Excited state proton transfer dynamics of an eminent anticancer drug, ellipticine, in octyl glucoside micelle†

Krishna Gavvala, Raj Kumar Koninti, Abhigyan Sengupta and Partha Hazra*

Photophysics and proton transfer dynamics of an eminent anticancer drug, ellipticine (EPT), have been investigated inside a biocompatible octyl-β-D-glucoside (OBG) micellar medium using steady state and time-resolved fluorescence spectroscopic techniques. EPT exists as protonated form in aqueous solution of pH 7. When EPT molecules are encapsulated in OBG micelles, protonated form is converted to neutral form in the ground state due to the hydrophobic effect of the micellar environment. Interestingly, steady state fluorescence results indicate the existence of both neutral and protonated forms of EPT in the excited state, even though neutral molecules are selectively excited, and it is attributed to the conversion of neutral to protonated form of EPT by the excited state proton transfer (ESPT) process. A clear isosbestic point in the time-resolved area normalized emission spectra (TRANES) further supports the excited state conversion of neutral to protonated form of EPT. Notably, this kind of proton transfer dynamics is not observed in other conventional micelles, such as, SDS, Triton-X and CTAB. Therefore, the observed ESPT dynamics is believed to be an outcome of combined effects of the local dielectric constant felt by EPT and the local proton concentration at the OBG micellar surface.

Introduction

Alkyl glucosides are the class of non-ionic surfactants, widely used in food, cosmetic and pharmaceutical products. Alkyl glucosides contain a hydrophilic part composed of a glucose moiety, and a hydrophobic part consisting of a hydrocarbon chain. These sugar surfactants are non-toxic and can be synthesized from renewable resources.1,2 Another important aspect of these surfactants is their biodegradable nature. The main reason for the biodegradability is the presence of glucoside linkage, which widely occurs in nature, and its formation and breakage are controlled by different glucosidases.3 These properties make them perfect substitutes of other surfactants, which are potentially dangerous to the environment. These biocompatible OBG surfactants are frequently used to study the dissolution and formation of biological membranes as well as the stabilization of proteins.4–8 Considering the biocompatible behavior of the alkyl glucoside, the micellar aggregate of these sugar based surfactants may be used as a potential drug delivery carrier. It is important to understand the effect of the micellar confinement on solubility and stability of drug in order to use it in the pharmaceutical field. Moreover, the dynamics study of drug molecules inside the glucoside micelle is very essential in order to gain insight into the effect of a confined environment on the stability of the drug molecules. In the present study, we have studied the modulation of proton transfer dynamics of a well known anticancer drug, ellipticine, when it is encapsulated inside one of the well studied glucoside micelles, namely, octyl-β-D-glucoside (OBG) micelle. We have chosen octyl-β-D-glucoside (OBG) because many properties such as the phase diagram, structural behavior of the OBG have been extensively studied by various techniques.9–21

Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole), a pyrido-carbazole alkaloid, a potential anticancer drug, was first isolated from the leaves of Ochrosia elliptica by Goodwin et al. in 1959.22 Ellipticine (EPT) intercalates in DNA and inhibits the activity of DNA topoisomerase-II, which results in inhibition of DNA replication and the transcription process of RNA.23–25 Due to the presence of basic pyridine nitrogen,26 EPT exists in two prototropic forms depending on the pH as well as environmental polarity (Scheme 1). It was found that EPT exists as neutral form in non-polar and hydrophobic media, and exhibits an emission peak in the range of 410–440 nm.27 Ellipticine exists as protonated form in hydrophilic medium and emits at ~530 nm; whereas in methanol and ethylene glycol the drug exhibits concomitant dual emission at ~430 nm and ~510 nm.27–29

