Spectroscopy and Photophysics of Lumiflavins and Lumichromes

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Received: October 9, 2003; In Final Form: January 9, 2004

Solvent effects on the spectroscopic properties of lumichromes and lumiflavins are presented. Fluorescence yields for lumiflavins are an order of magnitude larger than those for lumichromes, due to their lower nonradiative rate constants. Solvent effects on the absorption and emission band positions are explained on the basis of hydrogen-bonding interactions. TD-DFT calculations predicted that the lowest energy states are n,π* in the case of lumichromes, but π,π* in the case of the lumiflavins. The overall consistency of the predicted singlet−singlet and triplet−triplet transitions obtained for the various compounds studied, and the good correspondence between the predicted and measured transitions, indicate that the techniques applied provide an accurate description of the spectral properties of lumiflavins and lumichromes. The measured singlet oxygen yields have shown the lumichromes to be efficient singlet oxygen sensitizers.

Introduction

Iso- and alloxazines are closely related compounds, representing two classes of nitrogen heterocycles with active centers at N(10), N(5), N(3), and N(1), and at both carbonyl oxygens at C(2) and C(4). Isoalloxazines (10-substituted 2,3,4,10-tetrahydrobenzo[*g*]pteridine-2,4-dione) and especially flavins (7,8-dimethyl-substituted isoalloxazines) possess the yellow chromophore characteristic of flavoproteins, which are enzymes occurring widely in animals and plants. Since the discovery of the old yellow enzyme some 70 years ago, it has become clear that flavoproteins are involved in a very wide range of biological processes. Since the volume of data on ground and excited state properties of flavins is overwhelming, we will refer only to the symposium proceedings, entitled Flavins and Flavoproteins,[1] which illustrate both the wealth of available information and the progress that has been made in the photochemistry, structure, and functionality of flavins. The early interest in the photophysical and photochemical properties of alloxazines (benzo[*g*]pteridine-2,4(1*H*,3*H*)-diones) including lumichromes (7,8-dimethyl-substituted alloxazines) was mainly driven by their closeness to flavins, mostly as their photoproduct. It has been important to assess the toxicity of lumichromes as products formed by photochemical reactions of riboflavin. It has been shown that lumichrome, like riboflavin, is in fact nonmutagenic, nongenotoxic, and nonclastogenic.[2] Alloxazines can be associated to flavins in many organisms and may be involved in some biological processes.[3,4]

Alloxazines are structurally very closely related to isoalloxazines, but despite the structural similarity, alloxazines differ from isoalloxazines in physical and chemical properties. The main features of the spectroscopy and photophysics of both classes of compounds, iso- and alloxazines, are understood fairly well, but some details require further investigation. For example, the results on photophysics of alloxazines in their excited singlet and triplet states are scattered and are limited to selected solvents. Recently we have undertaken systematic studies to describe the photophysics of alloxazines, lumichromes, and isoalloxazines in a range of different solvents. The aim of the present paper is to characterize and reconcile the diverse photophysical and spectroscopic properties of iso- and alloxazines in five solvents, namely water, acetonitrile, 1,2-dichloroethane, 1,4-dioxane, and methanol.

The structures of all the compounds studied in this paper with atom numbering are shown in Figure 1.

Materials and Methods

Lumiflavin, lumichrome, and the solvents acetonitrile, 1,2-dichloroethane, 1,4-dioxane, and methanol, all from Aldrich, were used as received. Purified distilled, deionized unbuffered water was used in all experiments and the pH of all the aqueous solutions prepared was ca. 6. Solvent purity was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer. The lumichrome derivatives were available from previous work.[5,6]

Fluorescence decays were measured by using excitation at 355 nm and time-correlated single-photon counting detection on an IBH model 5000U fluorescence lifetime spectrometer. Time-resolved fluorescence measurements of lumichrome in 1,2-dichloroethane and acetonitrile were also conducted with a model C-700 fluorometer from PTI. The system utilizes a nanosecond flashlamp for excitation and a stroboscopic detection system.[7] Some of the measurements were also conducted by

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10.1021/jp037048u CCC: $27.50

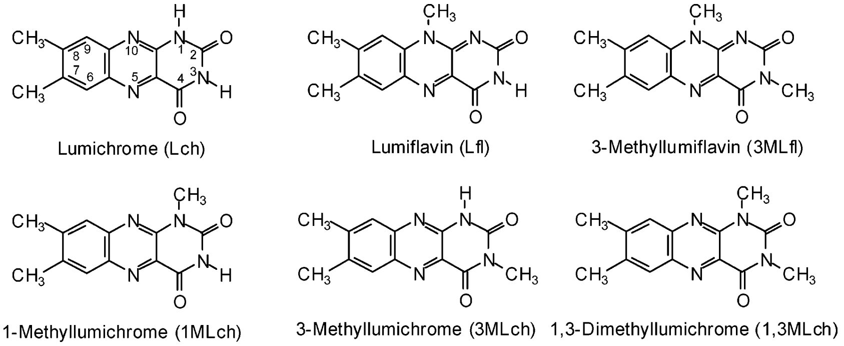

Figure 1. Structures of the compounds studied and their atom numbering.

using the frequency-doubled output of a mode-locked, synchronously pumped, cavity-dumped argon-ion/DCM dye-laser system, and detecting emission with a Hamamatsu microchannel-plate photomultiplier coupled to a time-correlated single-photon counting system. This measuring system has been described in detail previously.[8] Steady-state fluorescence spectra were obtained with a Jobin Yvon-Spex Fluorolog 3-11 spectrofluorometer, and UV−visible absorption spectra on a Varian Cary 5E spectrophotometer.

