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Effect of nano-confinement on the photophysics of lumichrome

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ABSTRACT

We have investigated the photophysics of lumichrome in the nano cage of reverse micelles by using UV–Vis absorption, fluorescence emission and picosecond time resolved emission spectroscopy. Different polar solvents were used such as water, methanol, dimethylformamide, ethylene glycol and glycerol to form the polar solvents pool of the reverse micelles. We have found that on increasing w value (w = [polar solvent]/[surfactant]), the quantum yield and fluorescence lifetime value gradually increases. Time-resolved area-normalised emission spectra (TRANES) analysis in reverse micelles shows the presence of an isoemissive point which indicates that presence of two emissive species of lumichrome in the reverse micelle.

1. Introduction

Lumichrome (7,8-dimethylalloxazine), consists of nitrogen heterocyclic compound is a member of biologically important flavins [1] and non-toxic molecule. It possesses both hydrogen bond donor and acceptor sites (N-atoms, O-atoms, N–H groups). Excited state proton transfer (ESPT) takes place via tautomerisation process [2–4]. Lumichrome is the photo degradation product of riboflavin (vitamin B_2) under neutral or acidic pH values [5,6]. In the presence of H-bond donating molecules (e.g.; pyridine, acetic acid) lumichrome forms two type of stable isomers, DMAL (7,8-dimethyl aloxazine) and DMIS (7,8-dimethyl isoalloxazine) in aqueous solution [7]. These two stable isomers undergo tautomerisation process involving N-1 and N-10 nitrogen atoms. DMAL tautomer is the predominant form in ground state whereas DMIS tautomer is formed via excited-state double proton-transfer reaction (ESDPT) [8]. Lumichrome was identified from sinorhizobium meliloti cells and catalyses the root respiration and shoot growth in alfalfa (Medicago sativa plants) [9]. Lumichrome is used as photosensitizers of singlet oxygen at pH 7 [10] and as a photo initiator in the polymerisation of 2-hydroxyethyl methacrylate (HEMA) in presence of triethanol amine (TEOHA) [11].

There are several reports available in the literature on the photophysics of lumichrome molecules [12–26]. Biczók et al. reported the binding of cucurbit[7]uril to an aqueous solution leading to the red shifted band of both absorption and emission spectra due to tautomerisation of proton transfer from N(1) to N(10) position in lumichrome [17]. Pill-Soon-Song et al. have reported that lumichrome emits two fluorescence bands at 440 and 540 nm, respectively in both pyridine-dioxan and acetic acid–ethanol mixtures. This is due to the excited state proton transfer from N-1 to N-10 positions [19,20]. Solvent dependency is observed in lumichrome molecule for both radiative and non radiative rate constants. Rate constants decrease with increasing solvent polarity and also increasing protic nature of the solvent as described by Sikorska et al. [21]. They have also studied the excited state double proton transfer (ESDPT) in the solid state of lumichrome dimer [22] and theoretical study of hydrogen-bonded complexes of lumichrome [23]. Recently Sikorski et al. have investigated the photophysical properties of lumichrome and its derivatives at different pH in aqueous solution and proposed the species exist in neutral, two monoanions and dianion form depending upon acid–base medium of the solution [25].

Reverse micelles are coated by the spherical aggregation of surfactant molecules, immersed in non polar solvents with polar head groups oriented inward which are in contact with water pool and the hydrophobic tails of the surfactant oriented outward into the nonpolar solvent. The polar solvent inside the reverse micelles core behave differently than the bulk solvents. The size of the reverse micelles is characterised by the ratio of polar solvent to surfactant concentration:

$$w = [\text{polar solvent}]/[\text{surfactant}]$$

Reverse micelles is an example of stable confined medium which are mainly used in biological system as well as several photophysical studies. We have used anionic surfactant aerosol OT (AOT, sodium di(2-ethylhexyl) sulfosuccinate) to form reverse micelles, and isooctane as non polar solvent. We have used water, methanol, dimethyl formamide (DMF), ethylene glycol (EG) and glycerol as polar solvents to form reverse micelles. The formations of reverse micelles by using the above polar solvents were reported in the literature [26–30]. In this Letter, we would like to report the influence of confinement on the photodynamics of lumichrome molecule in aqueous and non-aqueous reverse micelles. The goal of this Letter is to report the differences of the photodynamic of

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lumichrome in the nanoconfined environment of reverse micelles which is a suitable membrane mimic system, compared to that in neat polar solvents. Previously in reverse micelles the photodynamic of lumichrome derivative was reported by using different surfactant [12,13]. The photodynamic of other flavins molecules in AOT reverse micelle was reported in the literature [30]. The photodynamic of lumichrome in other polar solvents containing reverse micelles is not available in the literature. An isoemissive point in time-resolved area normalised emission spectra (TRANES) was observed in water, EG and glycerol reverse micelles confirming the presence of two different forms of lumichrome in the above polar solvents containing reverse micelles. We hope that this Letter will be helpful to understand the dynamical features of isoalloxazine type molecule in biological environments.

