ISSN 1070-3632, Russian Journal of General Chemistry, 2015, Vol. 85, No. 1, pp. 34–37. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © G.Yu. Vasil'eva, A.S. Yudina, V.N. Perminov, V.I. Porkhun, 2015, published in Zhurnal Obshchei Khimii, 2015, Vol. 85, No. 1, pp. 39–42.

Reactions of Flavosemiquinone Radicals in the Presence of Metal Ions

G. Yu. Vasil'eva, A. S. Yudina, V. N. Perminov, and V. I. Porkhun

Volgograd State Technical University, pr. Lenina 28, Volgograd, 400005 Russia
e-mail: galina-vas-vlg@yandex.ru

Received August 18, 2014

Abstract—Rate constant of disproportionation of flavosemiquinone radicals has been determined taking advantage of impulse spectroscopy. Yield of flavosemiquinone radical increases with addition of iron(II)-ammonium sulfate to riboflavin solution. Spectral-kinetic parameters of flavosemiquinone radical complexes with Zn^{2+} and Cd^{2+} have been obtained.

Keywords: impulse photolysis, metal salt, qiunoid compound, riboflavin, flavosemiquinone radical

DOI: 10.1134/S1070363215010065

Impulse photolysis of riboflavin in dimethylformamide. Mechanisms of formation and decay of semiquinone radicals in the course of photoreactions of various quinones with electron donors as well as selected metal ions have been elucidated in [1–6] by means of impulse photolysis and nuclei chemical polarization. Quinones (including heterocyclic ones) are good electron acceptors, being reduced into quinol via formation of semiquinone radicals.

It is known that many of flavoenzymes form flavosemiquinone radicals in good yield upon photoreduction. The radical intermediate has been demonstrated to contribute to catalytic action of the enzyme. Study of interaction of various radicals of flavinic compounds with metal ions is essential for understanding of the nature of active sites of metal-containing flavoproteins.

In this work we studied the influence of metal ions on mechanism of formation and kinetics of decay of flavosemiquinone radicals formed upon impulse photoexcitation of riboflavin. Riboflavin (vitamin B_2,FI) is a prosthetic group of important coenzymes like flavin adenine dinucleotide and flavin mononucleotide; it participates in many redox processes as a fragment of oxidoreductases.

Impulse photoexcitation of riboflavin induces reactions (1) and (2).

$$FI + h\nu \rightarrow FI_S^*, \quad (1)$$

$$FI_S^* \rightarrow FI_T^*. \quad (2)$$

Excited molecules FI* are reduced into semiquinone radical via reactions (3) and (4).

$$FI_S^* \xrightarrow{+H} FIH^{\cdot}, \quad (3)$$

$$FI_T^* \xrightarrow{+H} FIH^{\cdot}. \quad (4)$$

In the absence of suitable reducing agents, the FI* molecule is reduced via oxidation of the own ribityl side chain [Eq. (5)] upon impulse photoexcitation of oxygen-free solutions of FI DMF (5×10^{-5} mol/L, irradiation through the SZS-20 and ZhS-10 filters, λ 400–530 nm).

$$FIH \rightleftarrows FI^{\cdot -} + H^+. \quad (5)$$

Kinetics of the $FI^{\cdot -}$ radical decay follows the second order reaction rate law (6).

$$FI^{\cdot -} + FI^{\cdot -} \rightarrow \text{Products}. \quad (6)$$

In this work we determined the K_6/ε_{560} value of 1.35×10^5 cm/s. In order to estimate the K_6 value, molar absorptivity of flavosemiquinone radical-anion in DMF of $\varepsilon_{560} \approx 7.5 \times 10^2$ mol L^{-1} cm^{-1} can be used. Then K_6 was of $(1.0 + 0.5) \times 10^8$ L mol^{-1} s^{-1}. The reaction rate constant hereafter was determined as described elsewhere [7]. Spectral changes resulting from formation of intermediates in the course of impulse photoexcitation of the solutions in the presence of $CdCl_2$ are documented in Fig. 1.

