Journal of Photochemistry and Photobiology A: Chemistry 225 (2011) 113–116

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Short note

Absolute rate constants for 5-deazariboflavin triplet excited state in one-electron transfer processes

Mónica Barra*, Brett VanVeller, Chad Verberne

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

ARTICLE INFO

Article history:
Received 6 June 2011
Received in revised form 5 September 2011
Accepted 3 October 2011
Available online 10 October 2011

Keywords:
Flavins
Triplet-state quenching
Charge transfer
Laser-flash photolysis

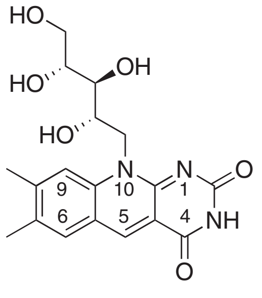
ABSTRACT

Laser-flash photolysis was employed to determine absolute rate constants for quenching of 5-deazariboflavin triplet excited state by amines and phenols in methanol solution. The observed reactivity order for triplet quenching by aliphatic amines parallels very well the corresponding trend in oxidation potentials for these reducing agents; quenching by aromatic amines is significantly more efficient. In the case of phenols, quenching rate constants render a Hammett reaction constant (ρ) value of -1.2 ± 0.1. This negative ρ value is in accord with a mechanism in which hydrogen-atom transfer from phenols to 5-deazariboflavin triplet excited state takes place via an electron–proton transfer sequence.

1. Introduction

The photochemical properties of flavins (that is, 10-alkyl-7,8-dimethylisoalloxazines) and corresponding deaza derivatives (in which the nitrogen atom in position 5 or 10 of the isoalloxazine ring is substituted by a CH group) have received a great deal of attention over the course of past decades due to their relevance in the elucidation of flavin-mediated biochemical processes [1–3]. Flavins are among the most important photosensitizers in biological systems [4], and are known to play a key role in photosynthesis [5] as well as in blue-light sensing photoreceptors [6], for example. A characteristic feature of flavins photochemistry is the variety of possible reaction pathways, which include photoaddition, photodealkylation, photodehydration and photoelectron transfer processes [1–3].

5-Deazariboflavin (**5DRF**) represents a popular flavin analog used in mechanistic studies of flavin-mediated reactions. Many of these studies are based on the use of **5DRF** semiquinone as a reductive agent of proteins, species that is generated photochemically via **5DRF** triplet state quenching in the presence of a hydrogen donor (typically EDTA) [7–10], yet little is known about the reactivity of the triplet excited state of **5DRF** (^{3}**5DRF***) itself. In the present paper, absolute rate constants for reaction between ^{3}**5DRF*** and (aliphatic or aromatic) amines and phenols in methanol solution are presented. The heterogeneous nature of biological systems is what motivates in part the interest in photochemical studies of flavins in non-aqueous media.



5DRF

2. Experimental

5-Deazariboflavin was synthesized according to the method of Carlson and Kiessling [11] and recrystallized from water: m.p. 278–282 °C (dec) (lit. 276–281 °C [12]); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.03 (s, 1H, NH), 8.82 (s, 1H, C^5–H), 7.92 (s, 1H, ArH), 7.84 (s, 1H, ArH), 5.14–3.42 (m, 11H, ribityl), 2.43 (s, 3H, $ArCH_3$), 2.32 (s, 3H, $ArCH_3$) ppm.

3-(Trifluoromethyl)aniline (3-CF_3PhNH_2), 4-(trifluoromethyl)aniline (4-CF_3PhNH_2), pyrrolidine (all from Aldrich, 99%), and methanol (EM Science, spectrophotometric grade) were used as received. Aniline ($PhNH_2$, Baker), diethylamine (BDH), piperidine (BDH), and triethylamine (Eastman) were distilled before use. DABCO (Aldrich), phenol (PhOH, Baker), and

---

* Corresponding author. Tel.: +1 519 888 4567x35948; fax: +1 519 746 0435.
E-mail addresses: mbarra@uwaterloo.ca, mbarra@sciborg.uwaterloo.ca (M. Barra).

