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Inter-Flavin Electron Transfer in the Ground State in the Absence of External Electron Donors and Acceptors

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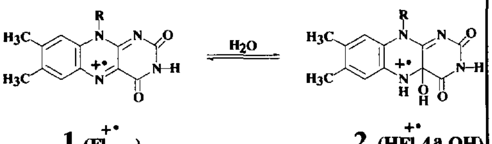
Abstract: *Inter-flavin electron transfer of N5-alkylflavinium cation 3 occurred in the ground state to primarily give the flavosemiquinone 4 and the superoxidized flavin 7. This provided insight into some chemistry of 7: a rapid addition followed by a (second) inter-flavin electron transfer of the derived 4^a-flavin adduct radical 6 giving 3 and, consequently, an additional formation of 4.* Copyright © 1996 Elsevier Science Ltd

$$2Fl \rightleftharpoons Fl_2 \xrightarrow{h\nu} {}^1Fl_2 \longrightarrow {}^3Fl_2 \longrightarrow Fl^{-\bullet} + Fl^{+\bullet} \qquad (1)$$

$$^3Fl + Fl \longrightarrow Fl^{-\bullet} + Fl^{+\bullet} \qquad (2)$$

$$Fl_{ox} \rightleftharpoons \overset{+\bullet}{Fl}_{sox} + e^- \qquad (3)$$



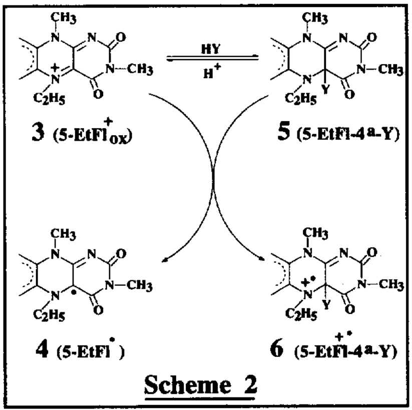
1 $(\overset{+\bullet}{Fl}_{sox})$

2 $(\overset{+\bullet}{HFl}\text{-}4a\text{-}OH)$

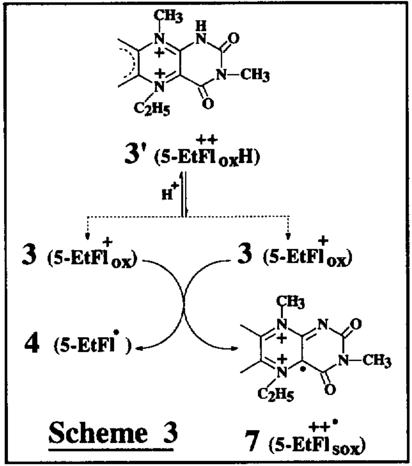
Scheme 1

In the flash photolysis of lumiflavin, excitation of a preformed dimer or more complex aggregate was formulated[1] to proceed through the singlet and triplet states resulting in a splitting of the dimer or aggregate to the flavosemiquinone anion and a flavin radical cation (Eq. 1). Formation of a flavin radical cation in aqueous solution can take place via photoionization.[2] The reaction of the flavin triplet states with another flavin molecule either in the triplet or ground state has been concluded[3] to occur via inter-flavin electron transfer to give the $Fl^{-\bullet}$ and $Fl^{+\bullet}$ radicals (Eq. 2). In the absence of an external electron donor, $Fl^{+\bullet}$ rapidly reacts with H_2O, but the product(s) so formed was (were) not identified. The authors[3] did not spectrophotometrically detect $Fl^{+\bullet}$, but Heelis and coworkers[4] later succeeded in determining the spectral characteristics of this radical which, in contrast with the earlier assumption,[3] did absorb significantly at $\lambda > 550$ nm. Regarding the oxidation state of the $Fl^{+\bullet}$ radical, the term "superoxidized" flavin (abbreviated by the subscript "sox") has been introduced[5] to emphasize the existence of an oxidation state higher than the one of the so-called normal "fully oxidized" flavin (abbreviated as Fl_{ox}; Eq. 3). The superoxidized flavin **1** and derived 4^a-hydroxy-4^a,5-dihydroflavin radical cation **2** are assumed to be key-intermediates in bacterial bioluminescence.[5-7] The existence of flavin derivatives related to **1** and **2** (cf. **7** and **6** [X= OH; OMe]) was proven by applying cyclic voltammetry,[8] while **6** or a derivative has appeared to accomplish the chemical hydroxylation of aromatic substrates.[9]

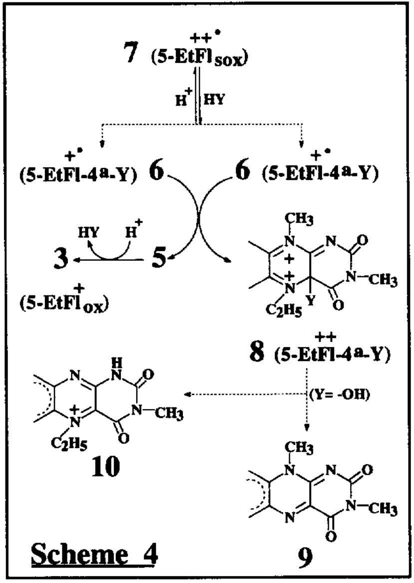
The present studies have shown that inter-flavin electron transfer comparable with the one represented by Eq. 2 may occur in the ground state when started from N5-alkylflavinium cations (e.g. **3**; Scheme 3).