At time 3×10^{-5} s an intermediate was formed, its absorbance spectrum being identical to that of $FI^{\bullet}$ formed in the presence of $CdCl_2$. However, absorbance spectrum of the intermediates at time 10^{-2} s in the presence of $CdCl_2$ was different from that of $FI^{\bullet}$. The changes of the absorbance spectrum were due to formation of new radical species at time 10^{-2} s, the $CdFI^{\bullet+}$ complex. Kinetics of decay of the intermediates revealed two stages: fast reaction (7) and slow reaction (8).

$$FI^{\bullet} + Cd^{2+} \rightleftarrows CdFI^{\bullet+}, \quad (7)$$

$$CdFI^{\bullet+} + CdFI^{\bullet+} \rightarrow \text{Products}. \quad (8)$$

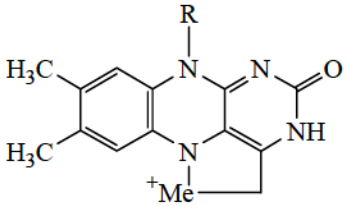
In this work we determined K_8/ε_{540} of 9×10^2 cm/s; as $\varepsilon_{540} \approx 3 \times 10^3$ mol L^{-1} cm^{-1}, $K_8 \approx (2.5 + 0.5) \times 10^6$ L mol^{-1} s^{-1}.

Reaction (7) yielding the complex is formally reversible. Study of the solutions absorbance at time 1×10^{-2} s as function of $CdCl_2$ concentration allowed determination of the equilibrium constant, $K_7 \sim 10^5$ mol^{-1}; hence, the equilibrium was strongly shifted towards the products.

Addition of $ZnSO_4$ (4×10^{-2} mol/L) into the studied solution induced formation of the complex as well; spectral and kinetic features of the $ZnFI^{\bullet+}$ complex were similar to those of $CdFI^{\bullet+}$.

Addition of the $CdCl_2$ or $ZnSO_4$ salts (10^{-2} mol/L) into aqueous solutions of riboflavin (pH 5) did not lead to the complexes formation: the absorbance spectra were identical to that of FIH*. The effect on the radicals decay resulted from the changes of dielectric permeability of the aqueous medium. Similar results were obtained in the case of *p*-benzosemiquinone radical: the anion-radical $Q^{\bullet-}$ formed complexes with copper ions, whereas the $QH^{\bullet}$ radical did not reveal the complex formation.

Coordination of $FI^{\bullet}$ with Cd^{2+} and Zn^{2+} ions can be represented by the following structure.



Flavosemiquinone radical acted as a bidentate ligand, capable of the complexes formation with metal ions bearing *d* electrons. The interaction between *d* (or hybrid) orbitals with the ligand π orbitals yielded a weak double bond. The unpaired electron was thus partially delocalized over the electronic shell of the metal ion. Study of the solutions containing $CdCl_2$ (5×10^{-3} mol/L), $CoSO_4$ (5×10^{-3} mol/L), $ZnSO_4$ (3×10^{-4} mol/L), or $(NH_4)_2Fe(SO_4)_2$ (3×10^{-4} mol/L) did not reveal formation of the complexes with the corresponding ions, due to either low reaction rate constant of the complex formation or low complex formation equilibrium constant.

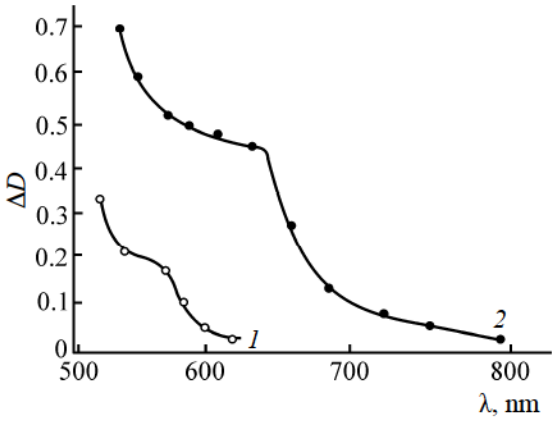


Fig. 1. Absorption spectra of intermediates formed during impulse photolysis of oxygen-free riboflavin solution (5×10^{-5} mol/L) in DMF at time (*1*) 5×10^{-5} and (*2*) 1×10^{-2} s.

The reaction rate constant of the complexes k_8 was about 50 times lower than that of the radicals decay k_6; hence, formation of the complex enhanced the radical stability. That was due to steric hindrance in the course of the radicals recombination and partial delocalization of the unpaired electron at the metal ion. As was earlier demonstrated for *o*-benzosemiquinone and phenoxyl radicals [8, 9], formation of the complex between the radical and the metal ion changed the redox potential. In view of biological redox reactions involving metal-containing flavoenzymes that meant that the system could span a wider range of the potentials, including the $FI \leftrightarrow FIH_2$ transition along with other ones: $FI \leftrightarrow MeFI^{\bullet+}$ and $MeFI^{\bullet+} \leftrightarrow FIH_2$.