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We have also constructed a time-resolved emission spectrum of both the neutral and protonated forms of EPT. Molecules are encapsulated in OBG micelles, which resulted in proton transfer being dramatically slowed down when the drug is incorporated in hydrophobic media. This work is to understand the effect of confinement on proton transfer state and time-resolved spectroscopic techniques. The focus of this work is to understand how ellipticine is transported to its target, it is very important to establish a relationship between the environment and the photophysical properties of EPT. For that purpose, the photophysical properties of the drug have been investigated in several confined and self-assembled systems such as cyclodextrins, cucurbiturils, micelles, reverse micelles, vesicles, bile salts and polymers. Although several attempts have been made on photophysical properties of the drug in many biological and biomimicking media, to date, excited state proton transfer dynamics of EPT has seldom been monitored in the aforementioned confined environments. Herein, we report the encapsulation of EPT drug in OBG micelles and the consequences of confinement on photophysical properties of EPT using steady state and time-resolved spectroscopic techniques. The focus of this work is to understand the effect of confinement on proton transfer dynamics of ellipticine. Interestingly, we have observed that proton transfer is dramatically slowed down when the drug molecules are encapsulated in OBG micelles, which resulted in proton transfer from both the neutral and protonated forms of EPT. We have also constructed a time resolved emission spectrum (TRES) and a time resolved area normalized emission spectrum (TRANES), which provide information about the existence of multiple emissive species in the excited state. Finally, these results are compared with other conventional micelles such as CTAB, SDS and Triton-X, and we have observed that any such excited state proton transfer process is absent in the above mentioned three micelles.

Experimental

Ellipticine (EPT), octyl-β-D-glucopyranoside (OBG), glucose, sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and Triton X-100 were purchased from Sigma Aldrich, and used without further purification. All the samples were prepared in phosphate buffer (10 mM), unless otherwise mentioned. Concentration of EPT in water was adjusted to ~10⁻⁵ M using a reported value of the molar extinction coefficient (ε₅₃₀ = 39 000 M⁻¹ cm⁻¹). Absorbance measurements were performed on a Perkin-Elmer UV-visible spectrophotometer (Lambda-45), and steady-state fluorescence spectra were recorded on a FluoroMax-4 spectrofluorometer (Horiba Scientific, USA). All time-resolved fluorescence measurements (both lifetime as well as anisotropy) were performed on a time correlated single photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH, UK). The detail description of the instrument is described elsewhere. Here, we have used a 375 nm diode laser for exciting drug molecules. The analysis of emission decays was done by IBH DAS6 analysis software. We have fitted both lifetime data with a minimum number of exponential. Quality of each fitting was judged by χ² values and the visual inspection of the residuals. The value of χ² ≈ 1 was considered as the best fit for the decays.

Results and discussion

Steady state measurements

Absorption spectra of EPT in buffer and in the presence of OBG are shown in Fig. 1a. The drug in buffer exhibits several peaks in the 250–450 nm region with a peak maximum at 300 nm and two shoulders at 350 nm and 420 nm. Earlier reports divulge that these are the characteristic peaks of protonated form of EPT. Upon gradual addition of OBG, the absorption profile of EPT does not change significantly until certain concentration (≤25 mM). At higher concentration of OBG (≥26 mM), noteworthy changes are observed in absorption spectra (Fig. 1a). The peaks representing protonated form (300 nm and 425 nm) are almost vanished and two new peaks appeared at ~280 nm and ~380 nm. These new peaks were previously observed in many instances, when EPT was incorporated in hydrophobic media, and therefore, the above observations infer that EPT experiences a hydrophobic environment above certain concentration of OBG (26 mM), and therefore ground state neutral form generates at the cost of protonated form of EPT. Interestingly, the concentration of OBG (≥26 mM), after which the changes in absorption spectra of EPT are observed, is in good agreement with the previously reported critical micellar concentration (CMC) of OBG.