Transient absorption measurements were performed by using two different nanosecond laser flash photolysis systems available in Barcelona and Loughborough, both with right-angle geometry. In Barcelona the LKS60 instrument from Applied Photophysics was used: the third harmonic (355 nm) of a Q-switched Nd:YAG laser (Spectron Laser Systems, UK; pulse width ca. 9 ns) was used for laser flash excitation. The measurements in Loughborough were performed with a nanosecond laser flash photolysis system as described previously elsewhere.[6]

Singlet oxygen luminescence experiments were carried out by excitation of the sensitizer with the third harmonic of a Lumonics hyperYAG HY200 Nd:YAG laser (355 nm, 8 mJ per pulse, 8 ns fwhm). The excitation energy was attenuated by using solutions of sodium nitrite in water. Detection was obtained on an EO-980P liquid nitrogen cooled germanium photodiode detector (North Coast Scientific), with a 1270 nm interference filter (Melles Griot) interposed between sample and detector to reduce detection of laser scatter and sensitizer emission, and to isolate the singlet oxygen phosphorescence. Data capture was with a 250 MS/s digitizing oscilloscope (Tektronix 2432A) and data analysis was done with Microcal Origin. Perinaphthenone (Aldrich) was used as a reference standard, $\phi_\Delta = 0.95 \pm 0.05$, independent of solvent.[9]

The electronic structure of lumiflavins and lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives has been studied by means of time-dependent density-functional theory (TD-DFT).[10] In this work, the TD-DFT calculations were performed by using the B3LYP hybrid method[11] in conjunction with a modest 6-31G* split-valence polarized basis set.[12] Excitation energies and transition intensities were calculated for the optimized ground-state geometries. Oscillator strengths were calculated in the dipole length representation. Calculations were performed by using the Gaussian 98 package of ab initio programs.[13] In the present study, T−T excitation energies and transition intensities were determined for the optimized geometry of the lowest triplet state (T_1). We have calculated the spectra using the unrestricted UB3LYP approach. Regarding the quality of the spectral predictions let us just note that the difference in the experimental transition energies in 1,4-dioxane solution between Lfl and Lch (22 680 and 26 390 cm^{-1}) is reproduced in the calculations (24 500 and 27 800 cm^{-1}) to within 500 cm^{-1}.

Results and Discussion

Lumiflavins and Lumichromes in Their Ground and Excited Singlet States. Lumiflavins, the first class of compounds examined in our studies, are represented by lumiflavin and 3-methyllumiflavin, 3MLfl, both existing in the isoalloxazine form. Lumiflavin and 3-methyllumiflavin have spectroscopic and photophysical properties very different from those of alloxazines. In absorption 3-methyllumiflavin shows two characteristic bands at about 345 (ca. 29 000 cm^{-1}) and 444 nm (ca. 22 500 cm^{-1}), the exact position of the former band depending on the solvent, while the latter shows little solvent dependence. The 3-methyl substitution has little effect on the absorption spectrum relative to the parent compound (Table 1). It is well-known that the methyl group in the N(10) position of isoalloxazines yields very closely structurally related iso- and alloxazines, possessing divergent spectral and photophysical properties. In particular, lumiflavin and 3-methyllumiflavin exhibit intense fluorescence. The maximum of the lumiflavin fluorescence is red-shifted by about 90 nm relative to that of the lumichrome, giving a broad, unresolved band with a maximum at about 530 nm (ca. 18 900 cm^{-1}). A summary of the photophysical parameters of the lumiflavins and lumichromes (alloxazinic form) is given in Table 1. The radiative and nonradiative decay constants for the lowest excited singlet state are calculated as $k_r = \phi_F/\tau_F$ and $\sum k_{nr} = (1 - \phi_F)/\tau_F$. Lumiflavin and 3-methyllumiflavin have fluorescence quantum yields 1 order of magnitude larger and correspondingly longer fluorescence lifetimes than the lumichromes; this can be entirely explained by their much lower nonradiative decay rates. In the case of lumiflavins, both radiative and nonradiative decay rates show little solvent dependence except in the case of water as solvent, where an increase in both radiative and nonradiative rate constants is observed. For lumichromes, a significant solvent dependence is seen for both radiative and nonradiative rate constants, with the rate constants decreasing with increasing solvent polarity and with increasing protic nature of the solvent. Note that iso- and alloxazines have a very limited solubility in a number of solvents, especially in the nonpolar solvents, where they are usually insoluble, having a limited solubility even in polar solvents.

A red shift in the λ_2 absorption band is observed for both lumichromes and lumiflavins with increasing polarity and hydrogen-donating ability of the solvent, which may be explained on the basis of hydrogen bonding. A hydrogen donor may bond at the N(10) and/or N(5) positions in lumichromes, and at the N(1) and/or N(5) positions in lumiflavin. Long-wavelength shifts of the λ_2 absorption band have been previously observed for lumichrome and riboflavin in the presence of the hydrogen-donating agent hexafluoro-2-propanol.[16−18] Analogy between the effects observed for alloxazines and isoalloxazines

TABLE 1: Spectroscopic and Photophysical Data for the Singlet States of Lumiflavins and Lumichromes[a]

solvent	compd	λ_2/nm	λ_1/nm	λ_F/nm	ϕ_F	τ_F/ns	$k_r/10^8\ s^{-1}$	$\Sigma k_{nr}/10^8\ s^{-1}$
Dx	Lfl	332	441 (15900)	531	0.19	9.1	0.21	0.89
	3MLfl	334	442 (12000)	531	0.20	8.4	0.24	0.95
	Lch	327	379 (8900)	445	0.027	0.45	0.60	21
	1MLch	328	381 (8100)	445	0.028	0.51	0.55	19
	3MLch	328	379 (8200)	443	0.026	0.47	0.55	21
	1,3MLch	328	382 (7600)	448	0.027	0.43	0.63	23
DCE	Lfl	345	447 (15100)	533	0.15	6.8	0.22	1.3
	3MLfl	344	448 (11100)	533	0.16	6.7	0.24	1.3
	Lch	344	382	440	0.026	0.61	0.43	16
	1MLch	334	382 (7600)	440	0.026	0.62	0.41	16
	3MLch	334	381	439	0.024	0.65	0.37	15
	1,3MLch	335	383	440	0.026	0.62	0.41	16
MeOH	Lfl	351	442 (12200)	531	0.13	6.8	0.19	1.3
	3MLfl	351	444 (9900)	533	0.15	6.3	0.24	1.3
	Lch	339	384 (7700)	453	0.032	1.04[b]	0.30	9.3
	1MLch	340	385 (7500)	453	0.037	0.94	0.35	10
	3MLch	340	383 (8000)	460	0.032	1.0[c]	0.32	9.7
	1,3MLch	340	386 (7500)	461	0.031	1.0[c]	0.31	9.7
AcN	Lfl	342	443 (13400)	533	0.16	7.7	0.21	1.1
	3MLfl	342	444 (10100)	531	0.17	7.1	0.24	1.2
	Lch	334	380 (8300)	437	0.028	0.64 0.7[d]	0.43	15
	1MLch	334	379 (7600)	437	0.027	0.63 0.5[d]	0.43	15
	3MLch	335	379 (8100)	436	0.026	0.64	0.41	15
	1,3MLch	335	373 (7200)	437	0.028	0.64	0.43	15
H_2O	Lfl	367	445 (10930)	530	0.14			
	3MLfl	364	444	520	0.15	4.5	0.33	1.8
	Lch	353	385 (7600)	479	0.088	2.7	0.32	3.4
	1MLch	354	386 (7200)	475	0.079	2.2	0.35	4.1
	3MLch	353	384 (7400)	470				
	1,3MLch	353	390 (7500)	471				

