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pH dependence of the absorption and emission behaviour of lumiflavin in aqueous solution

A. Tyagi, A. Penzkofer*

Fakultät für Physik, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany

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ABSTRACT

The spectroscopic behaviour of lumiflavin (7,8,10-trimethyl-isoalloxazine, oxidized form LF_{ox}) in aqueous solutions of pH range −1.08 to 14.6 is studied. Absorption spectra, fluorescence quantum distributions, quantum yields and lifetimes are determined. The ionization stage of ground-state LF_{ox} changes from cationic ($LF_{ox}H_2^+$) at low pH ($pK_c \approx 0.38$) via neutral ($LF_{ox}H$) to anionic (LF_{ox}^-) at high pH ($pK_a \approx 10.8$). The cationic, neutral, and anionic forms are identified by their different absorption spectra. $LF_{ox}H$ in neutral aqueous solution is reasonably fluorescent (fluorescence quantum yield $\phi_F \approx 0.29$, fluorescence lifetime $\tau_F \approx 5.2$ ns), while LF_{ox}^- is weakly fluorescent ($\phi_F \approx 0.0042$, $\tau_F \approx 90$ ps), and $LF_{ox}H_2^+$ is nearly non-fluorescent ($\phi_F \approx 3.6 \times 10^{-5}$, $\tau_F \approx 0.4$ ps).

A theory of the pH dependent equilibration of cationic, neutral and anionic molecules in the ground state and their dynamics in the excited state is developed. For lumiflavin in aqueous solution in the excited state no equilibrium distributions are reached between the cationic, neutral, and anionic forms. Some neutral excited lumiflavin transforms to the cationic ground-state form at low pH by intermolecular photo-induced proton transfer from H_3O^+ to $LF_{ox}H^*$. At high pH no photo-induced intermolecular proton transfer takes place.

1. Introduction

Lumiflavin (7,8,10-trimethyl-isoalloxazine, LF) is the fundamental molecule of the huge class of flavins with the most famous members riboflavin (RF), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) [1,2]. Lumiflavin is the dominant photoproduct of RF, FMN, and FAD in alkaline solution (pH > 9) [2–6]. It is obtained by photolysis of riboflavin in 1 M NaOH [4,7]. Ways of synthesis of lumiflavin are described in [8–10]. Lumiflavin is a photo-sensitizer of organic and biological molecules by radical formation (type I photosensitization) and singlet oxygen generation (type II photosensitization) [11–17].

The photo-physical behaviour of lumiflavin is described in [1,3,4,12,18–25]. Absorption spectroscopy [3,4,7,15,18,20,25], fluorescence spectroscopy [12,19,20], and triplet spectroscopy [12,19–22] were carried out for lumiflavin characterization. The pH dependence of fluorescence was studied [26,27]. Ionization equilibria between cationic, neutral and anionic forms in the ground state [28,29], singlet excited state [28], and triplet excited state [30] were determined. The photo-stability of lumiflavin in aqueous solutions [3,4,25], organic solvents [3–5], and in biological matter [11–15] was investigated. In alkaline solution at elevated temperatures lumiflavin was found to be thermally unstable by hydrolysis reaction [31,32]. Quantum chemical calculations on electronic structure, molecular properties, and spectroscopic parameters of lumiflavin were carried out [23,33–40]. Thereby lumiflavin served as prototype of flavins.

Despite the long period of lumiflavin investigation no detailed absolute absorption cross-section spectra, no absolute intrinsic fluorescence quantum distributions and quantum yields as well as no fluorescence decay curves over the experimentally accessible pH range from −1.08 (37% HCl) to 14.6 (4 M NaOH) are available. The mechanisms of fluorescence quenching of lumiflavin in neutral ($LF_{ox}H$), cationic ($LF_{ox}H_2^+$), and anionic form (LF_{ox}^-) have not been worked out. The difference in the constants of ground-state equilibrium, pK_c, and singlet excited equilibrium, pK_c^*, between cationic and neutral lumiflavin, and the equality of the constants of ground-state equilibrium, pK_a, and singlet excited equilibrium, pK_a^*, between neutral and anionic lumiflavin has not yet been satisfactorily explained.

