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Prototropism and dynamics of an anticancer drug in reverse micelles: Focus on the variation of pH in reverse micelles having $w_0 \geq 10$

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ABSTRACT

The modulations of the photophysics and dynamics of an archetypal anticancer β-carboline drug, namely, harmane (HM) within AOT reverse micelles (RMs) have been explored. To this end, particular focus is given on a pH-dependent study commensurate with the effect of increasing water content within the RM interior. Our results reveal markedly different photophysical behavior of the drug encapsulated within the RM compared to that in bulk aqueous medium. For example, our results show the presence (or absence) of the cationic (or neutral) emission of HM in RMs in pH values apparently much higher (or lower) than that of pure aqueous medium. The characteristic zwitterionic emission in strongly alkaline bulk aqueous solutions (e.g., pH 12.2 and 13.5) is also found to be lacking within the reverse micellar core, even in RMs with $w_0 \geq 10$. These data are further substantiated from the study of the dynamical aspects of the interaction scenario, that is, modulation of the fluorescence decay and rotational relaxation behaviors of HM entrapped within the RMs. Cumulatively, our results indicate the presence of a proton gradient across the reverse micellar water pool in which the interfacial regime appears to be more acidic in comparison to the central core. The results with alkaline solutions (e.g., pH 10.0, 12.2 and 13.5) suggest selective compartmentalization of the hydroxide ions leaving the effective pH of the water pool lower than that of the bulk aqueous solution.

1. Introduction

Because of their complex structural, dynamical and many fundamental properties largely distinct in comparison to those of bulk water, the water confined in microheterogeneous environments and/or residing at interfaces has formed the nucleus of many-faceted research endeavors in physicochemical, chemical and enzymatic/biological fields for years [1,2]. Reverse micelles are thermodynamically stable, nanoscopic, self-organized molecular assemblies that form upon dissolution of appropriate surfactant(s) in a suitable nonpolar solvent and typically used for probing the properties of confined water [1, 3–13]. The unique property of RMs of incorporating a small volume of polar solvent, typically water [3–14], within its nanoscopic interior has opened diverse windows of potential applications [15–21]. Apart from the study of water behavior within nanoconfinements [22,23], reverse micellar systems are also pushing the frontiers in investigation of complicated affairs such as the modulations of structure and dynamics of proteins and nucleic acids within nanoconfinement [24,25], development of safe-engineered drug delivery to specific sites [26], micellar catalysis [27], synthesis of well-designed molecular frameworks [28,29] and so forth. So far, the classic double-chained, anionic surfactant bis(2-ethylhexyl) sulfosuccinate (Aerosol-OT, abbreviated as 'AOT') has been the most commonly used surfactant for the preparation of RMs because of its well-known utility. The size of the intra-(reverse) micellar water pool can be controlled simply as a function of the molar concentration of added water through the following relationship [3–14]:

$$w_0 = [H_2O]/[AOT] \quad (1)$$

It is generally argued that in small RMs ($w_0 < 10$), the properties of the nanocompartmentalized water are remarkably different from those of bulk water [30,31]. However, the properties of the confined water start approaching those of bulk water with increasing hydration (that is, w_0) [32,33].

To this end, a host of molecular probes and various experimental techniques [34–40] have been exploited by researchers over the years in order to delineate the fundamental properties of nanoconfined water within RMs such as the dielectric constant, pH, ionic concentration, microviscosity, micropolarity etc. [7–13,34]. Many of these

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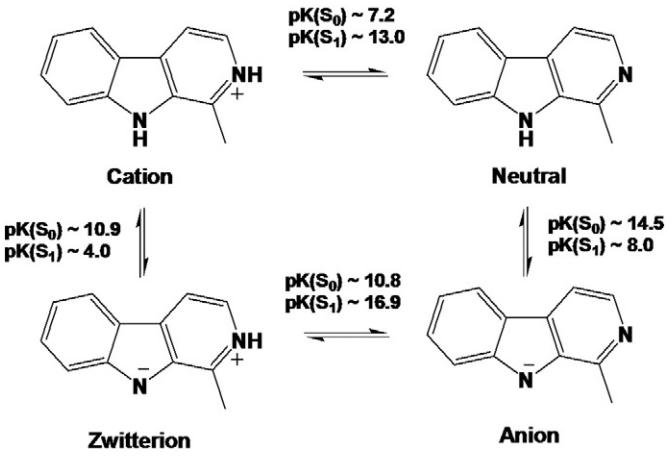
experimental results have also revealed the unique ability of AOT headgroups to buffer the entrapped water within the RMs [4,38–40], which in turn provides an additional edge favoring the use of AOT in studies related to fundamental properties of RMs.

The present work reports the study of photophysics and dynamics of a potential anti-cancer photosensitizer, namely, harmane (HM) within AOT RMs. Harmane represents one of the benchmark compounds of β-carboline drugs having wide-spectrum pharmacological and medicinal applications including their functionality as monoamine-oxidase enzyme inhibitors, interaction with neurotransmitters and neuromodulators of the Central Nervous System [41], photosensitizing activity toward various fungi, bacteria, viruses etc. [42,43]. The substantial modulation of electronic charge density distribution over the pyrrole and pyridine nuclei of HM following photoexcitation leads to complicated pH-dependent prototropic equilibria (Scheme 1) which operate differentially in the ground and excited-states [43–47].

