Singlet Oxygen Sensitisation by Excited State DNA

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Singlet oxygen quantum yields from the triplet excited states of DNA, nucleotides, dinucleosides, purine and pyrimidine bases in solution have been determined—for guanine-containing moieties no singlet oxygen was detected, and possible implications pertaining to DNA photodamage are discussed.

Previous studies[1] have shown that X-irradiating frozen solutions (80 K) of calf thymus DNA produces optical emissions. By comparison of both the emission spectra and their kinetics with those produced from UV excited nucleotides, the emissions have been assigned to phosphorescence from triplet guanine and thymine.

The formation of solute excited states by ionising radiation may occur by four different mechanisms, namely (*i*) direct excitation, (*ii*) ion-recombination, (*iii*) energy transfer from excited solvent and (*iv*) sub-excitation electrons.[2] If triplet excited state bases are produced then their reactivity with molecular oxygen needs to be established.

Two possible reactions of triplet excited bases with oxygen are electron transfer eqn. (1) and energy transfer eqn. (2).

$$^3\text{Base} + O_2 \rightarrow \text{Base}^{\bullet+} + O_2^{\bullet-} \quad (1)$$

$$^3\text{Base} + O_2 \rightarrow \text{Base} + O_2(^1\Delta_g) \quad (2)$$

Evidence that triplet state bases do participate in electron-transfer reactions has been obtained by fitting the rate constants for quenching of excited triplet bases by electron acceptors to the Rehm–Weller equation.[3,4] For this study, the triplet base energy was higher than the triplet state energy of the electron acceptors making triplet-triplet energy transfer a viable relaxation pathway.

Alternatively, the triplet state base could transfer energy to ground state oxygen *via* a type II photodynamic mechanism[5] resulting in the production of the highly oxidising species singlet oxygen, $O_2(^1\Delta_g)$ [eqn. (2)]. This is possible because the triplet states of DNA bases and nucleotides have sufficient energy (typically >300 kJ mol^{-1})[6] to generate $O_2(^1\Delta_g)$ [ground state $O_2 \longleftrightarrow O_2(^1\Delta_g)$ energy gap is 94.5 kJ mol^{-1}]. The singlet oxygen quantum yield (Φ_Δ) is related to the triplet quantum yield (Φ_T) by eqn. (3) where S_Δ is the fraction of

$$\Phi_\Delta = \Phi_T S_\Delta \quad (3)$$

triplet states quenched by O_2 giving $O_2(^1\Delta_g)$. However, despite adequate triplet quantum yields for bases, nucleosides and nucleotides,[7,8] $O_2(^1\Delta_g)$ generation and Φ_Δ of these species are unreported.

Solutions of DNA, the nucleotides 2′-deoxyguanosine 5′-monophosphate (GMP) and 2′-thymidine 5′-monophosphate (TMP), the dinucleosides thymidylyl(3′→5′)-2′-deoxyadenosine (T-A) and the bases guanine, thymine, adenine, cytosine and uracil in either MeCN or D_2O (10^{-4}–10^{-5} mol dm^{-3}) were irradiated with 248 nm attenuated light from a KrF Questek excimer laser (10 ns, 0–1.5 mJ per pulse). The 1270 nm luminescence from $O_2(^1\Delta_g)$ was detected at right angles to the laser beam, using a North Coast EO-817P liquid nitrogen cooled germanium photodiode, after passing through a 1270 nm silicon filter (30 nm bandpass). Values of Φ_Δ were determined by comparison of laser power/$O_2(^1\Delta_g)$ signal graphs using two standards, anthracene ($\Phi_\Delta^{MeCN} = 0.66$) and acridine ($\Phi_\Delta^{MeCN} = 0.88$) [note that the Φ_Δ of these compounds were determined prior to the above experiment by comparison to benzophenone as standard ($\Phi_\Delta^{MeCN} = 0.37$)[9] using a 351 nm XeF excimer laser excitation source].