1010-6030/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2011.10.007

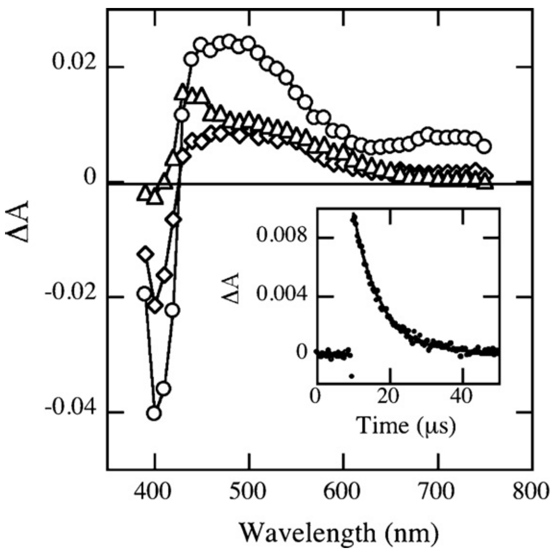


Fig. 1. Transient absorption spectra for 5-deazariboflavin (in N_2-purged methanol) recorded in the presence of 0.3 mM DABCO (○, 0.56 μs; Δ, 7.2 μs after laser pulse) or 0.67 mM triethylamine (◇, 14.8 μs after laser pulse). Inset: kinetic trace for 5-deazariboflavin (in N_2-purged methanol containing 0.1 mM DABCO) monitored at 700 nm.

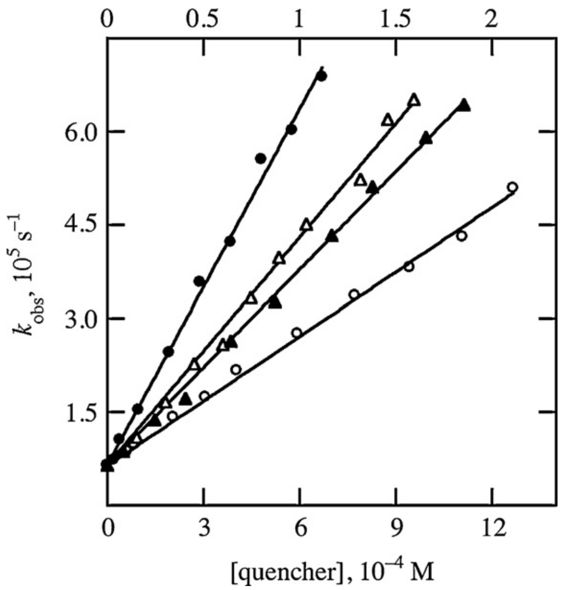


Fig. 2. Dependence of the observed pseudo-first-order rate constant for decay of 5-deazariboflavin triplet excited state as a function of quencher concentration: bottom scale, triethylamine (○) and DABCO (●); top scale, 3-(trifluoromethyl)aniline (△) and 2,4,6-trimethylphenol (▲).

4-*t*-butylphenol (4-*t*-BuPhOH, Acros) were recrystallized from petroleum ether before use. 4-Chloroaniline (4-$ClPhNH_2$, Baker) and 4-chlorophenol (4-ClPhOH, MCB) were recrystallized from methanol and hexane, respectively, before use. p-Anisidine (4-$MeOPhNH_2$), 2,4,6-trimethylphenol (2,4,6-Me_3PhOH), p-toluidine (4-$MePhNH_2$), 4-cyanophenol (4-CNPhOH, all from Aldrich), and 4-methoxyphenol (4-MeOPhOH, Acros) were recrystallized from water before use.

Kinetic traces corresponding to ^{3}**5DRF*** decay were obtained via laser-flash photolysis experiments using a Q-switched Nd:YAG laser (Continuum, Surelite I) operated at 355 nm (4–6 ns pulses, <15 mJ/pulse) for excitation. Further details on this time-resolved laser-flash photolysis system are reported elsewhere [13]. Solutions were contained in quartz cells constructed of 7 mm × 7 mm Suprasil tubing. Transient absorption spectra were collected under flow conditions to ensure the irradiation of fresh portions of sample by each laser pulse. All measurements were carried out at room temperature (21 ± 1 °C) using nitrogen-purged samples. Values for the observed rate constants were obtained by fitting the kinetic traces to a single exponential function using the general curve-fitting procedure of Kaleidagraph software (version 3.6.4) from Synergy Software. Reported values correspond to the average of two to three independent runs.