In this paper we work on these topics. The behaviour of the absolute absorption cross-section spectra, the absolute fluorescence quantum distributions, the absolute fluorescence quantum yields, and the fluorescence lifetimes of lumiflavin in aqueous solution are studied over the range from pH −1.08 to pH 14.6. The absorption and emission dependences are inter-

* Corresponding author at: Universität Regensburg, Fakultät für Physik, Universitätsstrasse 31, D-93053 Regensburg, Germany. Tel.: +49 941 943 2107; fax: +49 941 943 2754.
E-mail address: alfons.penzkofer@physik.uni-regensburg.de (A. Penzkofer).

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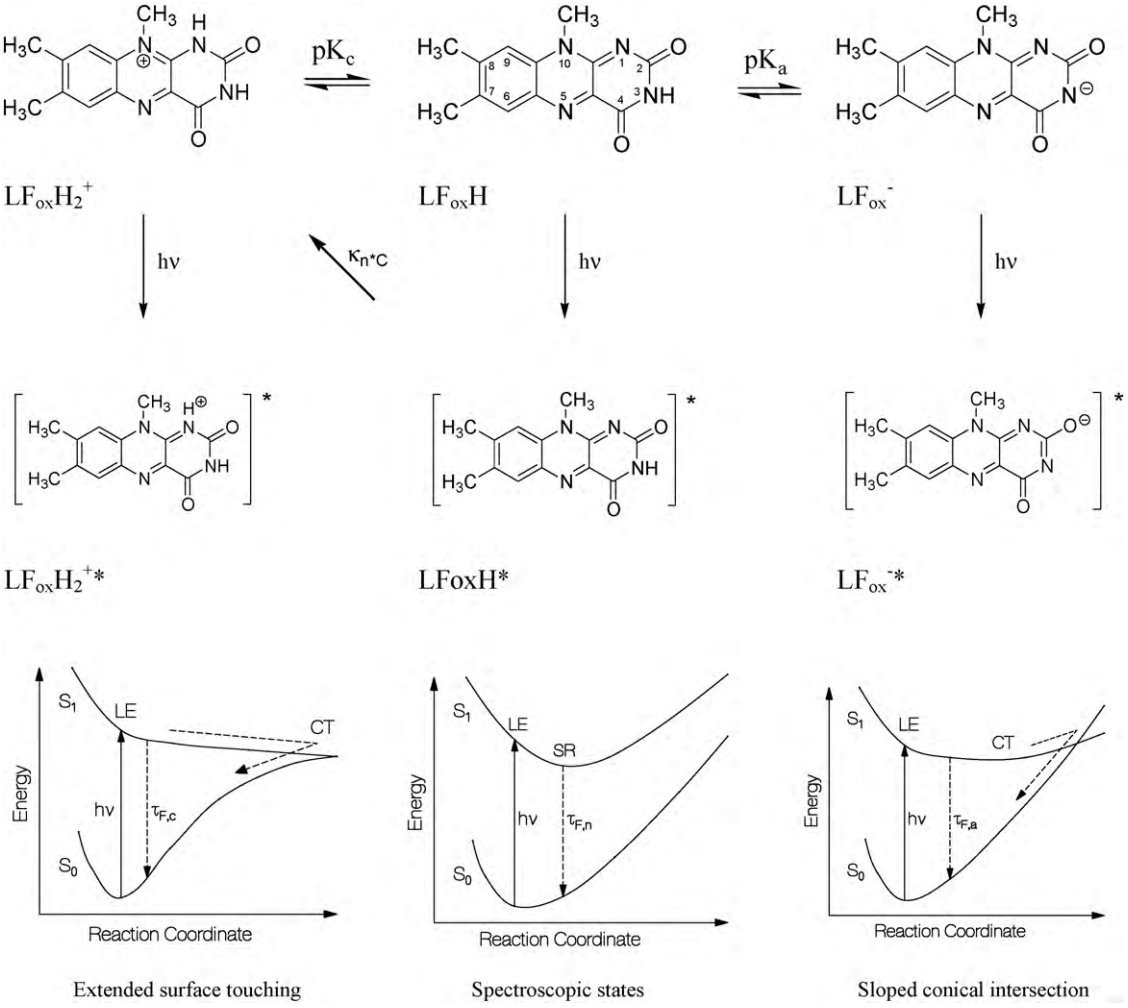