Results for the systems studied are shown in Table 1. Time-resolved $O_2(^1\Delta_g)$ luminescence signals were detected for triplet excited state cytosine, thymine, adenine and uracil in MeCN. Typically, the singlet oxygen lifetime (τ_Δ) calculated from these decays was 70 μs, consistent with literature values for τ_Δ in MeCN.[10] Similarly in D_2O, decays consistent with the expected τ_Δ (67 μs) for this solvent,[10] were observed for triplet excited state thymine, TMP and T-A. Representative $O_2(^1\Delta_g)$ decays are shown in Fig. 1. For the bases uracil and thymine in MeCN, the near equivalent values of Φ_Δ and Φ_T indicates that a type II photodynamic mechanism predominates. The Φ_Δ values of thymine, uracil, cytosine and adenine in MeCN were determined to be 0.07, 0.13, 0.02 and 0.03 respectively.

Table 1 Singlet oxygen generation properties for DNA, dinucleosides, nucleotides and purine and pyrimidine bases in either D_2O or MeCN. Accurate singlet oxygen quantum yields (Φ_Δ) for some bases in MeCN are shown, and estimated Φ_Δ for bases, nucleotides, dinucleosides and DNA in D_2O are also quoted. Values for triplet quantum yields (Φ_T) for thymine and uracil in MeCN and for thymine and TMP in water are taken from refs. 7 and 8 respectively

Compound	Solvent	$O_2(^1\Delta_g)$ signal	Φ_Δ	Φ_T
Thymine	MeCN	Yes	0.07 ± 0.010	0.06[7]
Uracil	MeCN	Yes	0.13 ± 0.010	0.20[7]
Adenine	MeCN	Yes	0.03 ± 0.005	—
Cytosine	MeCN	Yes	0.02 ± 0.005	—
Thymine	D_2O	Yes	≈0.01[a]	0.006[8]
Guanine	D_2O	No	<0.005[a]	—
2′-Deoxyguanosine 5′-monophosphate (GMP)	D_2O	No	<0.005[a]	—
2′-Thymidine 5′-monophosphate (TMP)	D_2O	Yes	≈0.01[a]	0.015[8]
Thymidylyl(3′→5′)-2′-deoxyguanosine (T-G)	D_2O	No	<0.005[a]	—
Thymidylyl(3′→5′)-2′-deoxyadenosine (T-A)	D_2O	Yes	>0.005[a]	—
DNA	D_2O	No	<0.005[a]	—

[a] Estimated Φ_Δ value by comparison of the $O_2(^1\Delta_g)$ signal with that of the DNA bases in MeCN for which accurate Φ_Δ were determined.

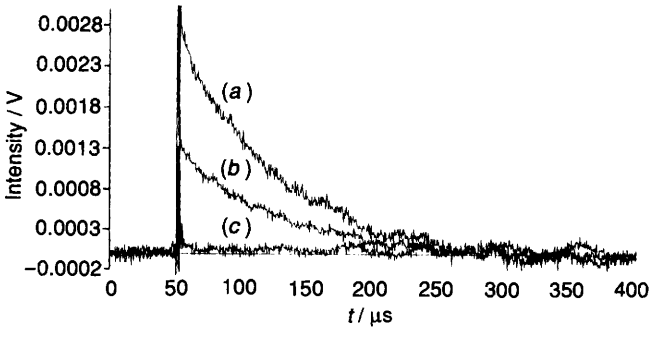

Fig. 1 Singlet oxygen luminescence decays of, (*a*): uracil in MeCN (*b*): thymine in MeCN and (*c*): GMP in D_2O. Addition of a specific singlet oxygen quencher, azide anion, to all samples gave traces analogous to (*c*). Further kinetic analysis of the data gave lifetimes for singlet oxygen (τ_Δ) of 70 μs in MeCN and 67 μs in D_2O (for samples generating singlet oxygen in this solvent) which are consistent with literature values.[10] All solutions were air saturated.

Further, the Φ_Δ for thymine and TMP in D_2O were estimated (by comparing signals to those obtained for the bases in MeCN) to be 0.01 and a signal just above the detectable limit of the instrument (equivalent to $\Phi_\Delta = 0.005$) was observed for the dinucleoside T-A. As well as τ_Δ measurements, validation of the $O_2(^1\Delta_g)$ signal with low Φ_Δ systems was achieved by addition of a specific $O_2(^1\Delta_g)$ quencher, namely azide.[5] For guanine, GMP, T-G and DNA in D_2O no detectable $O_2(^1\Delta_g)$ signal was observed. The types of curves obtained for guanine containing systems are illustrated by Fig. 1(*c*). The decay profile remained unchanged in the presence of azide.

