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Fate of anticancer drug ellipticine in reverse micelles in aqueous and methanolic environment: A photophysical approach

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ABSTRACT

The present investigation explored a detailed photophysics of an anticancer agent ellipticine, in AOT reverse micelle using steady state and time resolved spectroscopy. We observed that ellipticines are entrapped as a cationic species in AOT/hexane system. Increase in water content in reverse micelles, entraps more number of cationic species while increase in methanol content causes switch over of cationic ellipticine to a neutral species. Unlike in pure methanol, we did not observe any solvent assisted proton transfer in AOT/hexane/methanol system. This unique observation was explained by the inhomogeneity of methanol entrapped in AOT/hexane system.

1. Introduction

Ellipticine is a pyridocarbazole type plant alkaloid which exhibits cytotoxic activity against tumor cells. Methoxy ellipticine lactate and 2-methyl-9-hydroxyl ellipticinium acetate exhibit a significant biological activity [1]. The biological action of these pyridocarbazoles results in direct binding to DNA [2–7]. They induce protein associated DNA strand break by trapping Topoisomerase II [3–11].

Recently, ellipticine has attracted a number of scientists to investigate its photophysics in different solvents. In nonpolar solvent like hexane, ellipticine exhibits absorbance and emission maxima at 364 and 385 nm respectively [12]. The lifetime was reported around 15 ns [12]. In moderately polar solvents like dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) the emission takes place at around 425 nm with lifetime constant around 27 ns [12]. The long lifetime was attributed to strong hydrogen bonding between the charge transfer state of ellipticine and DMSO and DMF [12]. In methanol, ellipticine exhibits a dual emission at 435 and 535 nm. Miskolczy et al. attributed the long wavelength fluorescence band (535 nm) to the excited state proton transfer reaction by the solvent [13,14]. The rate of protonation in the excited state was reported to be $9.8 \times 10^7\ s^{-1}$. On the other hand, Banerjee et al. attributed the longer wavelength emission in methanol to the solvent assisted tautomerization from pyrol ring to pyridine ring. This report is in accordance with that reported by Cabo et al. [15]. The study of Banerjee et al. [16] suggests that excited state reaction involves solvent reorganization around ellipticine to form 'cyclic' solvated species which facilitates a rapid proton transfer and the two emission bands arise from the normal and tautomeric form. This is well established by excitation spectra of methanol and time resolved studies.

It has already been reported that ellipticine exists as protonated or deprotonated species in aqueous medium [17–22]. The major shortcomings in usage of neutral ellipticine as a pharmaceutical are its toxicity and low solubility in water, but cationic species, ellipticinium is more soluble in water than neutral ellipticine [20]. We can overcome the problem of low solubility of ellipticine in aqueous media by attaching the drug to polymer, peptide or micelle [23,24]. Ellipticine and its 9-methoxy analog have a net amphiphatic character, which gives ability to interact with the membrane [25]. Thus detailed knowledge of physical and chemical properties is necessary. In this context, reverse micelles (RM) provide an attractive model for bio-systems since they can mimic several important and essential features of biological membranes. The motivation of photophysical study of ellipticine in reverse micelles comes from its role as photosensitizer of DNA clevage [26]. It is already reported that in cytoplasm ellipticine exists in both neutral and protonated forms but in nucleus it exist as only protonated species [27]. Study of photophysical properties inside RM and its switchover from one species to another species will certainly help to understand the photophyscial behavior of ellipticine inside biological membrane and its role as photosensitizer trigger of nuclease activity inside tumor cell. One of the significant features of RMs is the presence of highly structured and nonhomogeneous water molecules, which represents an interesting model of water molecules present in biological systems such as membranes [24–30]. Enzyme-containing RMs may offer novel tools for biotechnology and for drug delivery through solubilization of lipophilic drugs [31]. Ellipticine forms a stable complex with Topoisomerase II [3–11]. In our previous effort, we studied the interaction of ellipticine in liposome environments [32]. From the viewpoint of future biophysical applications, it is necessary to make a vivid study of

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the photophysical processes of ellipticine in Aerosol-OT (AOT) reverse micelles.

2. Experimental

Sodium bis(2-ethylhexyl)sulfosuccinate (AOT) and hexane were purchased from Aldrich and were used without further purification. AOT was dried for 48 hours before use. Spectroscopic grade methanol from Merck and HPLC grade water from Rankem, India were used as received. We express water content (w_0) and methanol content (w_m) inside the reverse micelles using the following equation:

$$w_s = \frac{[\text{solvent}]}{[\text{AOT}]}$$

In a recent publication, Levinger et al. defined AOT/hexane/methanol system as a microemulsion, as this is a continuous system [33]. However, in this manuscript we would prefer the inhomogeneity of methanol in AOT/hexane/methanol system as reported by other groups [34–36].

Steady state absorption spectra were taken in a Varian UV–Vis spectrometer (Model: Cary 100). Emission spectra were taken in a Fluoromax-4p fluorimeter from Horiba Jobin Yvon (Model: FM-100). The samples were excited at 375 nm. All the measurements were done at 25 °C.

