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Fluorescence modulation and associative behavior of lumazine in hydrophobic domain of micelles and bovine serum albumin

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ABSTRACT

The photophysical behavior of the deprotonated form of lumazine (Lum-anion) was studied in biologically relevant surfactant systems like sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and TritonX-100 (TX-100) and also model water soluble protein, bovine serum albumin (BSA), using steady-state and time-resolved fluorescence spectroscopy in buffer solution of pH 12.0. The association constant values were calculated from modulated fluorescence intensity of Lum-anion in different medium. The interaction of non-ionic surfactant TX-100 was found to be about 10 times greater than SDS and CTAB. However, while the driving force of binding in SDS and/or TX-100 is mainly hydrophobic in nature, electrostatic interaction with the oppositely charged micellar head group is the predominant factor in CTAB. The thermodynamic parameters like enthalpy (ΔH) and entropy (ΔS) change, etc., corresponding to the binding of Lum-anion with BSA were estimated by performing the fluorescence titration experiment at different temperatures. Thermodynamically favorable and strong binding of Lum-anion ($K \sim 10^4\ M^{-1}$) into BSA is due to hydrophobic interaction in the ligand binding domain II. However, the binding mechanism is entirely different in presence of protein denaturing agent like urea and electrostatic interaction plays a major role under this condition.

1. Introduction

Pteridines in their multiple forms are widespread in biological systems and represent an important class of heterocyclic compounds with different roles ranging from pigments to cofactors for numerous redox and one-carbon transfer reactions [1,2]. Lumazines (pteridine-2,4(1,3H)-dione) are natural products from the metabolic degradation of pterins [3]. The function and properties of the lumazine protein isolated from blue light emitting bioluminescent photobacteria is similar to that of yellow fluorescence protein (YFP) [4]. The protein family consisting of lumazine synthases are known to form several quaternary structures and recently, been engineered for versatile applications ranging from catalysis of retro-aldol reaction [5], novel molecular encapsulation systems, etc. [6–8]. Lumazine derivatives are present in cells, since 6,7-dimethyl-8-ribityllumazine is the biosynthetic precursor of riboflavin (vitamin B2). Riboflavin is itself the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), essential cofactors for a wide variety of redox enzymes [9,10]. In a recent report, the use of lumazine was also demonstrated as a methanogen inhibitor [11]. The participation of pterins in photobiological processes has been suggested and/or demonstrated in the past decades and still continues to a topic of active research interest.

Lumazine presents different acid–base behavior in aqueous solutions. The only relevant equilibrium at physiological pH involves the neutral (acid) form and the monoanion (basic) form (Fig. 1), with a pK_a value of 8.0 ± 0.1 [12,13]. In recent communication, we have described the fluorescence behavior of neutral lumazine (Lum) with a combination fluorescence experiment and high level density functional theory (DFT) calculation [14]. Also, the photophysical behavior of the luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) and lumichrome (7,8-dimethylalloxazine), the heterocyclic systems containing similar structural motif to that in lumazine were reported in homogeneous as well as several bio-mimicking micro-heterogeneous media [15–18]. Whereas the photophysics and photochemistry of pterins have been studied in detail [19], little is known about the photochemical behavior of lumazines [20,21]; particularly, in biologically relevant heterogeneous media like micelles and/or proteins. The relatively high fluorescence quantum yield and long lifetime of 6,7-dimethyl-8-ribityllumazine, the prosthetic group of lumazine protein, makes it an attractive probe to study ligand–protein and protein–protein interaction and also protein dynamics using thermodynamically ideal, diluted protein solutions [22,23]. In the present paper, we report the results on the spectral modulation and binding behavior of mono-anionic lumazine (Lum-anion) in several surfactant media as well as in presence of model water soluble globular protein like bovine serum albumin (BSA) by steady state and time-resolved fluorescence techniques.

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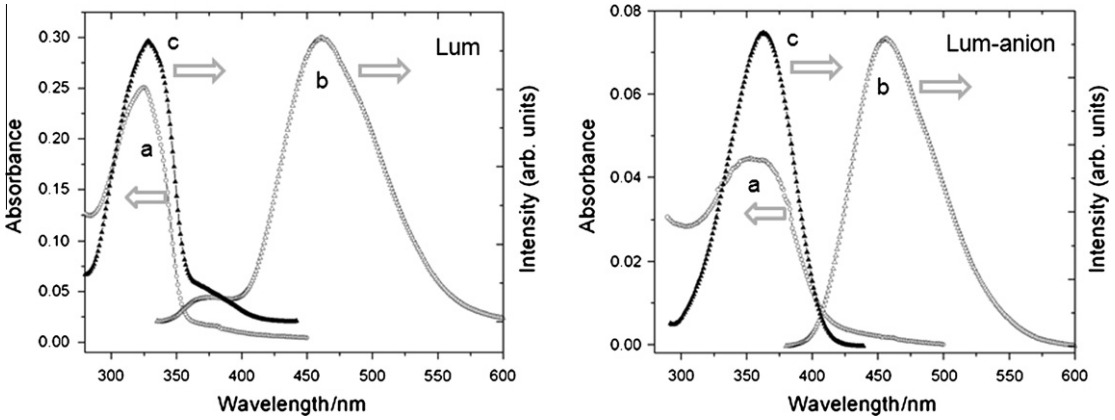

Fig. 1. Steady state spectral absorption (a), fluorescence emission (b) and excitation (c) spectra of $\sim 5.0 \times 10^{-6}$ mol dm^{-3} lumazine solution in neutral (Lum) and deprotonated (Lum-anion) form. The excitation wavelengths are 325 and 365 nm, respectively; whereas the excitation spectra were taken by monitoring the emission wavelength at 455 nm in both the cases.