[a] λ_1 and λ_2 are the positions of the two lowest energy bands in the absorption spectra, molar absorption coefficients in parenthes, λ_F is the fluorescence emission maximum, ϕ_F is the fluorescence quantum yield, τ_F is the fluorescence lifetime, k_r is the radiative rate constant, and $\sum k_{nr}$ is the sum of nonradiative rate constants. [b] From reference 14. [c] From reference 15. [d] From reference 6.

had led to a conclusion that hydrogen bonding at N(5) may be responsible for such behavior. It was suggested that hydrogen bonds in alloxazines are formed first at N(10), then at N(5), and at both carbonyl oxygens, C(2) and C(4),[18] while for isoalloxazines they are formed first at N(1), then at both carbonyl oxygens, C(2) and C(4), and finally at N(5).[19] Moreover, on the basis of molecular-orbital calculations, it was shown that hydrogen bonding in isoalloxazines at N(1) resulted in the blue shift of the λ_1 absorption band and in the red shift of the λ_2 band.[20] Hydrogen bonding at N(5) resulted in a red shift of both bands. Thus, we believe that the shift of the λ_2 absorption band results from hydrogen bonds at N(5) and, additionally, at N(1) for lumiflavin and N(10) for lumichrome. These H-bonding interactions exert a profound effect on radiative and nonradiative excited state decay rates; in lumichromes these are slowed relative to nonpolar or aprotic solvents. In lumiflavins these rates are relatively solvent insensitive, suggesting the N(10) interaction is important in determining intramolecular relaxation rates.

Spectroscopic properties of lumichrome and other alloxazines in different solvents have been the subject of a number of previous works.[21−24] Surprisingly, the data on the photophysics of lumichromes are very scattered and incomplete. This is especially true for any systematic results of the solvent effect on the singlet excited states of lumichromes; for example, data on fluorescence lifetimes are very limited. All the lumichromes exhibit two bands in the near-UV region with the maxima at about 334 (ca. 29 900 cm^{-1}) and 380 nm (ca. 26 300 cm^{-1}), which have been assigned to two independent π,π^* transitions.[23,24] Molar absorption coefficients and positions of the band maxima are listed in Table 1.

Fluorescence emission spectra of lumichromes exhibit a single band with a maximum at about 440 nm (ca. 22 700 cm^{-1}), the exact position depending on solvent. The wavelengths of the maxima are listed in Table 1, together with quantum yields and fluorescence lifetimes. The emission decay of lumichrome and its derivatives is described well by a single-exponential function. The data presented show that the decay of the singlet state is dominated by the rates of the nonradiative processes, these being more than an order of magnitude larger than those of the radiative processes. We can also note that all characteristics of the excited singlet states—ϕ_F, τ_F, k_r, and $\sum k_{nr}$—are essentially identical for the lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives, in each solvent, respectively. The recorded fluorescence lifetimes and quantum yields in polar acetonitrile, and nonpolar solvents 1,2-dichloroethane and 1,4-dioxane, show that solvent polarity does not significantly affect these properties. In polar protic solvents, water and methanol, the absorption and emission bands of all lumichromes studied undergo red shifts, the fluorescence quantum yields becoming higher and fluorescence lifetimes longer, if compared to lumichromes in aprotic solvents. A similar trend has been reported for lumichromes in some other protic solvents. For example, in ethanol the reported fluorescence lifetime is about 0.9 ns for Lch and 1MLch (1-methyllumichrome)[25] and in methanol a fluorescence lifetime of about 1.0 ns has been reported for all lumichromes.[26] However, in protic solvents the data for lumichromes should be interpreted with caution, because of possible phototautomerization between the alloxazine and isoalloxazine structures, for Lch and 3MLch (3-methyllumichrome). For lumichromes unsubstituted at the N(1), the excited-state proton transfer from N(1) to N(10) may form the corresponding isoalloxazine in methanol and aqueous solutions. The similarity of spectroscopic and photophysical data for Lch and 3MLch unsubstituted at the N(1) position, and those methyl-substituted at the same position, 1MLch and 1,3MLch (1,3-dimethyllumichrome), suggests that no double proton transfer within the hydrogen-bonded solvent

TABLE 2: Calculated (B3LYP/6-31G*) Singlet Energies, *E*, Starting from the Ground State and Corresponding Oscillator Strengths, *f* [a]