Scheme 2



Scheme 3

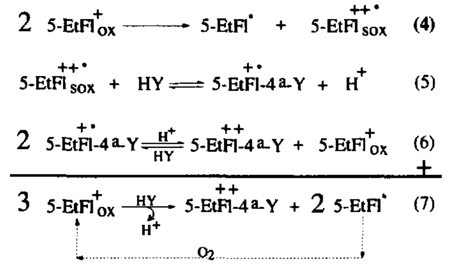


Scheme 4

Quantitative studies on the formation of the 5-ethylflavosemiquinone **4** have substantiated some chemistry of the superoxidized flavin **7**: a rapid addition (**7** + **HY** → **6** + $\mathbf{H^+}$; Y^-= HO^-, Cl^-, Br^-; Scheme 4) followed by a (second) inter-flavin electron transfer of the derived 4^a-flavin adduct radical **6** giving **3** and, consequently, an additional generation of **4**.

In previous studies on the spontaneous formation of the flavosemiquinone **4** (Scheme 2), the flavinium cation **3** has been considered to be the acceptor of an electron (**3** + e^- → **4**) donated by a 4^a-flavin adduct **5** (→ **6** + e^-) both in organic and aqueous solutions.[10] The flavinium cation **3** is in equilibrium with the dihydroflavin pseudobase **5** (Y= OH; pK'_a= 4.15 in 0.1 M citrate buffer). In aqueous solution, in the pH 2 - 7 range at 23 °C a relative initial rate / pH profile was found[10d] to be in agreement with the view depicted in Scheme 2. Consistently, the rate of the radical formation, as indicated by the appearance of **4**, decreased upon increasing the pH in the pH 4.1 - 7.0 range and upon lowering the pH in the pH 4.1 - 2.0 range.

However, further increase of the acidity up to 12 N, using H_2SO_4, $HClO_4$, HCl and HBr, caused a remarkable increase of the rate of the radical formation indicating the occurrence of a different pathway in which the flavin pseudobase was no longer involved. Although slower, spontaneous radical formation even occurred when **3** was shifted to the protonated species **3'** (Scheme 3) as indicated by the appearance of an alloxazinium-like absorption spectrum (Fig. 1; curve a → b → c) and by increased down-field shifts of the signals for the N^{10}-CH_3 and aromatic protons in the PMR-spectra [**3** (CD_3CN): δ= 1.93 (3, t, J= 8 Hz, C-Me); 2.57 (3, s, C-Me); 2.63 (3, s, C-Me); 3.41 (3, s, N-Me); **4.17 (3, s, N^{10}-Me)**; 5.00 (1, C-H); 6.05 (1, C-H); **7.94 (1, s, Ar-H)**; **8.20 (1, s, Ar-H)**. For **3'** (CD_3CN / 5% H_2SO_4): δ= 1.83 (3, t, C-Me); 2.71 (3, s, C-Me); 2.76 (3, s, C-Me); 3.51 (3, s, N-Me); **4.52 (3, s, N^{10}-Me)**; **8.34 (1, s, Ar-H)**; **8.53 (1, s, Ar-H)**]. Spontaneous formation of flavin radicals also took place in acidified organic solutions of **3**. Under some conditions, the reaction times could even be reduced to less than 30 minutes at room temperature (Fig. 2). The flavosemiquinone **4** is stable in the absence of O_2 thus allowing accurate analyses of it based on molecular extinctions established.[10b] The yields of **4**, formed anaerobically under variable experimental conditions in the absence of any additonal compound were found to be in the range of 64 - 69% (cf. Fig. 2, curve f). These results are in good agreement with the sequence of reactions formulated to proceed as summarized in Scheme 5 (Eqs. 4 - 6):



Scheme 5

(Eq. 4): A primary inter-flavin electron transfer of the flavinium cation **3** (Scheme 3) to give the flavosemiquinone **4** and, consequently, the superoxidized flavin **7**. (Eq.5): The addition of H_2O to the superoxidized flavin **7** leading to the formation of the radical cation **6** (Y= OH; Scheme 4). (Eq. 6): An inter-flavin electron transfer of **6** (Scheme 4) resulting in the flavinium cation **3** and a bicationic product **8** which was already observed in the electrochemical oxidation of **5** (Y= OH; OMe) by the release of 2 x 1 electron in two distinct steps.[8] (Eq. 7): As the important consequence of Scheme 5, the overall anaerobic transformation of the flavinium cation **3**, in the absence of other compounds, implies the formation of the flavosemiquinone **4** in a calculated, maximal yield of 66.7 % and of the bicationic product **8** in a calculated maximal yield of 33.3 %.