Emission profiles of EPT in phosphate buffer and in the presence of OBG are shown in Fig. 1b. EPT exhibits single emission maximum at 530 nm, which is believed to be originated from protonated form of the drug. With the gradual addition of OBG to the buffer containing EPT solution, the fluorescence intensity at 530 nm slightly increases along with a small hump in the 420–450 nm region. However, at concentration ≥26 mM, the 530 nm peak shoots up with a prominent new peak at 440 nm (Fig. 1b). The 440 nm peak also grows up and becomes comparable to the 530 nm peak at higher concentration of OBG. This kind of fluorescence switch of EPT from 530 nm in buffer to 440 nm in micelles is unique and can be visualized directly from the color change of solution from green to cyan (Fig. S1, ESI†). It is already well established that EPT residing in a hydrophobic...
environment emits the blue region (420–440 nm), and it is believed to be appeared from the neutral form of the drug. Thus, we believe that the newly appeared peak at 440 nm is coming from the neutral drug molecules. Hence, the appearance of blue emission at 440 nm demonstrates that conversion of the drug takes place from protonated to neutral form in the presence of an OBG micellar environment. However, if this switching of the form would be the case for the increment of neutral form peak intensity, then the intensity at 440 nm, which is a signature peak of neutral form of EPT, should have been increased at the cost of protonated form peak intensity at 530 nm. Astonishingly, the intensity at 530 nm concomitantly increases along with a 440 nm peak with the gradual hike in OBG concentration. Here it is necessary to mention that absorption results confirmed that transformation takes place from protonated to neutral form in OBG micelle (Fig. 1a). Moreover, the excitation spectra monitored at 440 nm and 530 nm are very similar (Fig. S2 and Note S1, ESI†). Based on above observations, we envisage that although in the ground state the population of neutral form of the drug increases in the micellar environment (probably due to decrease surrounding polarity of the drug), in the excited state some of the neutral form of drug molecules are converted to the protonated form by the excited state proton transfer process. As a result, the intensity of both the neutral as well as protonated form increases with OBG concentration.

The above results indicate that drug molecules are not buried completely inside the hydrophobic domain of the micelle, as protonated species in the excited state is not at all stable in the hydrophobic domain of the micelle. We guess that the drug molecules reside near the palisade layer of the micelle, where hydrophilic sugar head groups of the surfactant are present. In order to confirm the effect of glucose moieties present in the micelle, the drug fluorescence was monitored with increasing concentration of glucose. Neither intensity enhancement nor new peak appearance was observed in the emission spectrum of EPT up to 500 mM of glucose (Fig. S3, ESI†) and it suggests that the presence of sugar moieties is not solely responsible for the observed photophysics of the EPT. To verify the effect of a confined environment of the micelle, we have also collected the emission spectra of EPT in three different types of micelles, namely cationic, anionic and neutral micelles and the observed results are in good agreement with the literature reports. In the case of anionic SDS micelles, EPT exhibits a protonated peak at 530 nm, whereas it shows a peak at ~440 nm in CTAB micelle (Fig. S4, ESI†). Notably, although the TX-100 surfactant head group contains an –OH group, the micellar environment of TX-100 does not exhibit any protonated peak of ellipticine at 530 nm (Fig. S4, ESI†). Therefore, the appearance of both the peaks in the case of OBG micelles is believed to be an outcome of confined as well as polarity of the surrounding environments. Although we have proposed that excited state proton transfer is the origin of green emission in an OBG micellar environment, in order to gain deeper insight into the excited state proton transfer process we have performed time resolved measurements, which is discussed in the next section.

**Time-resolved measurements**

Time-resolved fluorescence measurement is an excellent technique to monitor the excited state dynamics of molecules and is a unique method to identify multiple emissive species present in a given system. Emission decay profiles of EPT in the absence and in the presence of OBG are collected at 440 nm and 530 nm in order to monitor the excited state dynamics of both neutral and protonated species, respectively (Fig. 2). As shown in Table 1, protonated EPT (at 530 nm) exhibits a bi-exponential decay profile in buffer having individual lifetime components of ~2 ns (87%) and ~6 ns (13%) with an average lifetime value of ~2.5 ns. In the present scenario, the assignment of the individual component is complicated, as our lifetime profile consists of only decaying components. In the case of methanol, where people observed dual emission, the decay profile at longer wavelength consists of a growth followed by a decaying component. Moreover, the growth component lifetime was shorter compared to that of the second component.

**Absorption (a) and emission (λex = 375 nm) (b) spectra of EPT in pH 7 buffer with increasing concentration of OBG (0 mM to 100 mM).**

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*Fig. 1*
profile in buffer is devoid of any growth component, it is difficult to assign individual components.

