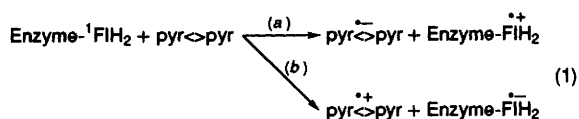# One-electron Reduction of 1,5-Dihydroflavins in Aqueous Solution: a Pulse Radiolysis Study

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One-electron reduction of 1,5-dihydroflavins with $e(aq)^-$ yields a three-electron reduced flavin species ($FlH_2{}^{\cdot-}$), which protonates with an associated pK_a value of 6.1, whereas the reaction of 1,5-dihydroflavins with $CO_2{}^{\cdot-}$ yields a similar, but not identical flavin species, which may be an adduct radical.

The enzyme DNA photolyase catalyses the photochemical conversion of pyrimidine dimers (pyr<>pyr) in DNA into pyrimidines (pyr), thus reversing the effect of far UV (200–300 nm) radiation. A general model has been proposed[1,2] according to which DNA photolyases function by formation of the reduced flavin excited singlet state (enzyme–$^1FlH_2$, FlH_2 = 1,5-dihydroflavin) at the catalytic centre. This species then transfers an electron to [eqn. (1a)] or from the pyrimidine dimers [eqn. (1b)] to generate a radical ion pair, resulting in dimer splitting, followed by charge neutralization.
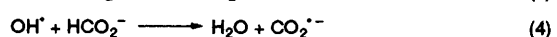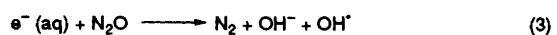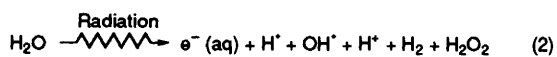
$$\text{Enzyme-}^1FlH_2 + \text{pyr}\!<\!>\!\text{pyr} \xrightarrow{(a)} \text{pyr}\!<\!>\!\text{pyr} + \text{Enzyme-}FlH_2{}^{\cdot+}$$
$$\xrightarrow{(b)} \text{pyr}\!<\!>\!\text{pyr}^{\cdot+} + \text{Enzyme-}FlH_2{}^{\cdot-} \tag{1}$$

Regarding the direction of electron transfer in eqn (1), molecular-orbital calculations predict that both the pyr<>pyr cation radical[3] and anion radicals[4] are prone to decay by ring splitting to constituent monomers. Intuitively, reduced flavins would be expected to be electron donors rather than acceptors. However, several lines of argument were proposed[5] in favour of electron abstraction by enzyme–$^1FlH_2$. Such a process cannot be ruled out as the electron affinity of FlH_2 would be increased markedly (ca. 2.5 eV) upon excitation.[6] Such a process of electron gain by $^1FlH_2$ would result in the formation of the as yet undetected species $FlH_2{}^{\cdot-}$ ($FlH_2{}^{\cdot-}$ = three-electron reduced flavin).

Recent studies employing picosecond flash photolysis[5] have detected a transient species (λ_{max} 400 nm) possibly resulting from electron-transfer between the excited state of the reduced flavin (enzyme–$^1FlH_2$) and the pyrimidine dimer. However, the nature of this species could not be established from its spectra.

Hence, a knowledge of the properties of the reduced flavin species $FlH_2{}^{\cdot-}$ could help to distinguish between the two different pathways of DNA photolyase action.

One-electron reduction of 1,5-dihydroFMN (FMN = flavin mononucleotide) (formed photolytically using EDTA as reductant) at pH 5.5 was carried out using the pulse radiolysis technique in argon saturated solution (containing formate anions as OH scavengers). A transient species was observed (λ_{max} 400 and 320 nm after correction for ground state depletion) immediately after the electron decay (ca. 2 μs after the pulse). However, under these conditions both $e^-(aq)$ and formate radicals ($CO_2{}^{\cdot-}$) are formed and so comparative experiments in nitrous oxide saturated solution were also carried out, where only $CO_2{}^{\cdot-}$ would be present, eqns. (2)–(4).

The formate radical anion was formed via reaction of the hydroxyl radical [formed in eqns. (2) and (3)] with formate anion [eqn. (4)]. The concentrations of formate anion were sufficient to prevent a significant reaction of EDTA with the hydroxyl radical.

