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Photoluminescence behavior of riboflavin and lumiflavin in liquid solutions and solid films

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ABSTRACT

The absorption and emission behavior of riboflavin and lumiflavin in water, tetrahydrofuran (THF), water–starch, THF–polystyrene, starch films, and polystyrene films was studied at room temperature. Absorption cross-section spectra, fluorescence quantum distributions, and fluorescence quantum yields were determined. For the starch films additionally phosphorescence and delayed fluorescence spectra as well as phosphorescence lifetimes and delayed fluorescence lifetimes were measured and their quantum yields of intersystem-crossing, intrinsic triplet-based phosphorescence quantum yields, T_1–S_0 radiative lifetimes, and S_0–T_1 absorption strengths were calculated. A method of absolute intrinsic luminescence quantum distribution and quantum yield determination for dye doped films on transparent plates with a fluorimeter is described.

1. Introduction

Riboflavin (vitamin B_2, lactoflavin, 7,8-dimethyl-10-ribityl-isoalloxazine) and lumiflavin (7,8,10-trimethyl-isoalloxazine) belong to the huge family of flavins [1–4]. Riboflavin plays an important role as cofactor in enzymes [1]. Lumiflavin is the fundamental molecule of the flavins and is often used for quantum chemical calculations [5–8]. It is the dominant photoproduct of riboflavin, flavin mononucleotide (FMN) and flavine adenine dinucleotide (FAD) in alkaline solution (pH > 9) [2].

The singlet–singlet absorption and fluorescence behavior of riboflavin and lumiflavin in liquid solutions are well documented (see [2,3,9–15] for riboflavin, and [4,12,15–18] for lumiflavin). The S_1–T_1 intersystem-crossing quantum yield ϕ_{ISC} [19–23], the T_1–S_0 phosphorescence lifetime τ_P [17,19,20,24–28], and the T_1–S_0 phosphorescence quantum yield ϕ_P [17,19,24] of riboflavin and lumiflavin have been investigated in aqueous and alcoholic solutions at room temperature and at 77 K. Reported results are collected in Table 1. T_1 state lifetimes were mainly determined by flash-photolysis studies (pump–probe measurement of triplet–triplet absorption [20,25,28,29], measurement of excitation pulse induced delayed luminescence [19,24]). The S_1–T_1 intersystem-crossing was studied by laser-induced optoacoustic spectroscopy [30], by triplet yield determination using electron paramagnetic resonance spectroscopy [19,31,32], by laser flash photolysis with a reference standard [20,23], by determination of quantum efficiency of singlet oxygen generation [33], by determination of EDTA (ethylene-diamine-tetraacetic acid) photo-reduction [21], and by a double-pulse excitation and fluorescence detection method [34].

In liquid solutions the phosphorescence efficiency is reduced by molecular diffusion allowing collisional bimolecular de-excitation [35] like triplet–triplet annihilation (P-type delayed fluorescence [36,37]), S_0–T_1 singlet–triplet electron transfer [38,39], and impurity quenching (mainly triplet quenching by dissolved molecular oxygen [40,41]). Under aerobic conditions (no de-aeration of liquid samples) the phosphorescence is strongly quenched by dissolved molecular oxygen and detection of phosphorescence emission is not accessible to conventional fluorimeter and phosphorimeter with minimum delay between pulsed excitation and gated detector switch-on of typically 100 μs because of short triplet state lifetime in the few microsecond to nanosecond range [20,27,35–37]. In air-impermeable solid-state hosts diffusion of molecules is hindered and phosphorescence studies with standard fluorimeter may become possible even at room temperature [36–38,42].

Here we study the photoluminescence (prompt luminescence = fluorescence, delayed luminescence = delayed fluorescence + phosphorescence) of riboflavin (Rib) and lumiflavin (LF) in starch films at room temperature (20 °C) under ambient

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Table 1
Intersystem-crossing quantum yield, ϕ_{ISC}, phosphorescence lifetime, τ_T, and phosphorescence quantum yield, ϕ_P, of lumiflavin (FL) and riboflavin (Rib) in various solvents. ana = anaerobe. aer = aerobe. RT = room temperature.

Flavin	Solvent	Temperature (°C)	ϕ_{ISC}	τ_T (ms)	ϕ_P	Reference
LF	H_2O, pH 2.2, aer	23	0.42	0.020		[20]
LF	H_2O, pH 6	RT	0.67			[20]
LF	H_2O, pH 7, ana	26		0.67		[26]
LF	H_2O, pH 7, ana	RT	0.43			[21]
LF	H_2O, distilled, ana	RT		1.5		[25]
LF	H_2O,pH 6.9, ana	RT		0.685		[28]
LF	Methanol, aer	RT		0.017		[27]
LF	Ethanol, aer	23	0.30			[20]
LF	Ethanol	−196		150	0.003	[17]
LF	Ethanol-methanol	−196	0.65		0.0053	[19]
LF	Ethanol-glycerol-H_2O	−196		112	6.7×10^{-4}	[24]
LF	Glycerol–water	−196		210		[19]
LF	Starch	20	0.415 ± 0.03	11.8 ± 0.7	0.0094	This work
LF	Polystyrene	20	<0.33	0.18 ± 0.05	$\approx 5 \times 10^{-5}$	This work
Rib	H_2O, pH 2.2, aer	23	0.40	0.019		[20]
Rib	H_2O, pH 6, aer	RT	0.67			[20]
Rib	H_2O, pH 7, ana	26		0.31		[27]
Rib	H_2O, pH 7, aer	RT	0.38			[35]
Rib	H_2O, pH 6.1, ana	RT	0.51			[22]
Rib	Methanol	RT	0.61			[23]
Rib	Methanol, aer	RT		0.0037		[27]
Rib	Ethanol	−196		170	0.004	[17]
Rib	Ethanol-methanol	−196	0.7		0.0071	[19]
Rib	Ethanol-glycerol-H_2O	−196		133	0.0012	[24]
Rib	Glycerol–water	−196		130		[19]
Rib	Starch	20	0.30 ± 0.03	9.1 ± 0.8	0.0025	This work