With the successive addition of OBG, the average fluorescence lifetime of EPT collected at 530 nm steadily increases until 26 mM of OBG. After 26 mM of OBG, the average lifetime shoots up to \( \sim 20 \) ns (Fig. 2 and Table 1). The increased average lifetime infers that the stability of protonated EPT is enhanced in the presence of an OBG micellar environment, as is evident that OBG forms micellar assembly after 26 mM. The most intriguing observation is the appearance of a \( \sim 4 \) ns growth component after 26 mM of OBG (\( \tau_3 \) in Table 1a). Moreover, the contribution of a growth component increases with OBG concentration. Notably, this kind of a long growth component of EPT was not observed in other confined environments, like reverse micelle, lipid bilayers, cyclodextrin/cucurbituril nano-cavity etc.,\(^{30-35}\) and in that sense the growth feature observed in the lifetime profile is unique. There are two possibilities for the appearance of a growth component in the fluorescence transient; either due to the slow solvation dynamics or due to the excited state reaction, such as a proton transfer process. In order to verify the first possibility, we have collected emission transients at longer wavelengths (red edge side) and the results depict no significant change in the growth component. This finding confirms that the growth component appeared in the decay profile is not because of solvation, but may be due to the excited state proton transfer process. To unveil the proton transfer mechanism, we have further collected fluorescence transients at 440 nm, which are believed to be originated from neutral form of the drug. In buffer medium, EPT exhibits tri-exponential decay at 440 nm with individual lifetime components of \( \sim 40 \) ps, \( \sim 290 \) ps and \( \sim 2.3 \) ns exhibiting an average lifetime of \( \sim 160 \) ps. It is evident from Fig. 1b that the intensity of EPT at 440 nm is negligibly small in buffer medium. Moreover, protonated EPT also contributes significantly towards the intensity at 440 nm, and therefore, we believe that the decay feature observed at 440 nm cannot be attributed solely to the neutral EPT molecules. However, we have collected the decay profile even at 440 nm in order to compare the dynamics of neutral EPT molecules generated in the presence of an OBG micellar environment. It is clear from the lifetime results that the average lifetime collected at 440 nm, which is believed to be originated from neutral drug, steadily increases with addition of OBG and jumps up to \( \sim 4 \) ns at \( \geq 26 \) mM of OBG (CMC of OBG micelle). It is interesting to notice that the growth component (\( \sim 4 \) ns) observed at 530 nm is in good agreement with one of the lifetime components collected at 440 nm (Table 1b). This confirms that protonated form generates at the cost of neutral form, and the conversion of neutral to protonated form takes place through the excited state proton transfer process. To confirm the presence of two different types of species in the

![Fig. 2](image-url) Time resolved fluorescence decays of EPT in pH 7 buffer with increasing concentration of OBG (0 mM to 100 mM) collected at 440 nm (a) and 530 nm (b) (\( \lambda_{\text{ex}} = 375 \) nm).

### Table 1: Fluorescence decay parameters of ellipticine in the presence of OBG (0 to 100 mM) collected at (a) 530 nm (\( \lambda_{\text{ex}} = 375 \) nm) and (b) 440 nm (\( \lambda_{\text{ex}} = 375 \) nm)

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<th>( \tau_2 ) (ns)</th>
<th>( \tau_3 ) (ns)</th>
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\( \tau_{\text{avg}} = a_1 \tau_1 + a_2 \tau_2 \).
excited state, we have further constructed a time resolved area normalized emission spectrum (TRANES), a recently developed technique to explore excited state components. The TRANES method is a one-step extension of the commonly used time resolved emission spectrum (TRES) analysis. TRES and TRANES of EPT between time 0.1 and 30 ns in the presence of OBG micelles are shown in Fig. 3. A clear isoemissive point at 480 nm is noticeable in TRANES, indicating the existence of equilibrium between neutral and protonated forms of the drug in the excited state. It is clear from TRANES that initial population is dominated by neutral species, however, at a sufficiently longer time-scale the protonated species becomes dominated compared to the neutral one. This further supports our conjecture that protonated species generate at the cost of neutral one in the excited state. It is already discussed that the lifetime of protonated species consists of a growth component (∼4 ns), which is believed to be the formation time-scale of protonated form. In TRANES, we have also noticed that the reasonable intensity of protonated form appears after 4 ns. Therefore, these results confirm that the proton transfer process takes place in OBG micelle on a ∼4 ns time scale. Earlier reports also elucidate that proton transfer dynamics can be slower in constrained environments. No isoemissive point is observed below CMC of OBG micelle and in the presence of glucose alone. Moreover, this kind of ESPT process is totally absent in other micelles, like SDS, CTAB and TX-100 micelles (Fig. S5 and Note S2, ESI†). Therefore, based on the above observations, we anticipate that OBG micelle provides some appropriate environment for the excited state proton transfer process, and we believe that the glucose moiety of OBG has some definite role in this overall ESPT process.