Radiation
$$H_2O \xrightarrow{\text{\Large\/\backslash\!/\backslash\!/\backslash\!/\backslash}} e^-(aq) + H^{\cdot} + OH^{\cdot} + H^+ + H_2 + H_2O_2 \tag{2}$$
$$e^-(aq) + N_2O \longrightarrow N_2 + OH^- + OH^{\cdot} \tag{3}$$
$$OH^{\cdot} + HCO_2{}^- \longrightarrow H_2O + CO_2{}^{\cdot-} \tag{4}$$

In this case only a weak transient absorption was observed (ca. 10% of that in argon, see later) and this was subtracted (after correction for the differing yields as defined by the G value) from the observed spectrum following the reaction of $e^-(aq)$ with 1,5-dihydroFMN. The resulting transient spectrum corrected for ground state depletion is shown in Fig. 1.

Monitoring the electron decay at 700 nm led to a rate constant of reaction of 1,5-dihydroFMN with $e^-(aq)$ of 6×10^9 mol^{-1} s^{-1}.

One-electron reduction of 1,5-dihydroFMN with $e^-(aq)$ was also carried out at pH 9.3 and a transient spectrum (λ_{max} 370 nm, Fig. 1) different to that at pH 5.5 was observed, again after correction for the small contribution from the reaction of $CO_2{}^{\cdot-}$ (see later). At pH 9.3 a rate constant of reaction of 1,5-dihydroFMN with $e^-(aq)$ of 3×10^9 mol^{-1} s^{-1} was determined. Essentially the same transient spectrum was observed from pH 9 to pH 12.

An attempt was made to determine the pK_a of the initial product of reaction of $e^-(aq)$ with dihydroflavin by monitoring the absorbance at 370 nm (Fig. 1, inset). However, measurements could not be made below pH 5 owing to the reaction of $e^-(aq)$ with H^+. A least-squares fitting process yielded a value of 6.1 ± 0.4 for the pK_a, with the large error limits reflecting the restricted range of pH values available.

Two-electron reduction of oxidised flavins by chemical, photochemical or radiolytical means has been well documented previously.[7,8] Three different protolytic forms are known,[8] namely the cation 1, neutral 2 and anionic species 3.

This study demonstrates that a further one-electron reduction of 1,5-dihydoflavin can be accomplished using the hydrated electron in neutral solution to give a spectrally distinct species. It is also clear that this species possesses a pK_a of 6.1. A plausible, but not proved, structural representation
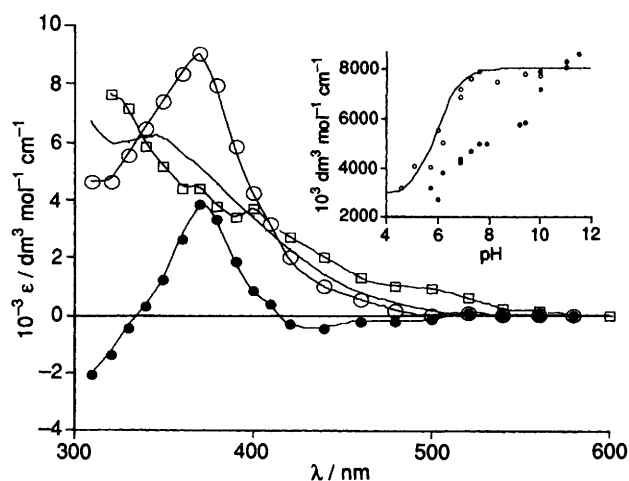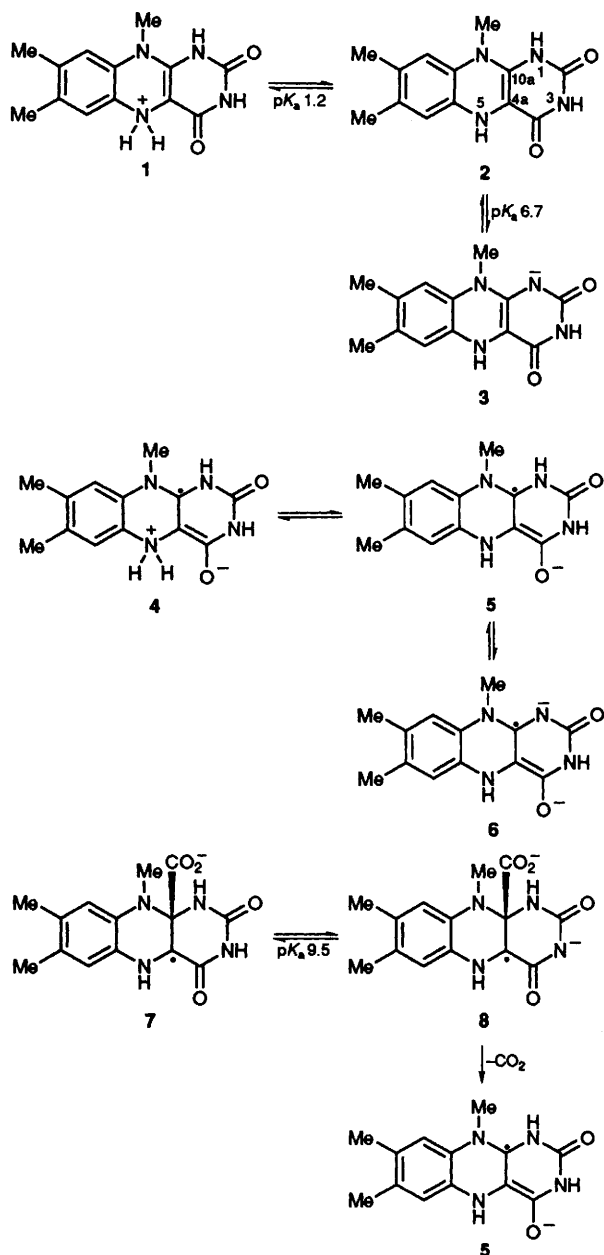

