine occurs to a significant degree (> 80% migration with < 10% contribution from the radicals of adenine and cytosine). In contrast, migration of the oxidative damage from cytosine to adenine is not significant (< 10% migration); the transient spectrum obtained from photoionisation of **3** (Fig. 2) is a composite spectrum containing components for **A9** and **C9** (identified by major optical absorption bands at 330 and 290 nm respectively) reflecting the distribution shown in Table 1. An interpretation of this result is that alternative chemical pathways for loss of the oxidising cytosine radical compete with electron transfer.

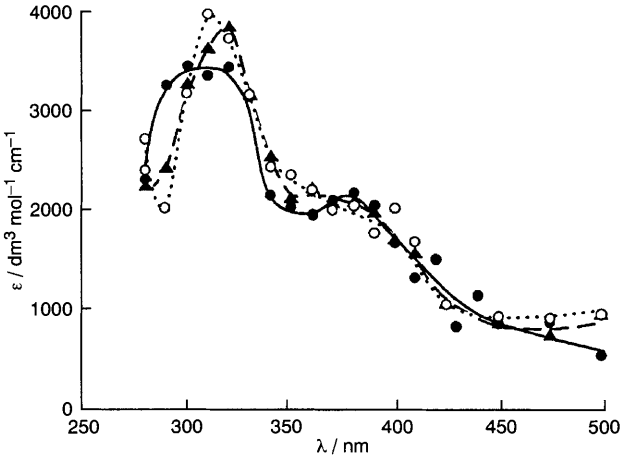

Fig. 1 Transient optical absorption spectra determined 5 μs after laser flash photolysis (193 nm) of an oxygenated aqueous solution of **G9** (–●–), **1** (-- ▲ --), **2** (···○···)

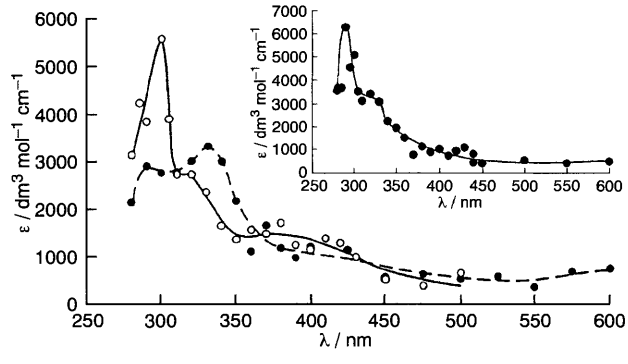

Fig. 2 Transient optical absorption spectra determined 5 μs after laser flash photolysis (193 nm) of an oxygenated aqueous solution of **A9** (--●--), **C9** (–○–) and **3** [insert spectra, (–●–)]

Oligodeoxynucleotides (**4**,**5**) containing adenine, guanine and cytosine were used to determine whether oxidative damage formed at adenine could be transferred to guanine *via* a linking chain of either one or two cytosine molecules. Based on the evidence above with **1**, **2** and **3**, radical cations of cytosine are predicted to transfer to guanine only. The most obvious spectral difference between the transient species of **4**, **5** and **G9** (Fig. 3) is in the spectral region 350–420 nm. The oxidation products of the guanine moiety absorb strongly in this region, whereas those of adenine and cytosine do not (see Fig. 2 for comparison). The

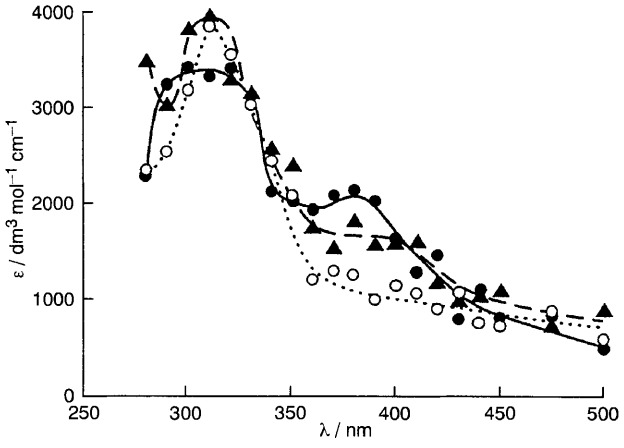

Fig. 3 Transient optical absorption spectra determined 5 μs after laser flash photolysis (193 nm) of an oxygenated aqueous solution of **G9** (–●–), **4** (-- ▲ --) and **5** (···○···)

dissimilarity of transient photoionisation spectra of **4** and **5** with that for **G9** indicates that there is incomplete migration of the oxidative damage to guanine. The magnitude of the spectral absorption bands of **4** and **5** in the region 350–420 nm is consistent with that predicted for insignificant migration of the oxidising radical from adenine to guanine *via* the cytosine moiety.

These preliminary results indicate that migration of oxidative damage in single stranded oligodeoxynucleotides occurs between certain neighbouring nucleic acid bases. This ability to migrate is severely hindered when separated by a third, less easily oxidised base, *e.g.* cytosine as shown in these models. The evidence suggests, for single stranded samples, that the distance of migration is only upto 2–3 nucleic acid bases.

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