Electron-transfer Quenching of Triplet State Thymine and Uracil

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Triplet thymine and uracil, generated by 249 nm laser flash photolysis in MeCN solution, are quenched by a variety of electron acceptors at rates corresponding to the Rehm–Weller relation, signifying an electron-transfer mechanism.

E.s.r. measurements of γ-irradiated solid DNA indicate charge separation occurs to give positive centres localised on guanine.[1] An enhanced yield of such centres in the presence of radiosensitizers (e.g. O_2 or nitroaryl compounds) would support the 'charge sequestration' model of radiosensitization of Adams,[2] but recent work[3] is at variance with earlier e.s.r. observations[4] supporting this mechanism. An alternative possibility is that electronically excited DNA bases transfer an electron to the electroaffinic drug, e.g. p-nitroacetophenone (PNAP). Smith has shown[5] that flash photolysis (white light) of PNAP–guanosine monophosphate mixtures in aqueous solution yields the spectrum of PNAP$^{\cdot-}$ via transfer of an electron from ground state guanosine to triplet state PNAP (^{3}PNAP). In an analogous study, Fisher and Land demonstrated fast attack of triplet 2-methylnaphthoquinone upon pyrimidines by electron transfer.[6]

The advent of powerful u.v. lasers enables the selective excitation of DNA bases to give workable absorptions of the triplet state bases. Solutions of thymine (2.7 × 10^{-4} mol dm^{-3}) and uracil (3.4 × 10^{-4} mol dm^{-3}) in MeCN were excited with 249.1 nm (15 ns, 200 mJ pulses) from an Oxford Lasers Model KX2 Kr/F$_2$ laser to give transients at 310 and 350 nm respectively, in agreement with the data of Salet and Bensasson.[7] The lifetimes of both triplets were reduced on addition of a wide variety of electron-accepting molecules, including the clinical radio-sensitizing drugs metronidazole and misonidazole. Correlations between rate constants and the thermodynamics of electron-transfer quenching of photoexcited species are made most commonly in the form of the Weller equation,[7] i.e. equation (1).

$$k_2 = k_{12}/\{1 + (k_{21}/\kappa^\circ)[\exp(\Delta G_{23}^\ddagger/RT) + \exp(\Delta G_{23}^\circ/RT)]\} \quad (1)$$

where $\Delta G_{23}^\ddagger = (\Delta G_{23}^\circ/2) + \{(\Delta G_{23}^\circ/2)^2 + [\Delta G_{23}^\ddagger(0)]^2\}^{\frac{1}{2}}$

and $\Delta G_{23}^\circ = F[E^\circ(D^{\cdot+}/D) - E^\circ(A/A^{\cdot-})] + \Delta E_{0,0} + w_p - w_r$
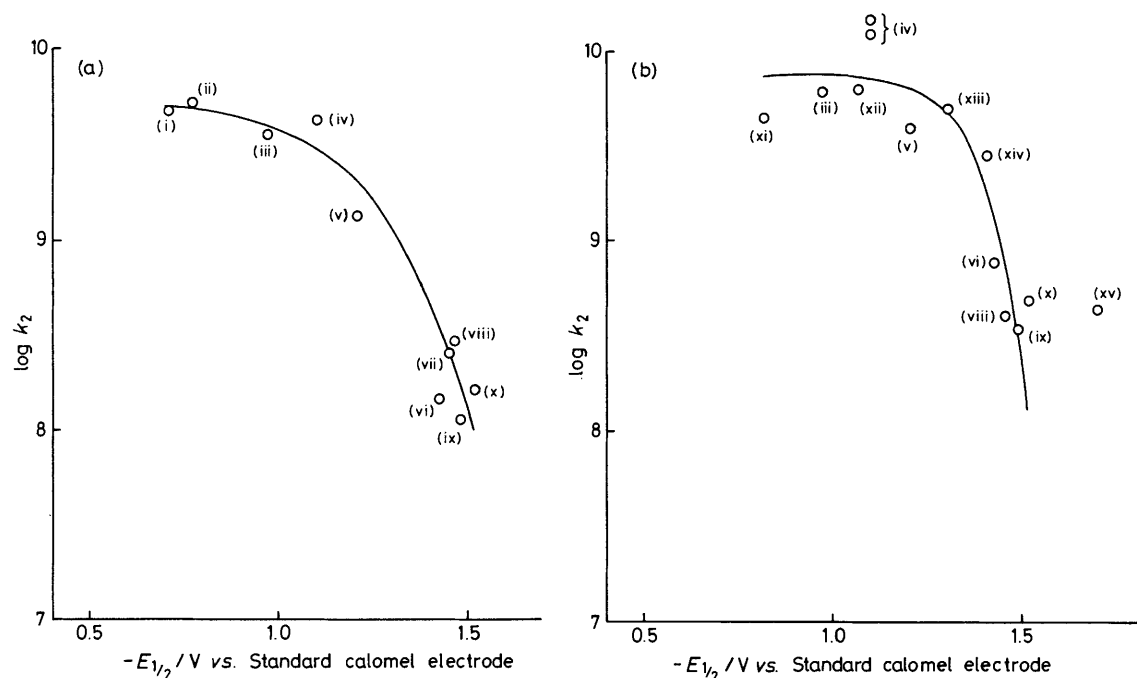