Furthermore time-resolved anisotropy measurements are performed to elucidate valuable information regarding the surrounding environment of the drug. The rotational relaxation of EPT in buffer takes place on a 120 ps timescale. However, the anisotropy decay profile in the presence of micellar medium exhibits noteworthy changes in the rotational diffusion time (Fig. 4). The anisotropy decay of EPT in OBG micelles exhibits a bi-exponential feature with correlation time constants of 120 ps and 2.6 ns with an average value of ∼970 ps. The 120 ps component is believed to be originated from free EPT molecules, whereas a 2.6 ns component can be assigned to the micelle encapsulated drug molecules. The increase in the relaxation time from 120 ps to 2.6 ns confirms the encapsulation of drug in OBG micelles, where EPT experiences a more restricted environment compared to buffer medium. This further suggests that rigidity also have some role in the ESPT process of the drug in the excited state. The τr value is used to determine the hydrodynamic volumes from the Stokes–Einstein relationship;

where \( D_r \) and \( \eta \) are the rotational diffusion coefficient and viscosity of the medium, respectively; \( V \) is the hydrodynamic molecular volume of the complex; and \( T \) is the absolute temperature.

![Fig. 3](a) Time resolved emission spectra (TRES) and (b) time resolved area normalized emission spectra (TRANES) of EPT in the presence of 100 mM of OBG.

![Fig. 4](Time resolved anisotropy decays of EPT in pH 7 buffer collected at 530 nm and in the presence of 100 mM of OBG collected at 440 nm in the emission spectrum (%ex = 375 nm).)
By using the above equation and assuming that the viscosity of the medium is the same as that of water, the evaluated effective hydrodynamic diameter of the micelle is 28.09 Å. This value is in good agreement with the reported hydrodynamic diameter.\(^{51}\)

Next we focus on the mechanism of excited state proton transfer in an OBG micellar environment based on all the experimental evidence. Absorption data indicate the formation of ground state neutral EPT molecules after CMC of OBG, whereas steady state emission results infer the presence of both neutral and protonated species in an OBG micellar environment. Excitation spectra monitored at 440 nm and 530 nm confirm that protonated species are formed exclusively in the excited state due to the excited state proton transfer (Fig. S2 and Note S1, ESI\(^+\)) process. Moreover, the lifetime results suggest the existence of both neutral and protonated species in the excited state, and the protonated species formed at the cost of neutral one on a ~4 ns time-scale. Time-resolved anisotropy results depict that the drug experiences a restricted environment in OBG micelle. Interestingly, this kind of excited state proton transfer dynamics is not observed in other conventional micellar environments like SDS, CTAB and TX-100. In the case of anionic SDS micelles, EPT exhibits a protonated peak at 530 nm, whereas in CTAB and TX-100 micelles it shows emission from neutral form at 440 nm. These results can be explained by the interplay between two effects. The first one is the local dielectric constant felt by the EPT, and the second is the local proton concentration at the micellar surface. It is known that the local dielectric constant for all these three micellar systems is around 35–40.\(^{52}\) Certainly, this environment does not favor charged species such as protonated form of EPT, and therefore, neutral form of EPT should be the stable species in all these micellar systems. The fluorescence spectra of EPT in TX-100 and CTAB micelles (Fig. S4, ESI\(^+\)) support this conjecture. Contrarily, in the SDS system the emission is observed from protonated form instead of neutral form of the drug. This observation can be explained by the negative micellar surface attracting protons and making the local interfacial proton concentration much higher than the bulk solution of pH 7.\(^{53,54}\)