Impulse photolysis of riboflavin in water. In the experiments covered in this section, aqueous (pH 7 or 5) and aqueous-propanolic (8 : 1 v/v, pH 4) solutions of riboflavin (3×10^{-5} mol/L) were used. Photoexcitation of the aerated solutions the radical was formed according to reactions (9) and (10).

$$R + M^{n+} \rightarrow R^+ + M^{(n-1)+}, \quad (9)$$

$$R + M^{n+} \rightarrow RM^{(n-1)+}. \quad (10)$$

Removal of oxygen from riboflavin solution as well as introduction of propanol (proton donor) did not lead

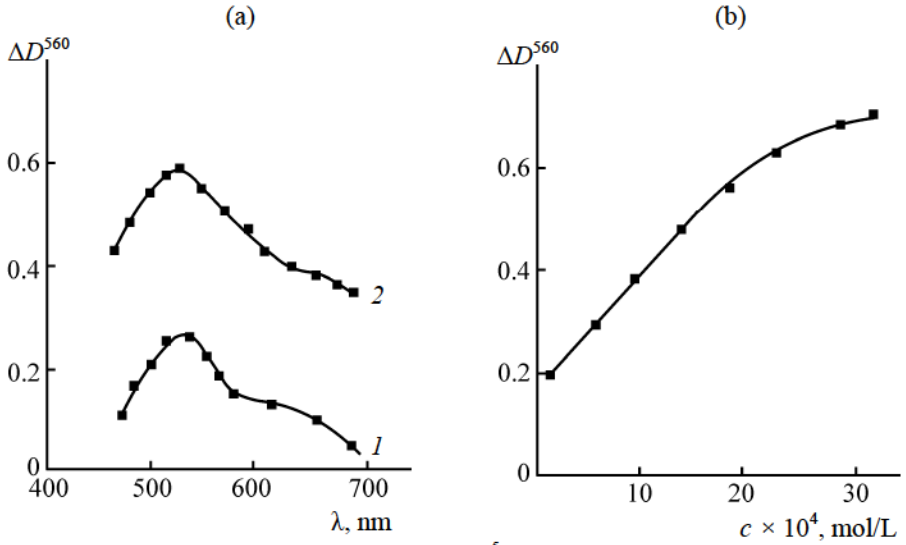


Fig. 2. (a) Absorption spectra of intermediates formed at time 3×10^{-5} s during impulse photolysis of aqueous-propanolic (8 : 1 v/v, pH 4) riboflavin solutions (3×10^{-5} mol/L). (b) ΔD^{560} of the solutions as function of $(NH_4)_2Fe(SO_4)_2$ concentration at time 3×10^{-5} s. (*1*) Pure riboflavin and (*2*) in the presence of $(NH_4)_2Fe(SO_4)_2$ (1.5×10^{-4} mol/L).

to significant increase of the radical yield (<10%). Hence, the major pathway of riboflavin photoreduction into FIH* under the experiment conditions was oxidation of ribityl side chain. Introduction of $(NH_4)_2Fe(SO_4)_2$ into aerated solution of riboflavin increased the FIH* yield (Fig. 2) and appearance of Fe^{3+} ions in the system; concentration of the latter was determined by means of spectrophotometry in the form of thiocyanate complex in acidic medium. The FIH* yield was seemingly increased due to oxidation of Fe^{2+}. Assuming $[FIH^*] - [FIH]_0 = [Fe^{3+}]$ ([FIH*] and $[FIH]_0$ standing for the radical concentration in the presence and in the absence of the iron salt, respectively) we determined the molar absorptivity of FIH*, ε_{560} 4.5×10^3 mol L^{-1} cm^{-1}. Major reaction of flavosemiquinone radicals decay was disproportionation [Eq. (11)].

$$FIH^{\cdot} + FIH^{\cdot} \rightarrow FI + FIH_2. \qquad (11)$$

Thus, $K_{11} = (3.5 + 0.5) \times 10^9$ mol/L for oxygen-free aqueous solution (pH 7).

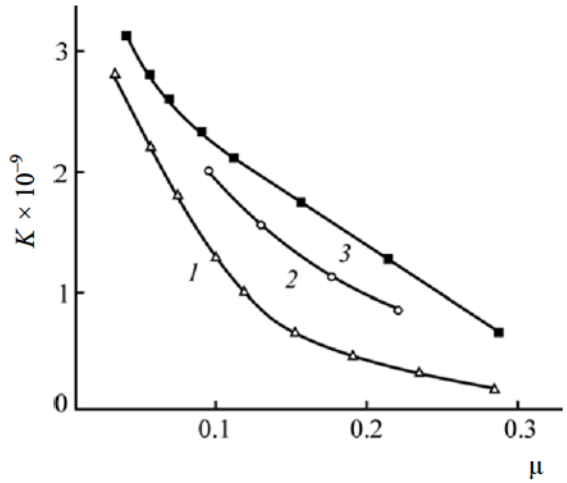


Fig. 3. Rate constant of flavosemiquinone radicals FlH* death as function of the aqueous solution ionic strength ($\mu \geq 0.1$, pH 5, 20°C). (*1*) K_2SO_4, (*2*) $ZnSO_4$, and (*3*) $(NH_4)_2Fe(SO_4)_2$.