conditions with a commercial fluorimeter (Cary Eclipse from Varian). The phosphorescence quantum distributions and quantum yields, the delayed fluorescence quantum distributions and quantum yields, and the phosphorescence lifetimes and delayed fluorescence lifetimes were determined. The quantum yield of S_1–T_1 intersystem-crossing ϕ_{ISC}, will be calculated from the measured delayed fluorescence quantum yield ϕ_{DF}, the fluorescence quantum yield ϕ_F, the fluorescence lifetime τ_F, the phosphorescence lifetime τ_P, and the energy difference between S_1 and T_1 states, $h\delta\nu_{S_1,T_1}$.

Comparative absorption and fluorescence studies were carried out on lumiflavin in Millipore water (here shortly named water or H_2O), tetrahydrofuran (THF), starch–water, polystyrene–THF, and polystyrene films. The spectroscopic studies on riboflavin were limited to water, starch–water, and starch since riboflavin is practically insoluble in THF, THF–polystyrene, and polystyrene.

There is great interest in the singlet and triplet photo-dynamics of flavins because of the flavin photo-sensor action in blue-light photoreceptors [43,44] (phototropins [45], cryptochromes [46], BLUF proteins [47], photoactivated cyclases [48,49]). Especially in the photo-cycle dynamics of phototropin, the flavin triplet state is involved in the flavin-C(4a)-cysteinyl adduct formation of the LOV domain (LOV = Light, Oxygen, Voltage) signaling state [45,50].

2. Experimental

The flavin dyes riboflavin (molar mass $M_{Rib} = 376.36\ g\ mol^{-1}$) and lumiflavin ($M_{LF} = 256.3\ g\ mol^{-1}$), the solvent THF, the polymers starch (from potatoes, treated with glycerol at 190 °C according to Zulkowsky [51], repeat unit: $C_6O_5H_{10}$, molar mass of repeat unit 162 g mol^{-1}, mass density $\rho_{ST} \approx 1.55\ g\ cm^{-3}$) and polystyrene (PS, repeat unit: C_8H_8, molar mass of repeat unit 104.15 g mol^{-1}, mass density $\rho_{PS} = 1.05\ g\ cm^{-3}$, average molar mass 35,000 g mol^{-1}) were purchased from Sigma–Aldrich and used as delivered. The solvent water was de-ionized in a Millipore water purifier and used in this form.

Stock solutions of LF in water and in THF, and of Rib in water were prepared with a concentration of 1×10^{-4} mol dm^{-3} (saturation concentration of LF in water and THF is lower, some un-dissolved LF was present). Starch was dissolved in water with a concentration of 20 g dm^{-3}. PS was dissolved in THF with a concentration of 80 g dm^{-3}. The dye solutions and the polymer solutions were mixed in a volume ratio of 1:1. For film preparation, 3 cm^3 of the mixed dye polymer solutions were poured over fused silica plates (25 mm diameter, 3 mm thickness) in Teflon hollow cylinders and dried in a drying cupboard at ≈65 °C for starch films and at ≈55 °C for the PS films. The thickness of the starch films was $d_f \approx 36$ μm, and the thickness of the PS films was $d_f \approx 215$ μm. The photoluminescence measurements were carried out at room temperature (20 °C) under aerobe conditions (no degassing of solvents).

The absorption spectra were measured with a spectrophotometer (Cary 50 from Varian). The fluorescence spectra, delayed fluorescence and phosphorescence spectra, delayed fluorescence and phosphorescence lifetimes were measured with a fluorimeter (Cary Eclipse from Varian). The photoluminescence spectra and delayed photoluminescence lifetimes were measured using a 90° angle arrangement (luminescence detection path perpendicular to light excitation path) and magic angle polarization conditions (excitation light polarized to the vertical direction, polarizer in detection path was oriented at an angle of 54.7° to the vertical) [52]. The applied fluorescence standard for fluorescence quantum distribution ($E_F(\lambda)$) and fluorescence quantum yield (ϕ_F) determination was rhodamine 6G in methanol ($\phi_F = 0.94$ [53]). The applied phosphorescence standard for phosphorescence and delayed fluorescence quantum distribution and quantum yield determination was erythrosine B in starch ($\phi_P = 0.06$ [54]). The films on fused silica plates were positioned vertical and oriented at an angle of 45° to the excitation light path (angle of incidence $\alpha = 45°$ for light excitation path, angle of exit $\alpha' = 45°$ for the emission detection path). The experimental arrangements for the photoluminescence measurements and the calculation of quantum distributions and quantum yields under these experimental conditions are described in Appendix 1.

The fluorescence lifetimes of the samples were measured using a mode-locked titanium–sapphire laser oscillator amplifier system