It is evident that a system containing a guanine moiety does not apparently sensitise $O_2(^1\Delta_g)$. This is particularly interesting in the case of guanine dinucleoside systems, *e.g.* T-G, because the triplet thymine residue has a lower energy triplet state,[6] *i.e.* ^{3}TMP = 315 kJ mol^{-1}; ^{3}GMP = 325 kJ mol^{-1}. Thus, if triplet state guanine is formed, triplet-triplet energy transfer would be expected to occur to give triplet state thymine, which we have established as being capable of forming $O_2(^1\Delta_g)$ by sensitisation. Lee and Rodgers[11] have recently shown that of the five nucleotides, only GMP (and also DNA itself) quenches dye-sensitised $O_2(^1\Delta_g)$. This would suggest to us that any $O_2(^1\Delta_g)$ produced from triplet state of guanine containing residues is rapidly quenched by the guanine base. If this is correct then a further mechanism for the photodamage of DNA may be envisaged where $O_2(^1\Delta_g)$ produced *via* a type II photodynamic mechanism from the DNA excited triplet state is quenched by a close proximity guanine residue. This leads to oxidative damage of the DNA chain with the damage initially being located on guanine sites.

We are now working on establishing the mode of interaction of triplet excited guanine with molecular oxygen. We must conclude from our results that this species does not generate $O_2(^1\Delta_g)$. However, we cannot rule out the possibility of triplet excited guanine sensitising $O_2(^1\Delta_g)$ but the $O_2(^1\Delta_g)$ formed rapidly reacting with ground state guanine.[11] The alternative to this is the electron transfer process given in eqn. (1). Preliminary UV 248 nm flash photolysis experiments in oxygenated aqueous solutions of GMP have found evidence for the formation of the $GMP^{\bullet+}$ radical, $\lambda_{max} = 320$ nm.[12] Further work investigating the yield of the $GMP^{\bullet+}$ radical in solutions of different oxygen concentrations is underway to establish these initial findings.

We thank the SERC for the suport of this work through a studentship to S. M. B. and the SERC Central Laser Facility for providing the facilities to carry out these experiments.

Received, 7th January 1994; Com. 4/00108G

References

1 G. J. Smith, *Radiat. Res.*, 1976, **68**, 163.
2 A. Singh, *Radiat. Res. Rev.*, 1972, **4**, 1.
3 T. J. Kemp, A. W. Parker and P. Wardman, *J. Chem. Soc., Chem. Commun.*, 1985, 1377.
4 T. J. Kemp, A. W. Parker and P. Wardman, *J. Chem. Soc., Perkin Trans. 2*, 1987, 397.
5 C. S. Foote, *Free Radicals in Biology*, ed. W. A. Pryor, 2, Academic Press, New York, 1976, pp. 85–133.
6 M. Daniels, *Photochemistry and Photobiology of Nucleic Acids*, ed. S. Y. Wang, Academic Press, New York, 1976, vol. 1, pp. 23–108.
7 C. Salet and R. V. Bensasson, *Photochem. Photobiol.*, 1975, **22**, 231.
8 C. Salet, R. V. Bensasson and R. S. Becker, *Photochem. Photobiol.*, 1979, **30**, 325.
9 S. K. Chattopadhyay, C. V. Kumar and P. K. Das, *J. Photochem.*, 1984, **18**, 293.
10 A. A. Gorman and M. A. J. Rodgers, *Handbook of Organic Photochemistry*, ed. J. C. Scaiano, CRC Press, Boca Raton, USA, 1989, vol. II, pp. 229–250.
11 P. C. C. Lee and M. A. J. Rodgers, *Photochem. Photobiol.*, 1987, **45**, 79.
12 L. P. Candeias, P. O'Neill, G. D. D. Jones and S. Steeken, *Int. J. Radiat. Biol.*, 1992, **61**, 15.