The so obtained value of K_{11} coincided (within the experimental accuracy) with diffusion constant of the radicals decay in water. Reaction (11) represents the interaction between polar particles; according to the Amis theory, the K_{11} value should go down with decreasing the solvent dielectric constant. Introduction of the metal salts into the solution led to decrease of the dielectric permeability and to decrease of K_9 (Fig. 2). Similar trend was revealed in the case of phenoxyl radicals [10].

Quenching of riboflavin fluorescense. Riboflavin fluorescence quenching with Fe^{2+} ions [iron(II)–ammonium sulfate) was studied in aqueous-propanolic medium. Average bimolecular reaction rate constant of the quenching was of $(2.5+0.5) \times 10^9$ L mol^{-1} s^{-1}. The FI_S^* lifetime was decreased with addition of the iron salt.

Paramagnetic ions are known as efficient FI fluorescence quenchers [10]; therefore, in the case of

aerated solutions the yield of flavosemiquinone radicals was expected to decrease. That was experimentally observed upon addition of $CuCl_2$ or $NiSO_4$ to the solution. However, the FIH* increased upon addition of iron(II)–ammonium sulfate (Fig. 3). That was probably due to electron transfer involving the FI electronic-excited state (12) or energy transfer onto the Fe^{2+} ion followed by Fe^{2+*} decay [Eqs. (13)–(15)].

$$FI_S^* + Fe^{2+} \xrightarrow{+H^+} FIH^{\cdot} + Fe^{3+}, \quad (12)$$

$$FI^* + Fe^{2+} \rightarrow FI + Fe^{\cdot 2+}, \quad (13)$$

$$FI^{*2+} \rightarrow Fe^{3+} + OH^- + H^{\cdot}, \quad (14)$$

$$FI + H^{\cdot} \rightarrow FIH^{\cdot}. \quad (15)$$

Mechanism of FIH* formation via reactions (12)–(15) seemed more probable as compared with the path (11). Energy transfer is among major mechanisms of quenching of FI fluorescence by Fe^{2+} ions, being spin-allowed and energetically favorable.

REFERENCES

1. Porkhun, V.I., Sviridov, B.D., and Nikiforov, G.A., *Zh. Obshch. Khim*., 1990, vol. 60, no. 1, p. 160.
2. Porkhun V.I., Rykov, S.V., and Nikiforov, G.A., *Zh. Obshch. Khim*., 1991, vol. 61, no. 2, p. 244.
3. Porkhun, V.I. and Rakhimov, A.I., *Russ. J. Phys. Chem.,* 2012, vol. 86, no. 12, p. 1915. DOI: 10.1134/S0036024412120242.
4. Porkhun, V.I. and Rakhimov, A.I., *Russ. J. Phys. Chem.,* 2012, vol. 86, no. 11, p. 1751. DOI: 10.1134/S0036024412110222.
5. Porkhun, V.I. and Rakhimov, A.I., *Khim. Fiz*., 2012, no. 12, p. 15.
6. Porkhun, V.I. and Rakhimov, A.I., *Russ. J. Gen. Chem*., 2011, vol. 81, p. 890. DOI: 10.1134/S1070363211050112.
7. Kuz'min, V.A., Renge, I.V., and Borisevish, Yu.E., *Russ. Chem. Bull*., 1982, no. 9, p. 1774.
8. Porkhun, V.I. and Rakhimov, A.I., *Russ. J. Gen. Chem*., 2010, vol. 80, no. 1, p. 150. DOI: 10.1134/S1070363210010238.
9. Porkhun, V.I. and Rakhimov, A.I., *Russ. J. Gen. Chem*., 2010, vol. 80, no. 1, p. 149. DOI: 10.1134/S1070363210010226.
10. Levin, P.P., Khudyakov, I.V., Kokrashvili, T.A., Darmanyan, A.P., and Kuz'min, V.A., *Dokl. Akad. Nauk SSSR*, 1982, vol. 262, no. 5, p. 1180.