Figure 1. Correlation of log k_2 with acceptor reduction potential[9] in MeCN for the quenching of (a) triplet excited thymine and (b) triplet excited uracil by electron acceptors. The highest concentration (mol dm^{-3}) used for each acceptor is given in parentheses, although a factor of at least 6 to 7 was employed in variation of each acceptor concentration. (a): (i), 1,4-Dinitrobenzene (1.0 × 10^{-4}); (ii), 2-methylnaphthoquinone (1.5 × 10^{-4}); (iii), misonidazole (4.0 × 10^{-5}); (iv), metronidazole (4.0 × 10^{-5}); (v), 4-nitrotoluene (1.9 × 10^{-4}); (vi), nitroethane (2.9 × 10^{-3}); (vii), 1-nitropropane (2.5 × 10^{-3}); (viii), nitromethane (2.0 × 10^{-3}); (ix), 1-nitrobutane (4.0 × 10^{-3}); (x) 1-nitropentane (3.2 × 10^{-3}). The full line-theoretical curve was derived from the Rehm–Weller equation using the parameters $E^\circ(D^{\cdot-}/D) = 1.80 \pm 0.05$ V and $\Delta G_{23}^\ddagger(0) = 16.5$ kJ mol^{-1}. N.b. Two further acceptors, tetranitromethane and 5-bromouracil are omitted because they do not accept electrons reversibly but by dissociative electron attachment. Their rates were near diffusion-controlled. (b): (iii)—(vi) and (viii)—(x) as for (a). (xi), 1,2-dinitrobenzene (1.4 × 10^{-5}); (xii), 1-chloro-4-nitrobenzene (7.6 × 10^{-5}); (xiii), fumaronitrile (1.6 × 10^{-4}); (xiv), 2,6-dimethyl-1-nitrobenzene (1.1 × 10^{-4}); (xv), 2-methyl-2-nitropropane (2.9 × 10^{-3}). The full line-theoretical curve was derived from the Rehm–Weller equation using the parameters $E^\circ(D^{\cdot-}/D) = 2.0 \pm 0.1$ V and $\Delta G_{23}^\circ(0) = 6.7$ kJ mol^{-1}.
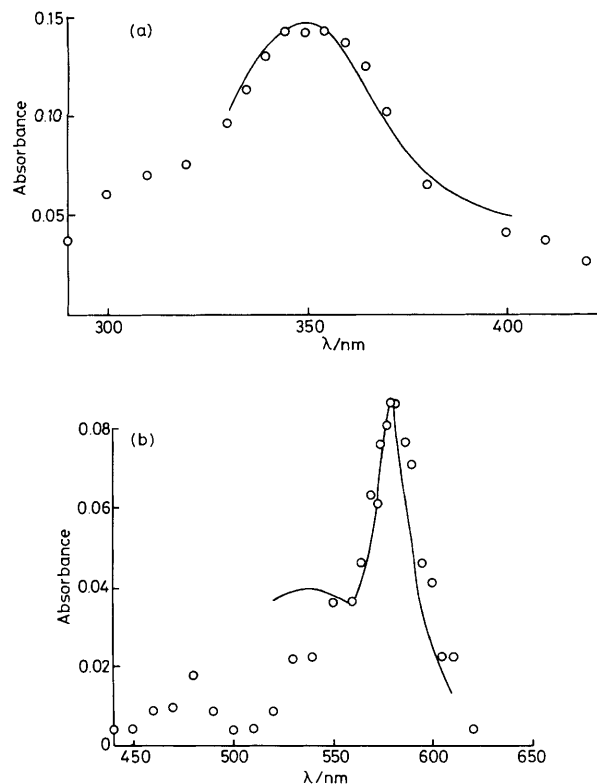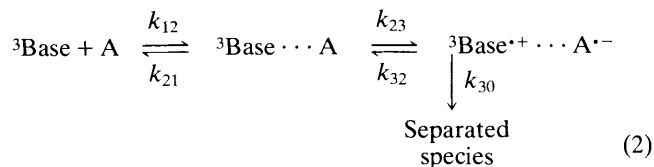
Figure 2. The transient spectrum ($\circ$) formed on 249 nm laser flash photolysis of thymine (2.6×10^{-4} mol dm^{-3}) with (a) $C(NO_2)_4$ (9.32×10^{-4} mol dm^{-3}) and (b) galvinoxyl (9.5×10^{-5} mol dm^{-3}), in MeCN. The full line-spectra of $C(NO_2)_3{}^-$ and the galvinoxyl anion were taken from refs. 11 and 12 respectively; the latter shows small additional absorption in the region 525—540 nm suggestive of an extra species.