Fig. 1 Transient absorption spectra observed after the reaction of $e^-(aq)$ with $FMNH_2$; pH 9.3, difference spectrum (●) and spectrum corrected for ground state depletion (○); pH 5.5, spectrum corrected for ground state depletion (□); spectrum of $FMNH^-$ at pH 9.3 (---). Inset: Variation of transient absorption coefficient at 370 nm as a function of pH after the reaction of $e^-(aq)$ (○) and $CO_2{}^{\cdot-}$ (●) with $FMNH_2$.

is shown below, **5**, for the species present at pH > 8. This species corresponds to the anion radical species, $FlH_2^{•-}$.

In principle, the pK_a observed at 6.1 could correspond to either a deprotonation or protonation. However, if it were a deprotonation [*e.g.* at N(1) to give structure **6**], this would represent essentially no change in the acidity of the N(1) position upon electron-gain. In contrast, if the pK_a represents a protonation, [*e.g.* at N(5) to give structure **4**], then the increase in basicity of five units would be entirely consistant with the increase in electron density.

The fate of the so-formed 'super reduced flavin' is unknown. Reduction of oxidised flavins beyond the normal two-

electron equivalence point has been demonstrated in steady-state radiolysis studies of flavins at pH < 2 or high redox potential flavins (7-chloro or 2-thio-flavins) at neutral pH.[8] In the latter case, the four-electron reduction was irreversible (with respect to oxygen) as also reported for the borohydride reduction [at the C(4) carbonyl function] of flavins.[9] Reduction at neutral pH apparently does not readily occur, however, this does not necessarily mean that the reversible redox couple $FlH_2/FlH_2^{•-}$ could not function at the enzyme active site.

The reaction of $CO_2^{•-}$ with 1,5-dihydroFMN was studied in more detail in N_2O saturated solutions. A slow reaction of $CO_2^{•-}$ was observed throughout the pH range 2–12, the rate constant of reaction of $CO_2^{•-}$ was 4×10^8 mol^{-1} s^{-1} at pH 2–5 and 10^8 mol^{-1} s^{-1} at pH 9.

The spectrum of the product of the reaction of $CO_2^{•-}$ with 1,5-dihydroFMN at pH 5 and 11 were very similar to those observed following the reaction of e$^-$(aq) at the same pH values. However, at intermediate pH values, the spectra observed between pH 7 and 10 are quite distinct from those observed with e$^-$(aq). A possible explanation is that the species are adduct radicals perhaps of a type shown below (**7**).

Radical addition to oxidised and one-electron reduced flavins has been documented previously for β-alcohol radicals *e.g.* [$^•CH_2CH(OH)Me$].[10] In contrast, radicals such as $CO_2^{•-}$ and the α-carbon radical $Me_2\overset{•}{C}OH$ usually carry out a one-electron reduction.[10] However, such a reduction is much less likely in the case of the two-electron reduced flavin and so addition may become the dominant process.

The main conclusion of this work is that reduced flavins can be further reduced to yield a spectrally distinct species whose properties at pH>8 (single sharp peak, λ_{max} 370 nm) are very similar to the transient intermediate observed during pico-second flash photolysis of the enzyme substrate complex of DNA photolyase enzyme (single sharp peak, λ_{max} 400 nm). The reduced flavin in the enzyme is thought to be in the anionic state *i.e.* the same form as at pH>7 in free solution.

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