The presence of a higher proton concentration around SDS micelles appears to favor the protonation of the excited EPT under the local environment conditions and to compensate for the low polarity. Interestingly, unlike the other micellar systems, in the excited state both neutral and protonated forms are observed in OBG micelles. Based on these observations we envisage that the presence of a glucose moiety and the polarity of a confined environment in OBG micelle play a major role in the conversion of neutral to protonated one in the excited state. It is already reported that the glucose molecules at the first hydration shell of the OBG micellar surface are projected inwards (away from bulk water) in the direction of the micellar hydrocarbon core, where the contact with water molecules is minimum.\(^{55}\)

Therefore, we anticipate that the drug molecules trapped in between the surfactants reside close to hydroxyl groups of glucose molecules, and therefore, excited state intermolecular proton transfer reaction takes place from glucose to drug molecules to yield excited state cationic form of the drug (Scheme 2). The local dielectric constant experienced by the drug molecule also contributes to the anomalous results obtained in OBG micelle. Though OBG micelles are neutral, the dielectric constant (~50)\(^{56}\) at the palisade layer of OBG micelle is higher than that of above mentioned three micelles due to the presence of plenty of –OH groups at the interface of OBG micelle. Therefore, the higher local dielectric constant sensed by the EPT molecule at the OBG micellar surface is also responsible for the stabilization charged species, such as protonated form of EPT in OBG micelle.

Our results suggest that EPT becomes less toxic in the presence of an OBG micellar environment, as absorption and emission results indicate that the solubility of the drug increases in the presence of an OBG micellar environment. Moreover, the drug loading to an OBG micellar environment can be easily monitored with the help of fluorescence switching from green to cyan color. Thus, considering biocompatible nature of OBG and above mentioned points, we believe that OBG micelle may act as a suitable drug delivery carrier for EPT. Most importantly, for the first time we have shown that the ESPT dynamics of ellipticine depends on both the polarity as well as surrounding proton concentration. The other important implication of our work is that one can predict a cellular environment with respect to polarity as well as proton concentration from the ESPT process of the drug.

![Scheme 2](image)

Scheme 2  Excited state proton transfer process of EPT in octyl-β-D-glucoside micelle.
Conclusion

In the present work, we have studied the photophysical and proton transfer dynamics of an eminent anticancer drug, ellipticine, inside a biocompatible octyl-β-D-glucoside (OBG) micellar medium using steady state as well as time resolved spectroscopic techniques. UV-visible absorption study reveals the conversion of protonated to neutral form of EPT, when OBG concentration reaches above critical micellar concentration. Interestingly, the emission at 530 nm (attributed to the protonated form of the drug) is also observed along with neutral form (emits at 440 nm), even when we selectively excite the neutral molecules. The above observation clearly demonstrates that the excited state proton transfer process inside the OBG micelle is responsible for the conversion of neutral to protonated form of the drug. Time resolved emission measurements depict a pronounced enhancement in the average lifetime of both neutral and protonated species collected at 440 nm and 530 nm, respectively, when EPT is encapsulated inside OBG micelles. Astonishingly, a rise component of ~4 ns is observed in the time-resolved emission decays collected at 530 nm above CMC of OBG, and it is attributed to the excited state proton transfer dynamics of EPT inside OBG micellar confinement. Time-resolved area normalized emission spectra confirm the existence of two species in the excited state, where neutral form transforms into the protonated form. This kind of proton transfer dynamics is absent in conventional micelles, like SDS, Triton X and CTAB, thereby indicating that the glucose molecules residing in the palisade layer of OBG micelle may have some role in the excited state proton transfer process. Based on all the above observations, we conclude that the observed ESPT dynamics is a combined effect of the local dielectric constant as well as the presence of glucose moieties at the palisade layer of the OBG micelle.

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