3. Results and discussion

Nanosecond laser-flash excitation of **5DRF** dissolved in methanol (with or without quencher present) leads to the formation of a broad transient absorption in the 430–750 nm. The initial spectrum (e.g., Fig. 1, open circles) agrees excellently with the spectra found in the literature and assigned to the triplet–triplet (T–T) absorption of **5DRF** dissolved in methanol [14] or aqueous solution [15]. Density functional theory calculations predict the lowest ^{3}**5DRF*** to have π,π^* character [14,15]; triplet π,π^* states are known to be fairly unreactive in radical-like reactions, consistent with the observation of ^{3}**5DRF*** in a hydrogen-atom donor solvent such as methanol ($\tau_T = 13 \pm 3$ μs (this work), 15 μs [14]), but reactive in charge (electron) transfer processes; in fact, single-electron transfer reactions have been reported for quenching of (singlet and triplet) excited states of flavins by different electron donors [1–3].

The transient absorption depicted in Fig. 1 for ^{3}**5DRF*** (open circles) is easily quenched upon addition of (aliphatic or aromatic) amines and phenols. Although significant signals overlap in the 390–450 nm region precludes unequivocal identification of the various transient species, it is noticed that triplet quenching by DABCO (Fig. 1, open triangles) leads to a relative increase in absorbance in the 390–430 nm region (where bleaching is initially observed) more significant than in the case of quenching by Et_3NH (Fig. 1, open diamonds); such an observation is consistent with absorption by DABCO radical cations ($\lambda_{max} \sim 440$ nm [16]). Similar results are seen with 4-$ClPhNH_2$ and 2,4,6-Me_3PhOH, whose oxidized transient forms absorb in the 390–460 nm region [17,18]. Since the absorption spectra of flavin triplets and corresponding reduced forms overlap considerably as well in the 500–600 nm region [19,20], kinetic traces for **5DRF** triplet decay were recorded at 700 nm (e.g., Fig. 1, inset), as a function of varying concentrations of (aliphatic or aromatic) amines and phenols. The pseudo-first-order rate constants (k_{obs}) determined experimentally in the presence of any of the reducing agents employed in this study show a linear dependence on the quencher concentration (Fig. 2 is representative), hence k_{obs} is related to the corresponding quenching rate constant k_Q according to $k_{obs} = k_o + k_Q$ [quencher], where k_o stands for the triplet decay rate constant in the absence of a quencher. Second-order rate constants were thus determined from the slope of k_{obs} vs. [quencher] plots; the resulting k_Q values are summarized in Table 1.

Comparison of k_Q values for aliphatic amines clearly indicates that reactivity increases with decreasing oxidation potential (E_{ox}) for corresponding amine, i.e., as the electron-donating ability of the quencher increases; the limited number of k_Q values obtained, however, precludes a statistically significant fitting to the Rehm–Weller equation. Comparison of k_Q values for aliphatic and aromatic amines also indicates that aromatic amines are more efficient quenchers than aliphatic amines of comparable oxidation potentials, as indeed observed in the case of triplet-state quenching of other flavins [19,21]. The difference in efficiency between aliphatic and aromatic amines has been interpreted in terms of a higher internal reorganization energy for the aliphatic

Table 1
Second-order rate constants for quenching of 5-deazariboflavin triplet excited states by amines and phenols in methanol solution.