	lumiflavin		3-methyllumiflavin		lumichrome		1-methyllumichrome		3-methyllumichrome		1,3-dimethyllumichrome	
$S_0 \rightarrow S_i$	$10^{-3}E/\mathrm{cm}^{-1}$	f	$10^{-3}E/\mathrm{cm}^{-1}$	f	$10^{-3}E/\mathrm{cm}^{-1}$	f	$10^{-3}E/\mathrm{cm}^{-1}$	f	$10^{-3}E/\mathrm{cm}^{-1}$	f	$10^{-3}E/\mathrm{cm}^{-1}$	f
$\rightarrow S_1$	24.5p **22.68**	0.191	24.4 **22.62**	0.188	27.6	0.002	27.5	0.001	27.7	0.002	27.5 **26.18**	0.083
$\rightarrow S_2$	24.9	<0.001	24.7	<0.001	27.8 **26.39**	0.066	27.6 **26.25**	0.080	27.7 **26.39**	0.071	27.5	0.003
$\rightarrow S_3$	26.6	0.001	26.5	<0.001	31.7 **30.58**	0.190	31.6 **30.49**	0.166	31.6 **30.49**	0.205	31.4	<0.002
$\rightarrow S_4$	31.0	0	29.6	0.053	31.9	<0.001	31.7	<0.001	31.7	<0.003	31.6 **30.49**	0.177
$\rightarrow S_5$	31.1 **30.12**	0.134	30.7	0	38.6	0.015	38.2	0.047	36.0	0.007	35.8	0.003
$\rightarrow S_6$	32.2	0.011	31.3 **29.94**	0.115	39.0	0	38.8	0	38.8	0	38.7	0
$\rightarrow S_7$	37.3	<0.001	36.9	<0.001	39.7	0	39.5	0.043	39.7	0	39.4	0
$\rightarrow S_8$	38.0	0.071	37.9	0.083	40.5	0.266	39.5	0	40.5	0.273	39.5	0.098
$\rightarrow S_9$	39.6	0.592	39.5	0.594	41.4	0	40.9	<0.001	41.5	0	40.9	0.583
$\rightarrow S_{10}$	40.0	0	40.1	0	42.1	0.284	41.2	0.538	41.9	0.353	41.0	<0.001
$\rightarrow S_{11}$	41.3	0.021	41.2	0.016	43.2	0.581	42.9	0.549	43.1	0.566	42.9	0.543
$\rightarrow S_{12}$	41.4	<0.001	41.4	<0.001	44.7	<0.001	44.4	<0.001	44.6	<0.001	44.2	<0.001
$\rightarrow S_{13}$	45.7	<0.001	44.3	0.007	48.0	0	47.7	0.153	47.6	<0.001	47.4	0.019
$\rightarrow S_{14}$	46.5	0.034	45.7	<0.001	48.1	0.139	47.9	<0.001	47.9	<0.001	47.5	0.158
$\rightarrow S_{15}$	47.6	0.633	47.3	0	49.8	<0.001	49.5	<0.001	48.0	0.169	47.8	<0.001

[a] Experimental values taken in 1,4-dioxane solutions are listed in bold type for comparison.

complex is possible in the S_1 state of either molecule in the present conditions.

The electronic structure of lumiflavin and lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives was studied by means of time-dependent density-functional theory (TD-DFT).[10] Recently, similar TD-DFT calculations have been performed for singlet and triplet absorption spectra of lumiflavin.[27−30] Both the previous results for lumiflavin and our results for lumiflavin and lumichromes demonstrate some very encouraging improvements as compared to previous semiempirical and ab initio calculations,[23,31] in that they succeeded in reproducing the correct order of the observed singlet excited states and oscillator strengths of the respective transitions. Moreover, to the best of our knowledge, the only theoretical predictions for similar compounds available in the literature were obtained by using semiempirical methods.[23,24] The results obtained in this study by the TD-DFT approach are presented in Table 2 and Figures 2 and 3.

We are aware of no experimental gas-phase spectra for the compounds presently examined. Thus we turn to spectra recorded in solvents, having in mind possible effects of the environment on the position and shape of the corresponding bands. However, the position of the lowest energy band is not much affected by polarity or the hydrogen bond donating ability of the solvent (vide supra). Positions of the second absorption band in polar acetonitrile and nonpolar 1,4-dioxane and 1,2-dichloroethane also confirm that polarity is not an important factor influencing the position of these bands. In polar protic solvents, water and methanol, the absorption and emission bands of both examined lumiflavins undergo red shifts, if compared to lumiflavins in acetonitrile or 1,4-dioxane. For both lumiflavins, the two calculated lowest energy transitions presented in Figure 2 are of π,π^* character at approximately 321 (31 100 cm^{-1}) and 408 nm (ca. 24 500 cm^{-1}), and the computed oscillator strengths confirm that only the π,π^* transitions should be observable. Note, however, that the π,π^* transitions are accompanied by two closely located n,π^* transitions at 323 (31 000 cm^{-1}) and 402 nm (24 900 cm^{-1}) of low oscillator strengths. For both lumiflavins, the lowest excited singlet state has the π,π^* character, in contrast to lumichromes, where the lowest calculated excited singlet state has the n,π^* character.

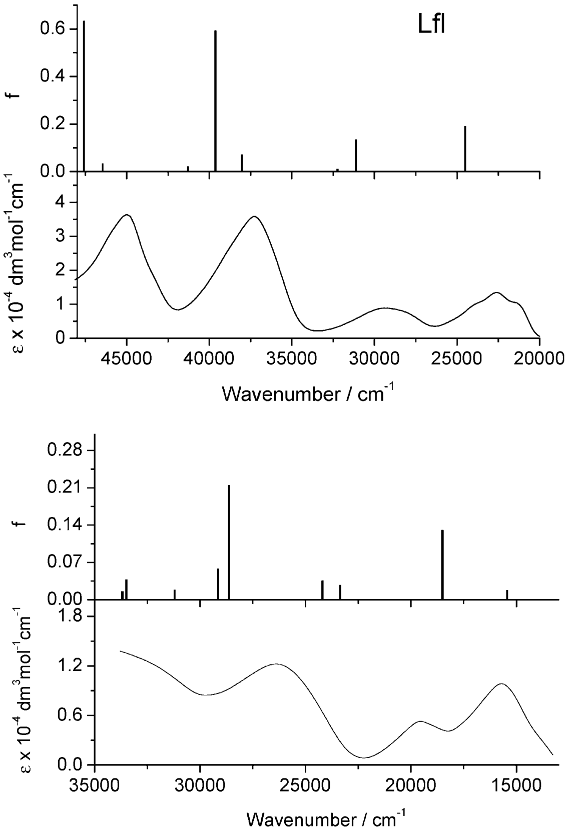

Figure 2. Top panel: Absorption spectrum of lumiflavin (Lfl) in acetonitrile solutions. Predicted transition energies and oscillator strengths (*f*) are indicated by solid vertical lines. Bottom panel: Calculated T−T transitions of lumiflavin (Lfl) obtained in the DFT calculations. Predicted transition energies and oscillator strengths (*f*) are indicated by solid vertical lines. For comparison, an experimental absolute transient absorption spectrum of Lfl in ethanol is given (excitation at 355 nm).