For the time resolved studies, we used a picosecond time correlated single photon counting (TCSPC) system from IBH (Model: Fluorocube-01-NL). The experimental setup for TCSPC has been described elsewhere [32]. The samples were excited at 375 nm using a picosecond diode laser (Model: Pico Brite-375L). The signals were collected at magic angle (54.70°) polarization using a photomultiplier tube (TBX-07C) as detector. The instrument response function of our setup is ~140 ps.

The amplitude weighted average lifetime was calculated using following equation

$$\langle \tau \rangle = \sum_{i=1}^{n} a_i \tau_i \quad (1)$$

where τ_i are the fluorescence lifetimes of various fluorescent components and a_i are the normalized pre-exponential factors. We used the same setup for anisotropy measurements. The time resolved anisotropy was described with the following equation:

$$r(t) = r_0 \sum_{i=1}^{n} a_{ri} \exp\left(-\frac{t}{\tau_{ri}}\right) \quad (2)$$

where $r(t)$ is the rotational relaxation correlation function. r_0 is the limiting anisotropy and τ_{ri} is the individual rotational relaxation time and a_{ri} is the normalized amplitude of rotational relaxation time.

3. Results

3.1. Steady state absorption and emission spectra

We took absorption and emission spectra in aqueous medium at different pH. Since the solubility of ellipticine is very less in aqueous medium (<10^{-6} M), so; absorption spectra particularly at longer wavelength is difficult of perception. Therefore, we would focus on excitation spectra of ellipticine in aqueous solution rather than absorption spectra. The emission spectra of ellipticine reveal that in acidic condition (pH ~ 2) the emission maximum takes place at 535 nm. On the other hand at pH 12, an additional band appears around 450 nm along with 535 nm band (Figure 1a). The excitation spectra at pH ~ 2 and pH ~ 12 were monitored at 535 and 450 nm, respectively (Figure 1b).

In hexane, the absorption band of ellipticine takes place at 364 nm. Addition of 0.1 M AOT to *n*-hexane produces two major absorption bands at 352 and 425 nm. The absorbance increases further with increase in w_0 value at 352 and 425 nm bands (Figure 2a). Surprisingly, we observe a reverse trend i.e. absorbance decreases with increase in w_m values at 425 nm wavelength in AOT/hexane/methanol system. Interestingly, two isobestic points appear at 297 and 409 nm which imply that more than one kind of species exist in the ground state in AOT/hexane/methanol system (Figure 2b).

The emission maximum of ellipticine in n-hexane appears at 385 nm. Addition of 0.1 M AOT to this solution shifts the emission band to 500 nm. The quantum yield increases from 0.15 to 0.28. With increase in the w_0 values, the emission spectra are found to be red shifted followed by a decrease in the quantum yield (Figure 3a). Addition of methanol to AOT/hexane system diminishes the intensity at 500 nm band while another emission band grows up at 442 nm. Surprisingly, at highest methanol content the emission band at 500 nm almost disappears and the emission band at the shorter wavelength (442 nm) becomes the primary band. We also observe an isoemissive point at around 466 nm (Figure 3b).

3.2. Time resolved studies

We measured fluorescence lifetime of ellipticine in aqueous medium at different pH. At pH ~ 10, the lifetime of ellipticine is bi-exponential and the time components are 0.182 ns (95%) and 5.6 ns (5%). At pH ~ 12 the lifetime is almost single exponential with a component of around 180 ps. At pH ~ 7, the lifetime is tri-exponential and consists the components of 0.182 ns (16%), 2.0 ns (80%) and 5.55 ns (4%). The lifetime did not change on going from pH ~ 7 to pH ~ 2.

In hexane the lifetime of ellipticine was reported earlier [12]. We took time resolved decays at 505 nm varying w_0 and w_m values. At w_0 = 0, the average lifetime is around 22 ns. The components are 1.10 ns (14%) and 25.35 ns (86%). The results are summarized in Table 1. On increasing w_0 values, longer component decreases and at highest water content (w_0 = 32) it becomes 9 ns (65%) while the shorter component decreases from 1 ns to 0.459 ns (35%). In AOT/hexane/methanol system, at highest methanol content (at w_m = 16) the longer component decreases to 11.23 ns (80%) and the shorter component is reduced to 0.606 ns (20%). The lifetime components are shown in Figure 4a and b, respectively.

We took time resolved anisotropy decays in hexane at 410 nm and in AOT/hexane system at 505 nm varying w_0 (Figure 5a) and w_m values (Figure 5b). In pure hexane, ellipticine exhibits a single exponential decay with time constant 145 ps. In AOT/hexane, the anisotropy decay becomes bi-exponential with the shorter (τ_{r1}) and longer (τ_{r2}) time constants of 1.00 and 2.93 ns, respectively (Table 2). The average rotational relaxation time decreases beyond w_0 = 4. Interestingly, decrease in rotational relaxation is massive in AOT/hexane/methanol system. Table 2 reveals that addition of methanol enormously enhances the amplitude of fast component from 27% to 81% at w_0 = 2. At w_m = 16, fast component dominates and anisotropy decay becomes almost single exponential (Figure 5b).

4. Discussions

Ellipticine has pK_a value around 7.40 in aqueous medium [37]. So; in aqueous medium ellipticine may exist as a protonated and deprotonated species depending on the pH of the medium [20–22] (Scheme 1). The protonation takes place at the nitrogen of