2. Materials and method

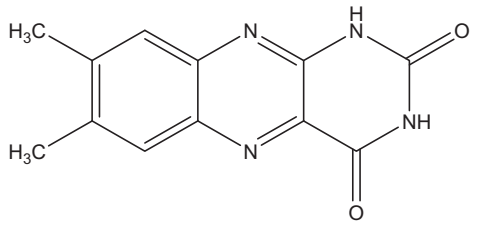
Lumichrome (Scheme 1) was procured from Sigma–Aldrich and used as received without any further purification. AOT (sodium di octyl sulfosuccinate, 99%, Sigma–Aldrich) was purified and dried under vacuum at 298 K for more than 48 h using a process described elsewhere [31]. Millipore water was used for preparation of water reverse micelle. Methanol, isooctane was purchased from Spectrochem, India. DMF (HPLC grade) and glycerol (ACS grade) were purchased from RANKEM, India. Ethylene glycol was purchased from CDH, India. The concentration of probe molecules was maintained 2×10^{-5} M and that of AOT was 0.1 M for all measurements.

The steady state absorption and emission spectra were measured by using UV–Vis absorption spectrophotometer (Model: UV-2550, SHIMADZU) and Fluoromax-4P spectrofluorometer (HORIBA JOBIN YVON). All the steady state measurements were carried out at 298 K by using Jeoitech refrigerated bath circulator (Model: RW0525G). The fluorescence quantum yields are measured assuming the quantum yield of quinine sulphate in 0.1(N) H_2SO_4 as 0.546 [32] by using the following equation:

$$\phi_s = \frac{I_s A_r n_s^2}{I_r A_s n_r^2} \phi_r \tag{1}$$

where subscript s and r denotes the sample and reference respectively. I stand for the integrated area under the emission band, A stands for the absorbance of the solution at the excitation wavelength and n stands for the refractive index of the solvent at 298 K.

Fluorescence lifetimes (τ_f) were determined by using picoseconds time correlated single-photon counting (TCSPC) technique. We have used time-resolved fluorescence spectrophotometer from Edinburgh Instruments (Model: Lifespec-II, U.K.). We have used a picosecond diode laser at 375 nm as an excitation source. The instrument response function (IRF) of our system is ~75 ps. The fluorescence transients were detected in magic angle (54.7°) polarisation using Hamamatsu MCP PMT (3809U) as detector. The decays were analysed using F-900 decay analysis software. The observed fluorescence time-resolved decays were fitted after deconvoluting IRF by using the following equation:



Scheme 1. Schematic representation of lumichrome (7,8-dimethyl alloxazine).

$$R(t) = A + \sum_{i=1}^{N} B_i \exp(t/\tau_i) \tag{2}$$

where B_i is the pre-exponential factors with the characteristic lifetimes τ_i and A is the background.

The amplitude weighted average lifetime was calculate as $\langle\tau\rangle = \sum_{i=1}^{N} C_i \tau_i$, where, $C_i = \frac{B_i}{\sum_{i=1}^{N} B_i}$.

The fluorescence anisotropy decays [$r(t)$] were measured by using the same instrument (Model: Lifespec-II). For the anisotropy measurement, the emission intensities at parallel ($I_{//}$) and perpendicular ($I_{\perp}$) were collected alternatively by fixing the time for both the decays. Motorised polarizer's were used to collect the parallel and perpendicular decays. Then the following equation was used to get the value of $r(t)$

$$r(t) = \frac{I_{II}(t) - G I_{\perp}(t)}{I_{II}(t) + 2G I_{\perp}(t)} \tag{3}$$

G is the correction factor for detector sensitivity to the polarisation direction of the emission. The F-900 software was used to fit the anisotropy decay. All experiments were carried out three times to check the reproducibility of the data. In the case of steady state absorption and fluorescence measurement, the temperature was kept fixed at 298 K by using peltier-controlled cuvette holders from Quantum Northwest (Model: TLC-50).

3. Results and discussion

3.1. Steady state results

In presence of neat solvents, lumichrome shows two absorption peaks. The first absorption peak is around 280–350 nm range whereas the second absorption peak appears around 350–420 nm [33]. In aqueous solution, lumichrome shows two absorption peaks, one at 353 nm and a shoulder at 385 nm [14]. This is due to two independent π–π^* transitions based on semiempirical calculations [34,35]. Lumichrome entrapped in the aqueous reverse micelles (water/AOT/isooctane reverse micelle) shows two absorption peaks at 338 and 382 nm respectively (Figure 1a). Here the absorption peak is blue shifted compared to neat water. With gradual addition of water in the reverse micelle core lumichrome exhibits red shift of first absorption peak but the position of the second absorption peak remains unchanged indicating that the probe is encapsulated within the water pool of the reverse micelles. Valle et al. [30] observed two absorption peaks of flavins molecules in water/AOT/n-hexane reverse micelles, around 360–375 nm and around 442–450 nm range. With gradual addition of water inside the reverse micelles, the emission maxima become red shifted as shown in Figure 1b and at $w = 30$, it appears at 458 nm. The fluorescence quantum yield (φ_f) increases with increase of w value (Table 1).

In methanol/AOT/isooctane reverse micelles, we observed two absorption peaks. In the methanol reverse micelles the absorption peak of lumichrome is red shifted and emission peak is blue shifted compared to that in neat methanol. With gradual addition of methanol in the reverse micelles core, the absorption peak becomes red shifted [Figure S1(a)]. The emission peak is also red-shifted from 440 nm ($w = 1$) to 445 nm ($w = 5$) as shown in Figure S2(a). The fluorescence quantum yield (φ_f) gradually increases with increasing w values. In case of DMF/AOT/isooctane reverse micelles, two absorption peaks observed at 331 and 382 nm respectively. Here, the two absorption peaks position remain almost same at higher w values. But the emission peak undergoes red-shift with increase in w value from 437 nm (at $w = 1$) to 445 nm (at $w = 3$) as shown in Figure S2(b). In the DMF reverse micelles the absorption peak and emission peak of lumichrome is red shifted compared to that in