Quencher	E_{ox}, V[a,b]	k_Q, 10^8 M^{-1} s^{-1}	Quencher	E_{ox}, V[a,c]	k_Q, 10^8 M^{-1} s^{-1}	Quencher	E_{ox}, V[a,d]	k_Q, 10^8 M^{-1} s^{-1}
Et_2NH	0.628	1.43 ± 0.06	4-CF_3PhNH_2	0.723	33.0 ± 0.4	4-CNPhOH	2.57	0.70 ± 0.03
Piperidine	0.668	2.1 ± 0.1	3-CF_3PhNH_2	0.698	36.6 ± 0.4	4-ClPhOH	2.23	2.4 ± 0.1
Pyrrolidine	0.597	2.8 ± 0.1	4-$ClPhNH_2$	0.535	69 ± 3	PhOH	2.10	4.9 ± 0.2
Et_3N	0.436	3.45 ± 0.03	$PhNH_2$	0.445	56 ± 1	4-*t*-BuPhOH	2.00	11.8 ± 0.4
DABCO	0.264	9.5 ± 0.1	4-$MePhNH_2$	0.305	57.9 ± 0.8	2,4,6-Me_3PhOH	1.85[e]	31.4 ± 0.3
			4-$MeOPhNH_2$	0.125	63.0 ± 0.3	4-MeOPhOH	1.67	37 ± 1

[a] Oxidation potential vs. Ag/AgI.
[b] Measured in DMSO solution [28].
[c] Measured in DMSO solution [29].
[d] Measured in CH_3CN solution [30].
[e] Value for 2,4,6-tri-*t*-butylphenol.

quenchers [19]. A limiting value of ca. 6×10^9 M^{-1} s^{-1} is obtained for triplet-state quenching of **5DRF** by aromatic amines in methanol solution (Table 1, average of last four entries). This value is comparable to the limiting rate constants reported for triplet-state quenching of 7,8-dimethylalloxazine (lumichrome), lumiflavin and riboflavin ($5–7 \times 10^9$ M^{-1} s^{-1} [19,21]). Limiting rate constants for triplet-state quenching of flavins lower than the normally accepted diffusion control value (ca. 1×10^{10} M^{-1} s^{-1}) have been attributed to a decrease in the electronic matrix element related to the frequency factor for the unimolecular rate constant of the triplet-state quenching process [19].

As in the case of (aliphatic) amines, rate constants for ^{3}**5DRF*** quenching by phenols increase with decreasing oxidation potential (increasing electron-donating ability) of corresponding quenchers (Table 1). Interestingly, log(k_Q) values for 4-substituted phenols show an excellent linear correlation with Hammett substituent constants σ^+ [22] (Fig. 3); the negative ρ value of -1.2 ± 0.1 resulting from the slope is certainly indicative of the electrophilic nature of ^{3}**5DRF***. The increase in reactivity for quenching by phenols with decreasing oxidation potential and decreasing Hammett substituent constants is consistent with a net phenolic H-atom transfer process involving an initial electron transfer followed by a fast proton transfer, as previously proposed for triplet-state quenching of 10-methylisoalloxazine [23] and flavin mononucleotides [24]. As in the case of π,π^* triplet-state quenching of carbonyl compounds by phenols [25–27], these reactions are thought to involve the formation of a hydrogen-bonded exciplex, in which the partially protonated triplet is more easily reduced by the partially ionized phenol.

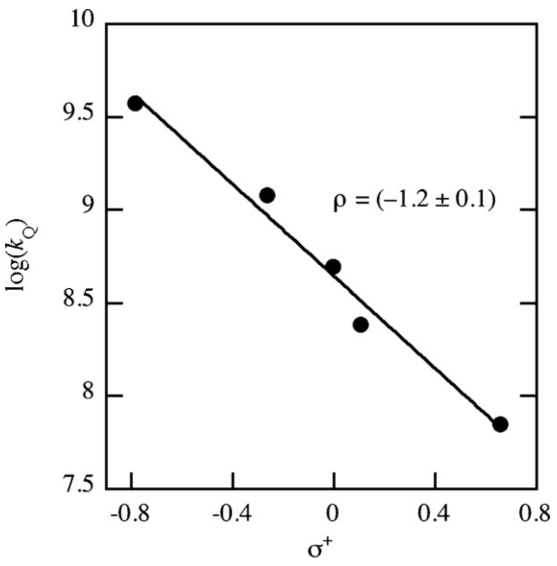


Fig. 3. Hammett plot for quenching of 5-deazariboflavin triplet excited state by phenols in methanol solution ($R = 0.991$).