For all lumichromes, the two calculated lowest energy transitions presented in Figure 4 are of π,π^* character, located

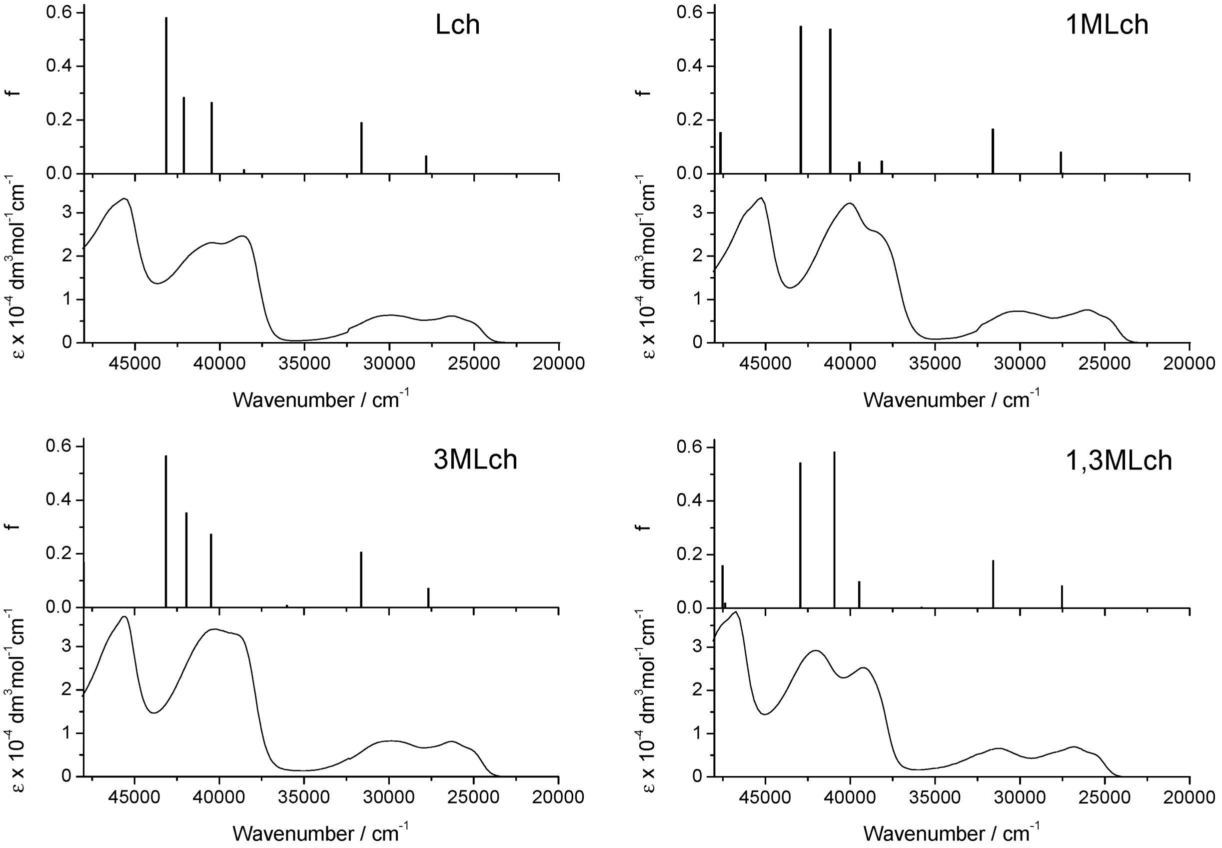

Figure 3. Absorption spectra of lumichrome (Lch), 1-methyllumichrome (1MLch), 3-methyllumichrome (3MLch), and 1,3-dimethyllumichrome (1,3MLch) in acetonitrile solutions. Predicted transition energies and oscillator strengths (*f*) are indicated by solid vertical lines.

at approximately 316 (31 700 cm^{-1}) and 359 nm (ca. 27 800 cm^{-1}), and accompanied by two closely located n,π* transitions at 313 (31 900 cm^{-1}) and 362 nm (27 600 cm^{-1}) of low oscillator strengths. Hence, the observed transitions are of π,π* character. The difference between the predicted and observed transition energies is about 1500 cm^{-1}. As is the case for many aza-aromatics, lumichrome possesses close-neighboring n,π* and π,π* (calculated $\Delta E = 200$ cm^{-1}) singlet excited states, having a lowest excited singlet state of n,π* character. On the basis of the data in Table 2 for lumichromes it may be expected that the first excited singlet state is of n,π* character in nonpolar solvents, but π,π* in protic media. This so-called level inversion arises from the sensitivity of the energy of n,π* transitions to the hydrogen bond donor ability of the solvent. In contrast, the energies of π,π* transitions are not strongly influenced by changes in the hydrogen bond donor properties of solvents, as is the case for the longest wavelength absorption band and fluorescence emission of lumiflavins. This interpretation based on the level inversion is supported experimentally by the marked decrease of the $\sum k_{\mathrm{nr}}$ occurring for Lch and 1MLch in water vs the aprotic solvents. However, if level inversion occurs, large differences in the radiative rate constants in different solvents should be observed. Inspection of the data presented in Table 1 shows that the radiative rate constant for lumichromes is nearly independent of the nature of the solvent. An alternative explanation for the photophysical data presented in Table 1 is based on the so-called *proximity effect*.[32] This phenomenon is believed to be a consequence of vibronic interaction between close-lying n,π* and π,π* singlet states and does not therefore require state inversions to account for solvent-dependent photophysical properties. Such an interpretation based on the *proximity effect* is entirely consistent with our latest TD-DFT calculations for hydrogen-bonded complexes between lumichrome and methanol, and between lumichrome and acetic acid. For such complexes between hydrogen-bonding agents and lumichrome at the N(1)−H group and N(10) atom, the lowest excited singlet state has π,π* character but is located only 200 cm^{-1} below the second excited singlet state, which is of n,π* character. Finally, it is well-known, that as the hydrogen bond-donating ability of the solvent increases, the energy of a π,π* excited singlet state decreases to a greater extent than that of the ground state. For lumichromes, the lowest π,π* absorption band shifts to longer wavelength by only about 7 nm, while the π,π* fluorescence transitions are more solvent sensitive, and the fluorescence spectrum shifts by about 40 nm from acetonitrile to water.[6,26]