2. Materials and methods

2.1. Chemicals

The neutral lumazine (Lum) was received from Sigma–Aldrich Chemical Pvt. Ltd. (product no. L-3307) and used without any further purification. The analytical grade type – II water was obtained from Elix 10 water purification system (Millipore India Pvt. Ltd.). All the experiments were carried out at ambient temperature of 293 ± 1 K in buffer solution of pH 12.0 obtained by mixing 100 ml of 0.05 M Na_2HPO_4 with 53.8 ml of 0.1 M NaOH and the volume was adjusted to 200 ml. The analytical grade reagents were obtained from Sisco Research Laboratory (SRL), India. As discussed in the previous section, only the deprotonated form of lumazine (Lum-anion) exists in the solution mixture at this working pH. The surfactants sodium dodecyl sulfate (SDS, product no. 86201-0), cetyltrimethylammonium bromide (CTAB, product no. 85582-0), and TritonX-100 (TX-100, product no. T8787) were procured from Sigma–Aldrich (India) and all were used as received. Essentially fatty acid and globulin free, ⩾99% (agarose gel electrophoresis), lyophilized powder form of bovine serum albumin (BSA, Sigma, cat. no. B4287) was also used as received. The stock solution of the surfactants/protein was prepared by mixing required amount of the substrate in buffer. The final solution pH was controlled with Systronics μ-pH system 361 and adjusted for slight pH variation due to the mixing of the substrates, if any, by adding required amount of HCl or NaOH. The chromophore concentration (~5 μM) was very low to avoid any aggregation and kept constant during spectral measurements; whereas, the final concentration of the heterogeneous media was adjusted by adding the required volume from the stock solution. All the solutions were prepared afresh and kept for 30 min for settling before the spectroscopic measurement.

2.2. Experimental procedure

Steady-state absorption spectra were recorded on a Perkin–Elmer model Lambda25 absorption spectrophotometer. Fluorescence spectra were taken in a Hitachi model FL4500 spectrofluorimeter and all the spectra were corrected for the instrument response function. Quartz cuvettes of 10 mm optical path length received from PerkinElmer, USA (part no. B0831009) and Hellma, Germany (type 111-QS) were used for measuring absorption and fluorescence spectra, respectively. For fluorescence emission, the sample was excited at 365 nm unless otherwise mentioned, whereas excitation spectra were obtained by monitoring at the respective emission maximum. In all cases, 5 nm bandpass was used in the excitation and emission side. Any possible contribution of inner filter effect due to attenuation of the incident light by the quencher is negligible, since none of the components providing the heterogeneous environments have any significant absorption at the excitation wavelength. Nevertheless, the observed fluorescence intensity (F_{obs}) was corrected for any possible attenuation of excitation intensity in presence of surfactant and protein medium by using the following equation [24,25]:

$$F(\lambda_E, \lambda_F) = F_{obs}(\lambda_E, \lambda_F) \times \frac{A(\lambda_E)}{A_{tot}(\lambda_E)} \quad (1)$$

where A represents the absorbance of free Lum-anion and A_{tot} is the total absorbance of the solution at the excitation wavelength (λ_E). All steady-state data obtained from at least three separate experiments were averaged and further analyzed using Origin 6.0 (Microcal Software, Inc., USA).

Fluorescence quantum yields $\left(\phi_f^i\right)$ were calculated by comparing the total fluorescence intensity under the whole corrected fluorescence spectral range with that of a standard (quinine bisulfate in 0.5 M H_2SO_4 solution, $\phi_f^s = 0.546$ [26]) with the following equation using adequate correction for solution absorbance (A) and solvent refractive index (n) [27].

$$\phi_f^i = \phi_f^s \cdot \frac{F^i}{F^s} \cdot \frac{1 - 10^{-A^s}}{1 - 10^{-A^i}} \cdot \left(\frac{n^i}{n^s}\right)^2 \quad (2)$$

The relative experimental error of the measured quantum yield was estimated within ±10%. The temperature variation experiments were carried out by attaching a circulatory thermostat bath (MLW, Germany, type U2C) to the cell holder.

Fluorescence decay analysis were performed in a LED based fluorescence lifetime spectrometer (Photon Technology International) equipped with a stroboscopic detector. Fluorescence lifetime measurement using the stroboscopic method is becoming increasingly popular [28–30] over the standard time correlated single photon counting (TCSPC) and/or phase modulation techniques due to its fast, low cost and easy operating condition, without compromising the accuracy [31]. The kinetic traces were fitted with a sum of exponential decay function by a reconvolution procedure with the lamp pulse profile obtained from scattering of a colloidal suspension of coffee diary whitener using Felix32 software supplied by PTI.