In summary, absolute rate constants for triplet-state quenching of **5DRF** by amines and phenol in methanol solution are reported. As observed with other flavins, aromatic amines are significantly more effective quenchers than aliphatic amines of similar oxidation potential. On the other hand, the negative Hammett reaction constant value obtained for triplet-state quenching by phenols is in accordance with the proposed coupled electron/proton transfer for this type of reaction. Moreover, rate constants obtained for phenols clearly indicate that tyrosyl side chains in flavoproteins would provide an efficient pathway for triplet flavin degradation.

Acknowledgement

Thanks are due to the Natural Sciences and Engineering Research Council (NSERC) of Canada for partial funding.

References

[1] S. Fukuzumi, T. Tanaka, Flavins and deazaflavins, in: M.A. Fox, M. Chanon (Eds.), Photoinduced Electron Transfer, Part C, Elsevier, Amsterdam, 1988, pp. 636–687.
[2] P.F. Heelis, The photochemistry of flavins, in: F. Müller (Ed.), Chemistry and Biochemistry of Flavoenzymes, vol. 1, CRC Press, Boca Raton, 1991, pp. 171–193.
[3] E. Silva, A.M. Edwards (Eds.), Flavins: Photochemistry and Photobiology, Royal Society of Chemistry, Cambridge, 2006.
[4] M.A. Muñoz, A. Pacheco, M.I. Becker, E. Silva, R. Ebensperger, A.M. Garcia, A.E. De Ioannes, A.M. Edwards, Different cell death mechanisms are induced by a hydrophobic flavin in human tumor cells after visible light irradiation, J. Photochem. Photobiol. B: Biol. 103 (2011) 57–67.
[5] M. Medina, Structural and mechanistic aspects of flavoproteins: photosynthetic electron transfer from photosystem I to NADP$^+$, FEBS J. 276 (2009) 3942–3958.
[6] A. Losi, Flavin-based blue-light photosensors: a photobiophysics update, Photochem. Photobiol. 83 (2007) 1283–1300.
[7] G. Tollin, J.K. Hurley, J.T. Hazzard, T.E. Meyer, Use of laser flash photolysis time-resolved spectrophotometry to investigate interprotein and intraprotein electron transfer mechanisms, Biophys. Chem. 48 (1993) 259–279.
[8] J.T. Hazzard, S. Govindaraj, T.L. Poulos, G. Tollin, Electron transfer between the FMN and heme domains of cytochrome P450BM-3: effects of substrate and CO, J. Biol. Chem. 272 (1997) 7922–7926.
[9] I.F. Sevrioukova, J.T. Hazzard, G. Tollin, T.L. Poulos, Laser flash induced electron transfer in P450cam monooxygenase: putidaredoxin reductase–putidaredoxin interaction, Biochemistry 40 (2001) 10592–10600.
[10] J. Santagostini, M. Gullotti, J.T. Hazzard, S. Maritano, G. Tollin, A. Marchesini, Inhibition of intramolecular electron transfer in ascorbate oxidase by Ag$^+$: redox state dependent binding, J. Inorg. Biochem. 99 (2005) 600–605.
[11] E.E. Carlson, L.L. Kiessling, Improved chemical syntheses of 1- and 5-deazariboflavin, J. Org. Chem. 69 (2004) 2614–2617.
[12] P. Smit, G.A. Stork, H.C. van der Plas, J.A.J. den Hartog, G.A. van der Marel, J.H. van Boom, Synthesis of 5-deazariboflavin adenine dinucleotide (5-dFAD) using a modified triester approach, Recl. Trav. Chim. Pays-Bas 105 (1986) 538–544.
[13] A.M. Sanchez, M. Barra, R.H. de Rossi, On the mechanism of the acid/base-catalyzed thermal cis–trans isomerization of methyl orange, J. Org. Chem. 64 (1999) 1604–1609.
[14] M. Insińska-Rak, E. Sikorska, J.L. Bourdelande, I.V. Khmelinskii, W. Prukała, K. Dobek, J. Karolczak, I.F. Machado, L.F.V. Ferreira, A. Komasa, D.R. Worrall, M. Sikorski, Spectroscopy and photophysics of flavin-related compounds: 5-deazariboflavin, J. Mol. Struct. 783 (2006) 184–190.
[15] S. Salzmann, V. Martinez-Junza, B. Zorn, S.E. Braslavsky, M. Mansurova, C.M. Marian, W. Gärtner, Photophysical properties of structurally and electronically

modified flavin derivatives determined by spectroscopy and theoretical calculations, J. Phys. Chem. A 113 (2009) 9365–9375.