The meaning of these terms is detailed in the original paper of Rehm and Weller[8] and in later reviews, but in brief k_2 is the second-order quenching rate constant and the other subscripts refer to the individual stages of the electron transfer, viz. equation (2).

$$^3\text{Base} + A \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} {}^3\text{Base} \cdots A \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} {}^3\text{Base}^{\cdot+} \cdots A^{\cdot-} \quad \downarrow k_{30}$$

$$\text{Separated species} \qquad (2)$$

$\Delta G_{23}^{\ddagger}(0)$ is the intrinsic barrier to electron transfer (when ΔG_{23}° equals zero) for the series of reactions when one reactant (in our case ^{3}Base) is not varied while $\Delta E_{0,0}$ is the spectro-

scopic energy of ^{3}Base. The two parameters adjusted to optimise the fit were $\Delta G_{23}^{\ddagger}(0)$ and $E^{\circ}(D^{\cdot+}/D)$ while k_{12} was taken as 1.0×10^{10} dm^3 mol^{-1} s^{-1}. The fits, shown in Figure 1, indicate that A acts as an electron acceptor in the quenching process rather than an energy acceptor, although both reactions are thermodynamically feasible in view of the large values of $\Delta E_{0,0}$ for ^{3}Base for thymine and uracil.

In certain cases, where $A = C(NO_2)_4$ and galvinoxyl, experiments conducted where >90% of the incident 249.1 nm photon pulse was directly absorbed by the base, gave evidence for the species $A^{\cdot-}$ or its breakdown product, e.g. $C(NO_2)_3{}^-$ and the galvinoxyl anion respectively,[10—12] (Figure 2). The fractions of triplet base producing negative ions were, for $C(NO_2)_3{}^-$, 0.69 ± 0.03 (from thymine) and 0.42 ± 0.03 (from uracil) and for galvinoxyl, 0.023 ± 0.002 (from thymine) and 0.0130 ± 0.0005 (from uracil); presumably the ready dissociation of $C(NO_2)_4{}^{\cdot-}$ accounts for the higher level of charge separation in this case.

Evidently electroaffinic radiosensitizers *can* function by accepting charge from excited DNA in a redox-controlled manner, although further work is necessary to quantify the likely redox relationship in the biological environment.

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