# One-electron Oxidation of the Flavin Triplet State as Studied by Laser-Flash Photolysis

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One-electron oxidation of the flavin triplet state occurs in its reaction with tetranitromethane.

Photoreactions of flavins have been extensively studied, with both intramolecular and intermolecular photoreduction being observed.[1] Such reactions involve a one-electron or two-electron (equivalent) reduction of the flavin triplet state. We report here, in contrast, a one-electron oxidation of the lumiflavin (**1**) triplet state in its reaction with the oxidant, tetranitromethane (TNM).

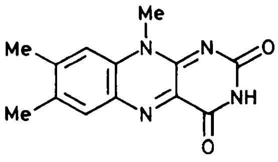
Flash photolysis of lumiflavin in anaerobic aqueous solution (pH 7) in the presence of TNM ($10^{-3}$ M) reveals the transient absorption of the well known neutral flavin triplet state[2] ($^3$Fl) immediately after the pulse (Figure 1). The triplet state decays over 2 μs (*cf.* τ 20 μs in the absence of TNM) to reveal a new transient absorption (Figure 1, *t* 2 μs, $\lambda_{max.}$ 340 and 650 nm). The build-up of the intense short wavelength absorption at 340 nm matches the decay of $^3$Fl (Figure 1, inset). By comparison with literature data,[3] the transient species absorbing at 340 nm can confidently be assigned to the nitroform radical [$C(NO_2)_3^-$] produced upon one-electron reduction of tetranitromethane. The transient absorbing at 650 nm does not resemble the spectrum of known flavin species (*e.g.* flavin semireduced radical) and can be assigned to the one-electron oxidised radical ($Fl^{\cdot +}$) [equation (1)].

$$^3Fl + C(NO_2)_4 \rightarrow Fl^{\cdot +} + C(NO_2)_3^- + NO_2 \qquad (1)$$

Under the above conditions, greater than 90% of the initially formed triplet species have reacted with TNM. Hence using the extinction coefficient[2] of $^3$Fl ($\varepsilon_{690}$ 4400 dm$^3$ mol$^{-1}$ cm$^{-1}$) an estimate of $\varepsilon_{650}$ 2300 dm$^3$ mol$^{-1}$ cm$^{-1}$ for $Fl^{\cdot +}$ can be made.

Variation of the pH in the range 3—8 showed a marked pH dependence in the reactivity of the flavin triplet state (monitored at 700 nm) towards TNM (Figure 2), revealing an apparent kinetic p$K_a$ of *ca.* 4.9. This value corresponds closely with the known p$K_a$ of the lumiflavin triplet state (4.45[2]), showing that the reactivity of the neutral triplet state ($^3$Fl) is far greater than that of the protonated form ($^3FlH^+$).

Thermodynamic calculations[4,5] have previously explained the differing reactivity of $^3$Fl versus $^3FlH^+$ towards electron donors. A similar approach applied to one-electron loss by the flavin suggests that the p$K_a$ for protonation of the oxidised flavin radical ($Fl^{\cdot +}$) is less than that for $^3$Fl (*i.e.* <4.45). Consistent with the above considerations, at pH values less



(**1**)

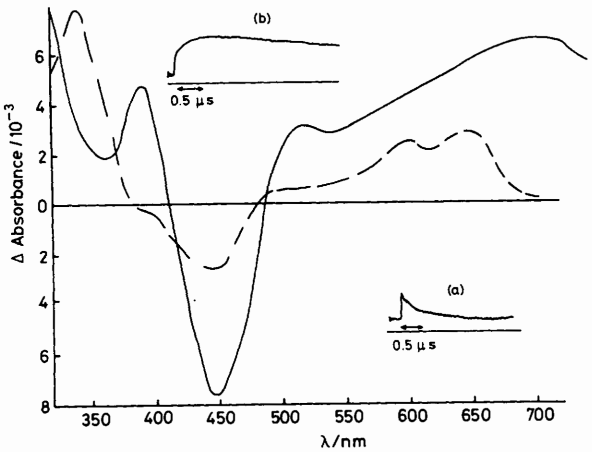


**Figure 1.** Transient spectra obtained upon laser flash photolysis of lumiflavin ($5 \times 10^{-5}$ M) in nitrogen saturated aqueous solution, plus TNM ($10^{-3}$ M) pH 7 (excitation at 353 nm): (——) spectra obtained immediately (0.1 ns) and (- - -) 2 µs after the laser pulse. Inset: oscilloscope traces showing (a) the decay of $^3Fl$ at 700 nm, (b) the build-up of $[C(NO_2)_3^-]$ absorption at 340 nm.

than 4, the reaction of $^3FlH^+$ with TNM yields a quite different residual transient spectrum to that at pH 7 ($\lambda_{max.}$ 340 and 560 nm). The new species ($\lambda_{max.}$ 560 nm) can be tentatively assigned to the protonated flavin oxidised radical ($FlH^{\bullet 2+}$).

The observation of the $Fl^{\bullet +}$ radical is of interest owing to its involvement in the triplet–triplet and triplet–ground state reactions [*e.g.* equation (2)] proposed to account for flavin photodegradation in the absence of added electron donors.[6]

$$^3Fl + Fl \xrightarrow{H^+} FlH^{\bullet} + Fl^{\bullet +} \qquad (2)$$

The spectral features of $Fl^{\bullet +}$ reported here could account for the failure to detect the absorption of $Fl^{\bullet +}$ in such reactions

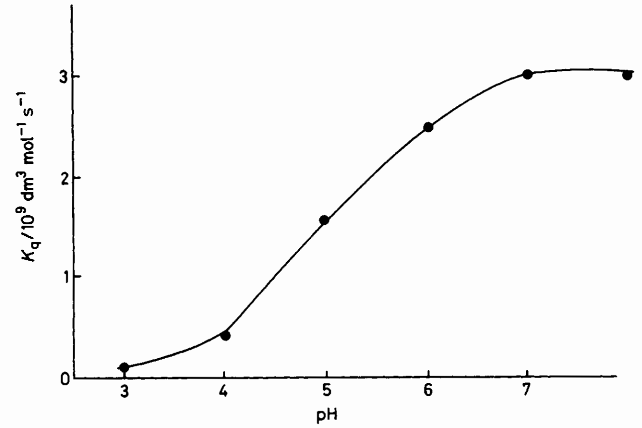


**Figure 2.** The pH dependence of the bimolecular rate constant of quenching ($K_q$) of $^3Fl$ by tetranitromethane.

due to overlap with the absorption of $FlH^{\bullet}$ and its relatively low extinction coefficient.

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