[16] A.M. Halpern, D.A. Forsyth, M. Nosowitz, Flash photolysis of saturated amines in acetonitrile solution at 248 nm: formation of radical cations, J. Phys. Chem. 90 (1986) 2677–2679.

[17] K. Othmen, P. Boule, B. Szczepanik, K. Rotkiewicz, G. Grabner, Photochemistry of 4-chloroaniline in solution. Formation and kinetic properties of a new carbene, 4-iminocyclohexa-2,5-dienylidene, J. Phys. Chem. A 104 (2000) 9525–9534.

[18] T.A. Gadosy, D. Shukla, L.J. Johnston, Generation, characterization, and deprotonation of phenol radical cations, J. Phys. Chem. A 103 (1999) 8834–8839.

[19] G. Porcal, S.G. Bertolotti, C.M. Previtali, M.V. Encinas, Electron transfer quenching of singlet and triplet excited states of flavins and lumichrome by aromatic and aliphatic electron donors, Phys. Chem. Chem. Phys. 5 (2003) 4123–4128.

[20] S.P. Vaish, G. Tollin, Flash photolysis of flavins. IV. Some properties of the lumiflavin triplet state, Bioenergetics 1 (1970) 181–192.

[21] M.V. Encinas, S.G. Bertolotti, C.M. Previtali, The interaction of ground and excited states of lumichrome with aliphatic and aromatic amines in methanol, Helv. Chim. Acta 85 (2002) 1427–1438.

[22] C. Hansh, A. Leo, R.W. Taft, A survey of Hammett substituent constants and resonance and field parameters, Chem. Rev. 91 (1991) 165–195.

[23] I. Ahmad, G. Tollin, Solvent effects on flavin electron transfer reactions, Biochemistry 20 (1981) 5925–5928.

[24] K.H.K. Olsen, L.H. Skibsted, Quenching of triplet-excited flavins by flavonoids. Structural assessment of antioxidative activity, J. Org. Chem. 74 (2009) 7283–7293.

[25] W.J. Leigh, E.C. Lathioor, M.J. St Pierre, Photoinduced hydrogen abstraction from phenols by aromatic ketones. A new mechanism for hydrogen abstraction by carbonyl n,π^* and π,π^* triplets, J. Am. Chem. Soc. 118 (1996) 12339–12348.

[26] M. Yamaji, T. Itoh, S. Tobita, Photochemical properties of the triplet π,π^* state, anion and ketyl radicals of 5,12-naphthacenequinone in solution studied by laser flash photolysis: electron transfer and phenolic H-atom transfer, Photochem. Photobiol. Sci. 1 (2002) 869–876.

[27] A.M. Ribeiro, A.R. Bertoti, J.C. Netto-Ferreira, Phenolic hydrogen abstraction by the triplet excited state of thiochromanone: a laser flash photolysis study, J. Braz. Chem. Soc. 21 (2010) 1071–1076.

[28] W.-Z. Liu, F.G. Bordwell, Gas-phase and solution-phase homolytic bond dissociation energies of H–N$^+$ bonds in the conjugate acids of nitrogen bases, J. Org. Chem. 61 (1996) 4778–4783.

[29] F.G. Bordwell, X.-M. Zhang, J.-P. Cheng, Bond dissociation energies of the N–H bonds in anilines and in the corresponding radical anions. Equilibrium acidities of aniline radical cations, J. Org. Chem. 58 (1993) 6410–6416.

[30] F.G. Bordwell, J.-P. Cheng, Substituent effects on the stabilities of phenoxyl radicals and the acidities of phenoxyl radical cations, J. Am. Chem. Soc. 113 (1991) 1736–1743.