Lumiflavins and Lumichromes in Their Lower Triplet States. The electronic structure of lumiflavins and lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives has been studied by means of the time-dependent density-functional theory in their triplet states and compared to experimental T−T absorption spectra. TD-DFT calculations have been used to predict different spectral, photophysical, and photochemical properties of lumiflavin.[28−30,33] In fact, there exist few theoretical predictions of the UV−vis T−T electronic spectra of lumiflavins and alloxazines. Recently, similar TD-DFT calculations have been made for the singlet and triplet absorption spectra of uracil and lumiflavin.[27] In contrast to our study, in which direct comparison of theoretical and experimental transitions is made, the authors[27] have compared the calculated transitions of lumiflavins to the flavin mononucleotide, FMN, spectrum in aqueous solutions.

It is predicted, on the basis of TD-DFT (B3LYP/6-31G*) calculations, that the first excited triplet states are of π,π* character for lumiflavins and lumichromes, and are well separated, by more then 2000 cm^{-1}, from their second excited

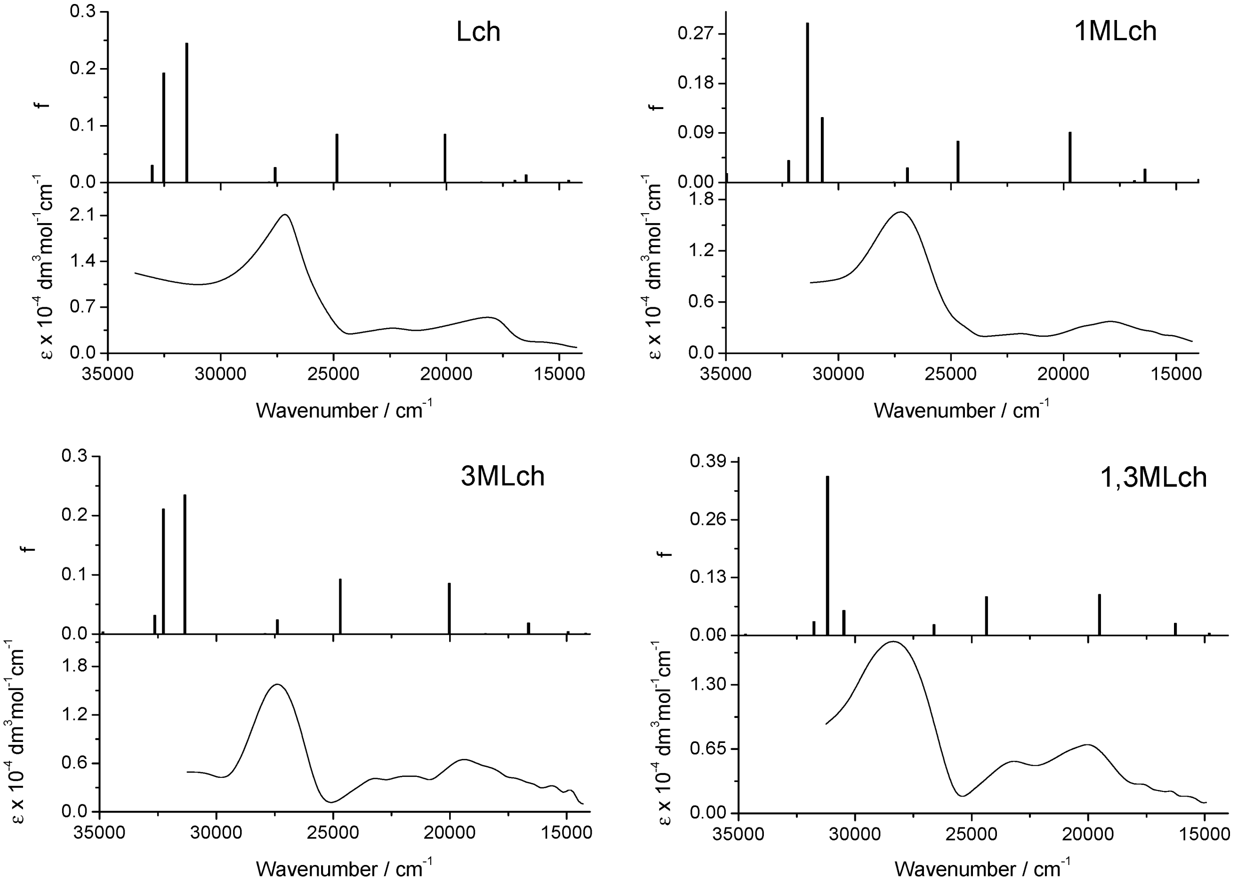

Figure 4. Predicted T−T transitions of lumichrome (Lch), 1-methyllumichrome (1MLch), 3-methyllumichrome (3MLch), and 1,3-dimethyllumichrome (1,3MLch) obtained in the DFT calculations: transition energies and oscillator strengths (*f*) are indicated by solid vertical lines. For comparison, experimental absolute transient absorption spectra of Lch in ethanol, and of 1MLch, 3MLch, and 1,3MLch in acetonitrile, are given (excitation at 355 nm).

triplet state. Corresponding energies are as follows: 20 430 cm^{-1} for Lch, 20 580 cm^{-1} for 1MLch, 20 390 cm^{-1} for 3MLch, and 20 510 cm^{-1} for 1,3MLch, and for lumiflavins 16 670 cm^{-1} for Lfl and 16 600 cm^{-1} for 3MLfl. In the present study, T−T excitation energies and transition intensities were determined for the optimized geometry of the lowest triplet state (T_1), and the results are shown in Table 3 and Figures 2 and 4. Detectable transitions of lumiflavin shown in the Figure 2 are located at about 15 400, 18 500, 23 400, 24 200, and 28 600 cm^{-1}. An experimental absolute transient absorption spectrum of lumiflavin in ethanol, also shown in Figure 2, exhibits bands around 15 750, 19 630, and 26 270 cm^{-1}. The theoretical bands are obviously blue-shifted relative to the experimental spectrum. Note, however, that we are comparing the experimental spectrum in ethanol to the calculations referring to isolated gas-phase molecules. The solvent effect has been elucidated by comparing gas-phase uracil spectra with that in aqueous solution [27] where the uracil gas-phase absorption bands are blue-shifted by about 1200−2500 cm^{-1}, although there are no known absorption spectra of lumichromes and lumiflavins in the gas phase. There exist other possible sources of deviations between theory and experiment, thus an overall spectral prediction accuracy of ±1500 cm^{-1} for singlets and ±2500 cm^{-1} for triplets should be expected even for much smaller molecules. Note that there are several lower energy transitions (see Table 3), which could not be observed in the experimental spectrum because of current limitations of the flash-photolysis setup.