Fig. 1. Top row: structural formulae of cationic, neutral, and anionic lumiflavin in the oxidized (flavoquinone) redox state (taken from [2]) with thermal equilibrium paths (pK_c and pK_a). Middle row: possible structural formulae of cationic, neutral, and anionic lumiflavin in the excited state. Bottom row: illustrative potential energy curves for ground state and first excited state of cationic, neutral, and anionic lumiflavin. Transitions are indicated.

preted in terms of photo-physical and photochemical interactions (photo-physical fluorescence quenching of $LF_{ox}H$ at neutral pH, intra-molecular charge transfer for $LF_{ox}H_2^+$ and LF_{ox}^-). The pK values of cationic–neutral equilibrium (pK_c) and neutral–anionic equilibrium (pK_a) in the ground state are extracted from the absorption dependences. In the excited state no ionization state equilibria are reached within the short fluorescence lifetimes. Therefore no equilibration constants, pK_c^* and pK_a^*, are accessible. Instead, pH values pK_{Fc}^* and pK_{Fa}^* of mid-point fluorescence efficiency between cationic and neutral species as well as between neutral and anionic species will be determined by pH dependent fluorescence quantum yield measurements. A theory of the pH dependent equilibration of cationic, neutral and anionic molecules in the ground state and their dynamics in the excited state will be developed. The inequality $pK_c < pK_{Fc}^*$ will be explained by intermolecular proton transfer deactivation of neutral excited lumiflavin $LF_{ox}H^*$ at low pH ($LF_{ox}H^* + H^+ \rightarrow LF_{ox}H_2^+$).

The structural formulae of lumiflavin in the flavoquinone (oxidized) redox state in neutral form ($LF_{ox}H$), in cationic form ($LF_{ox}H_2^+$), and in anionic form (LF_{ox}^-) are shown in the top row of Fig. 1. The flavosemiquinone (semi-reduced) redox state ($LFH_2^\bullet$) and the flavohydroquinone (fully reduced) redox state ($LF_{red}H_3$) of lumiflavin are not present under our experimental conditions (aerobic solutions, no added reducing agents) [1,2,41]. Therefore the complex photo-dynamics of semi-reduced and fully reduced flavins in their neutral, cationic, and anionic form [42–44] is not addressed here.

2. Experimental

Lumiflavin (LF) was purchased from Sigma–Aldrich and used as delivered. The dye was dissolved in aqueous solutions of different pH. At lowest pH (−1.08) LF was dissolved in concentrated hydrochloric acid (37 wt.% HCl). In the range of pH −0.3 (2 M HCl) to pH 3 (10^{-3} M HCl) differently concentrated aqueous HCl solutions, and in the range of pH 11 (10^{-3} M NaOH) to 14.6 (4 M NaOH) differently concentrated NaOH solutions were used. A citric acid/NaOH/NaCl buffer (Fixanal from Aldrich) was used for pH 4. For pH 6, pH 8, and pH 10 self-prepared 10 mM sodium phosphate buffers with 10 mM NaCl were used. All measurements were carried out at room temperature under aerobic conditions.

The absorption spectra were measured with a commercial spectrophotometer (Cary 50 from Varian). The fluorescence emission spectra were recorded with a commercial fluorimeter (Cary Eclipse from Varian) under magic angle conditions. The spectra were corrected for the spectral sensitivities. For absolute intrinsic fluorescence quantum distribution and quantum yield calibration the dyes riboflavin in water ($\phi_F = 0.26$ [45]) and POPOP (1,4-di(5-phenyloxazolyl)benzene) in ethanol ($\phi_F = 0.85$ [46]) were used as reference standards.