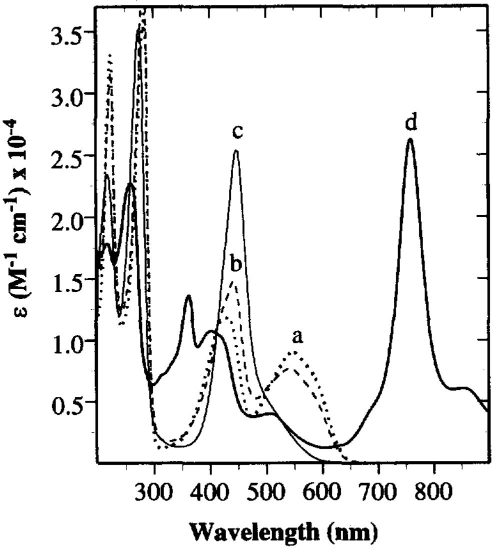


Figure 1. (a) Molecular absorbance of **3** as obtained from a 5 x 10^{-5} M solution in MeCN, λ_{max} at 224, 283, 432 and 548 nm. (b) Spectrum of a mixture of species **3** and **3'** in 12 N H_2SO_4. (c) Molecular absorbance of **3'** in 11.6 N $HClO_4$ (complete protonation), λ_{max} at 219, 275 and 448 nm.
(d) Spectrum of the new chloride in 12 N H_2SO_4 with λ_{max} at 363, 397, 511, 761 and 853 nm used as criteria for the purity of the compound.

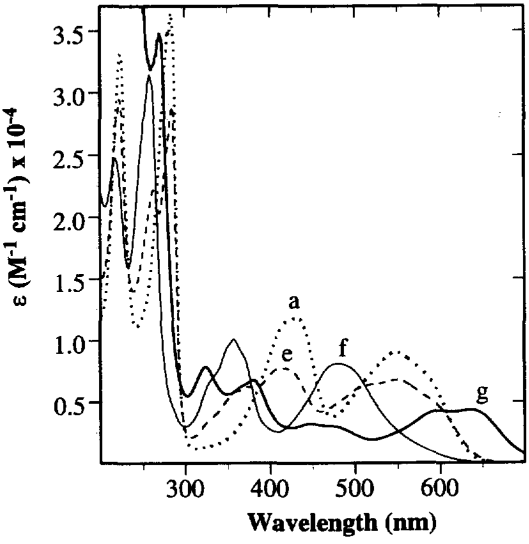


Figure 2. (a) See Figure 1; (e) Spontaneous change of spectrum at 23 °C , 3 min after the addition of conc HCl (HCl / flavin ratio= 10). (f) Spectrum of a mixture of protonated flavosemiquinone **4** and product(s) derived from **8** at the end of the spontaneous, anaerobic process, 30 min after the addition of HCl. (g) Spectral change following the addition of NEt_3 (0.1 vol %) to convert the protonated flavosemiquinone to non-protonated **4** revealing its accurate yield (65.5%).

The electron-deficient nature of **8**, in these experiments *formally* derived from the flavin adduct by the loss of two electrons seems to be supported by the structures of the products of a ready decomposition leading to 3-methyllumiflavin **9** and 5-ethyl-3-methylalloxazinium cation **10** (Scheme 4). In order to increase the yield of **8**, 5-EtFl$^{\bullet}$ was recycled to 5-EtFl$^{+}{}_{ox}$ by air-oxidation (Eq. 7). When both species **4** and **3** were no longer detected, the solution of **8** or a derived product was still found able to achieve the hydroxylation of an aromatic substrate.[9]

In comparable acidic environment, the presence of Cl^- or Br^- caused a considerable increase of the radical formation rate. We suggest that this enhancing anion effect is due to a faster additon of Cl^- (Br^-) to **7** to provide different radical cations **6** (Eq. 5; Y= Cl, Br). In the presence of H_2O, an exchange of Cl^- (Br^-) by HO^- may readily occur in a further stage of the process.

From a more concentrated solution ($\approx 10^{-1}$ M) of **3** in MeCN / HCl, a new solid chloride was isolated which rapidly decomposed in alcoholic and aqueous solutions to give 3-methyllumiflavin **9** as a major product. The decomposition slowed down in 12 N H_2SO_4 to give a remarkable absorption spectrum with high absorbances at higher wavelengths (Fig. 1, curve d). The new compound has reversible redox properties. Tentative mass spectral studies showed the presence of a peak at m/e 334 suggesting the covalent binding of a chlorine atom by the flavin. This peak probably represents a fragmentation of a dimer of a yet unknown oxidation level, in the sense that no conclusion is yet to be drawn whether the product has been derived from **6** or **8**. It is emphasized that a similar spectrum can be obtained in the absence of Cl^- indicating the existence of more than one product with a comparable structure. Dimerization implies the relative stabilization of an intermediate which is also of interest in connection with the early view[1] that inter-flavin electron transfer would take place in a dimer (Eq. 1) or more complex aggregate. The structure elucidation of the new chloride is in progress.

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