Figure 4 shows experimental T−T spectra of lumichromes in acetonitrile solutions. The theoretical spectra successfully reproduce energies and oscillator strengths of these T−T transitions, within the limitations discussed above. In our recent publication, we have reported the first results of TD-DFT calculations on 1-methyllumichrome.[34] To the best of our knowledge, the spectra reported in Figure 4 are among the first theoretical T−T spectra available, describing excitation from the lowest triplet state of lumichromes, and of alloxazines in general. Note that previous theoretical approaches used for similar compounds were limited to semiempirical methods.[23,24] The T−T excitation energies and transition intensities were determined for the optimized geometry of the lowest triplet state (T_1), and the results are shown in Table 3 and Figure 4. These results are particularly important when we have to discriminate between the triplet excited state and other possible species, readily formed by photoexcited alloxazines in different environments. The detectable transitions of lumichrome, Lch, shown in the Figure 4 and located at about 16 500, 20 100, 24 900, 27 600, and 31 500 cm^{-1} are quite close to the available experimental values: 15 750, 18 180, 22 300, and 27 000 cm^{-1}. The difference between predicted and observed transition energies is higher than that for the singlet states, amounting to about 2000 cm^{-1} for the lowest, with a tendency to increase for the higher energy T−T transitions. Several lower energy transitions could not be detected (see Table 3), as we already mentioned.

The absolute experimental triplet−triplet absorption spectra of Figures 2 and 4 were calculated by correcting transient spectra for the ground-state depletion, using previously measured values of the triplet extinction coefficient at 370 nm[6,35] and the steady-state absorption spectra taken in the respective solvents. In the

TABLE 3: Calculated (UB3LYP/6-31G*) Triplet Excitation Energies, *E*, Starting from the Lowest Triplet State and Corresponding Oscillator Strengths, *f*[a]

	lumiflavin		3-methyllumiflavin		lumichrome		1-methyllumichrome		3-methyllumichrome		1,3-dimethyllumichrome	
$T_1 \rightarrow T_i$	$10^{-3}E$/cm^{-1}	f	$10^{-3}E$/cm^{-1}	f	$10^{-3}E$/cm^{-1}	f	$10^{-3}E$/cm^{-1}	f	$10^{-3}E$/cm^{-1}	f	$10^{-3}E$/cm^{-1}	f
$\rightarrow T_2$	7.89	0	7.79	0	6.94	0.008	6.65	0.010	6.91	0.009	6.44	0.011
$\rightarrow T_3$	8.06	0.006	8.19	0.005	7.04	0	6.85	0	7.08	0	6.64	0
$\rightarrow T_4$	8.10	0	8.27	0	11.9	0	11.7	0	11.8	0	11.4	0
$\rightarrow T_5$	12.9	0.010	10.9	0.004	14.6	0.004	14.0	0.005	14.2	0.001	13.8	0.004
$\rightarrow T_6$	13.6	0	13.4	0	16.5 **15.75**	0.013	16.4 **15.38**	0.024	14.9	0.004	14.8	0.005
$\rightarrow T_7$	15.4	0.018	15.5	0	17.0	0.004	16.8	0.003	16.6 **15.63**	0.019	16.3	0.027
$\rightarrow T_8$	18.2	<0.001	17.9	<0.001	18.5	<0.001	18.0	<0.001	18.5	<0.001	17.9	<0.001
$\rightarrow T_9$	18.5 **15.75**	0.131	18.6	0.125	19.9	0	19.7 **17.86**	0.091	19.5	0	19.5 **20.00**	0.092
$\rightarrow T_{10}$	23.4 **19.63**	0.027	23.2	0.024	20.1 **18.18**	0.085	20.2	0	20.0 **19.23**	0.085	19.6	0
$\rightarrow T_{11}$	24.2	0.035	24.2	0.038	24.9 **22.30**	0.085	24.7 **22.22**	0.075	24.7 **21.74**	0.092	24.4 **23.26**	0.087
$\rightarrow T_{12}$	26.3	0.001	26.2	0.0006	27.6	0.026	26.9	0.027	27.4 **23.26**	0.024	26.6	0.024
$\rightarrow T_{13}$	28.6 **26.27**	0.214	28.6	0.235	27.9	<0.001	27.5	<0.001	27.9	<0.001	27.7	<0.001
$\rightarrow T_{14}$	29.1	0	29.0	0.052	30.8	0	30.5	0	30.9	0	30.5	0.056
$\rightarrow T_{15}$	29.1	0.058	29.2	0	31.5 **27.00**	0.245	30.7 **27.03**	0.118	31.3 **27.30**	0.235	30.6	0
$\rightarrow T_{16}$	31.2	0.018	30.8	0.017	32.5	0.192	31.4	0.289	32.3	0.211	31.2 **28.57**	0.358

[a] Experimental values are listed in bold type for comparison.

TABLE 4: The Quantum Yields of Photosensitized Production of Singlet Oxygen, ϕ_Δ, and the Singlet Oxygen Lifetimes in Methanol, τ_Δ

compd	ϕ_Δ	τ_Δ/μs
lumichrome	0.85	9.8 ± 1.0
1-methyllumichrome	0.86	10.0 ± 0.5
3-methyllumichrome	0.93	10.3 ± 1.3
1,3-dimethyllumichrome	0.91	9.4 ± 1.1
lumiflavin	0.48	10.4 ± 2.1
3-methyllumiflavin	0.53	9.5 ± 1.4

calculations, we assumed that only two absorbing species exist after the first singlet excited state has decayed, namely, the ground-state and the first triplet excited-state molecules. Note that triplet extinction coefficients measured by the triplet−triplet energy transfer from benzophenone have relative uncertainties of 10%.

Singlet Oxygen Measurements and the Triplet Yields. Although triplet state population in iso- and alloxazines has been demonstrated, the amount of available data regarding singlet oxygen generation is rather limited.[36,37] However, it has been proposed that lumichrome may play an important role in the photodegradation of polyamidehydroxyurethane polymers in aqueous solution,[38] and that singlet oxygen may be involved. Recent studies have shown that lumichrome may act as an efficient photoinitiator of free-radical polymerization of 2-hydroxyethyl methacrylate in the presence of triethanolamine.[14] A further point of interest is the possibility of using iso- and alloxazines to sensitize the photooxidation of substituted phenols in water.[39]

To shed further light on this and to investigate further the nonradiative relaxation pathways, we measured photosensitized singlet oxygen formation by monitoring the characteristic $O_2^*(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-)$ emission at 1270 nm. The quantum yields and lifetimes of singlet oxygen, ϕ_Δ and τ_Δ, formed by triplet photosensitization were determined by exciting air-saturated lumiflavin and lumichrome samples in methanol at 355 nm, and are presented in Table 4. The emission lifetime values presented in Table 4 are typical for singlet oxygen in methanol solutions.[40]

The values of ϕ_Δ reported in Table 4 for lumichromes in methanol are higher than our reported values for a set of alloxazines, including lumichrome and 1-methyllumichrome, in acetonitrile solution.[6] In acetonitrile it was shown that the efficiency of singlet oxygen production from quenching of the triplet state f_Δ^T was unity within the error of the experiment. On this basis the quoted singlet oxygen quantum yields can be regarded as approximating intersystem crossing yields; alternatively, if $f_\Delta^T < 1$, then the ϕ_Δ may be regarded as a lower limit for the intersystem crossing yield. Inspection of the data in Table 1 reveals that for the pair Lfl and Lch in all solvents, radiative rate constants are comparable, although nonradiative rates differ significantly. In methanol, on the basis that $\phi_\Delta = \phi_T$ (i.e. f_Δ^T is unity), values of k_{isc} are calculated as 0.62 and 7.9×10^8 s^{-1}, respectively. This difference in intersystem crossing rates can be rationalized on the basis of the nature of the excited states, which emerges from the TD-DFT calculations. For lumichromes in aprotic solvents, for which a higher $^1\pi,\pi^*-{}^1n,\pi^*$ electronic energy gap leads to a reduced *proximity effect*, the disposition of $^1n,\pi^*$ relative to $^3\pi,\pi^*$ appears to have an important influence on the rate of intersystem crossing, consistent with the selection rules for such processes. For lumichromes in protic solvents, with strong mixing between $^1\pi,\pi^*$ and $^1n,\pi^*$ states, the intersystem crossing process becomes less favored. For lumiflavins in protic and aprotic solvents the lowest excited triplet and singlet states are both π,π^* in nature (vide supra). As noted previously, lumichromes possess close-lying n,π^* and π,π^* states and the possibility of either the *proximity effect* or a state inversion in strongly hydrogen-bonding solvents must be borne in mind. On the basis of the singlet oxygen data, however, the presence of the low-lying n,π^* states clearly acts to influence the rate of intersystem crossing in the case of lumichromes. The above hypothesis is supported by the available ϕ_T data for lumichrome and lumiflavin. Assuming the lumichrome lowest singlet state has n,π^* character in aprotic solvents and is strongly vibronically coupled to the very close lying excited state of π,π^* character in protic solvents, one can expect the quantum yield of triplet formation to vary as a function of the solvent nature. Indeed, the quantum yield of triplet formation by lumichrome in acetonitrile, ethanol, and water was reported to be 0.71,[6] 0.61, and 0.41,[41] respectively. In comparison, for lumiflavin, where the lowest singlet and triplet excited states both have π,π^* character, a ϕ_T of 0.41 was reported in ethanol.[41] As has been mentioned previously and as is shown in Table 1, the radiative rate constant is practically solvent independent, which suggests that the *proximity effect* has an important influence on the photophysics of lumichromes.

The values of ϕ_Δ reported in Table 4 for lumiflavin and 3-methyllumiflavin are significantly smaller, if compared to lumichromes. However, it can be noted that the previously reported values for lumiflavin are higher than those in Table 4, for example it has been reported that ϕ_Δ for lumiflavin is 0.85 in acetonitrile and 0.8 in ethanol[36] and references therein. However, for the riboflavin molecule, structurally closely related to lumiflavin, the reported values are very close to our value for lumiflavin: 0.49 in water at pH =7.4, 0.48 in CH_3OD, and 0.47 in CH_3OH,[36] and references therein. The reason for the difference between our value and that reported for lumiflavin in ethanol is unclear, although for lumiflavin and analogues significant photochemical instability was noted in methanol, with both the solution absorbance and the apparent singlet oxygen quantum yield increasing with increasing irradiation doze. The values quoted in the Table 3 were obtained by changing the solution following each laser pulse to minimize these effects.

Conclusions

Studies of the solvent effects on spectroscopic properties of lumichromes and lumiflavins have shown differences and similarities. Fluorescence yields for the lumiflavins are an order of magnitude larger than those for the lumichromes, attributable entirely to smaller nonradiative rate constants, since the respective radiative rate constants are of similar magnitudes. Solvent changes in absorption bands can be explained on the basis of hydrogen-bonding interactions. TD-DFT calculations show that the lowest energy states are n,π^* in the case of lumichromes, but π,π^* in the case of the lumiflavins. The overall consistency of the predicted S−S and T−T transitions obtained for the various compounds studied, and a good correspondence between the predicted and the measured transitions, indicates that our theoretical understanding has reached a stage when we can reliably predict the spectral properties of lumiflavins and lumichromes using the TD-DFT approach.

Acknowledgment. The interdisciplinary grant from A. Mickiewicz University and University of Economics, Poznań, Poland, No. 51103-506, to M.S. and E.S. is gratefully acknowledged. The calculations were performed at the Poznań Supercomputer Centre (PCSS).

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