The present work is focused on photophysical characterization of HM in AOT RMs with particular emphasis on large RMs, that is, $w_0 \geq 10$ over a wide range of pH. The contrasting photophysical and dynamical behaviors of HM within the RM-encapsulated state in comparison to those in bulk homogeneous media unveil that the nature of the nanoconfined water sampled by the entrapped HM moleculesin large RMs (that is, $w_0 \geq 10$) remains substantially different from that of bulk water. Our results while apparently contrary to many existing literature reports [32,33,48–50], underscore the pivotal roles of pH gradient across the water pool confined in RMs, relative differences in migration of various ions, and selective compartmentalization of hydroxide ions in reverse micellar core (for pH > 7).

2. Experimental

Harmane (HM), the surfactant AOT and hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used as procured from Sigma-Aldrich Chemical Co., USA. The solvents assayed, namely, 1,4-dioxane, benzene and *n*-heptane were used as received from Spectrochem, India (Spectroscopy grade) after keeping over molecular sieves (E-Merck Ltd., 5 Å)for >48 h. The surfactant (AOT) was extensively dried in vacuum desiccator prior to use. The 0.1 M solution of AOT was prepared by dissolving appropriately weighed quantity of AOT in *n*-heptane (spectroscopy grade) obtained from Spectrochem, India. The water of required pH was prepared by adding calculated amounts of aqueous solution of HCl or NaOH in triply distilled deionized Milli pore water followed by verification of the pH on Elico, LI120 pH-meter. For adjusting the w_0 in AOT reverse micellar interior, calculated amounts of water (of required pH) was added to the solution of AOT prepared in *n*-heptane, and the solution was then inverted very slowly followed by vortexing for ~10 s. The reverse micellar solutions containing the molecular probe HM were finally equilibrated for ~10 min without agitation before measurements. The absorption and fluorescence spectral data were acquired on Hitachi UV–Vis U-3501 spectrophotometer and Jasco FP-8500 fluorometer, respectively with appropriate background correction. The picosecond-resolved fluorescence decays were obtained on FluoroCube-01-NL spectrometer by the technique of Time-Correlated Single Photon Counting (TCSPC) [51]. The light sources used for photoexcitation of the samples were a Laser-diode at $\lambda_{ex} = 375$ nm (Model: DD375L-9395 with IRF ~15 ps) and a nanoLED at $\lambda_{ex} = 336$ nm (Model: DD340-9466 with IRF ~100 ps). Dynamic light scattering (DLS) measurements were carried out on a Malvern Nano-ZS instrument equipped with a thermostated sample chamber (Laser source: 4 mW He-Ne laser having $\lambda = 632.8$ nm, scattering angle = 173°). The instrumentations and experimental methods/procedures are further described in detail in the Supporting information.



Scheme 1. pH-Dependent prototropic equilibria of HM (pK values in aqueous medium).

3. Results and discussion

3.1. Photophysical behavior of HM in bulk medium: pH-dependent prototropism

3.1.1. Absorption and fluorescence spectroscopic studies

The absorption and fluorescence spectra of HM in bulk aqueous medium at various pH values are displayed in Fig. 1. In aqueous solution of pH 7.2 the absorption profile of HM is comprised of two distinct bands at λ_{abs} ~340 nm and ~370 nm representative of the neutral and cationic species of HM, respectively, Fig. 1a [43–47,52]. In tune with this result, in alkaline aqueous solution (pH 10.0, 12.2, 13.5) the characteristic absorption band of the neutral (λ_{abs} ~340 nm) form of HM is found to predominate (Fig. 1a) [43–47,52]. The outline of the absorption spectra are understandable considering the fact that the Cation ⇋ Neutral ground-state prototropic equilibrium is characterized by $pK_a^{CN}(S_0)$ ~7.2 (Scheme 1) which accounts for the population of two species in the ground-state, namely, the cationic (in acidic pH) and the neutral form (in alkaline pH). The plausibility of formation of the anionic structure from the neutral form of HM in the ground-state is reasonably negated with a view to the exceedingly high $pK_a^{NA}(S_0)$ value of ~14 [43,53,54].

Based on earlier spectroscopic and computational results the lowest-lying excited electronic states of β-carboline compounds have been assigned as ^1L_a and ^1L_b states [55–59]. In direct analogy to the reported literature (absorption band position as well as calculated oscillator strength) the bands in the 330–370 nm wavelength regime and in the shorter wavelength regime (below 300 nm) can be attributed to ^1L_b ($S_1 \leftarrow S_0$) and $_1L_a$ ($S_2 \leftarrow S_0$) electronic transitions, respectively [55–59]. The microheterogeneous environment within reverse micelles does not correspond to an appropriate environment for the study of complex electronic transitions such as $S_2 \leftarrow S_0$ (rather the study of such transitions would require suitably designed experimental technique(s) and condition(s)). Thus, at the moment we chose to abstain from making specific comments on the absorption band at the shorter wavelength region which is also beyond the aim and scope of the present work.

The characteristic pK_a values corresponding to various prototropic equilibria of HM are typically reversed in the excited-state (S_1-state): $pK_a^{CN}(S_1)$ ~13.0 and $pK_a^{NA}(S_1)$ ~8.0 [43,53,54]. Drawing on this, it is reasonably understandable that the cationic species (λ_{em} ~440 nm) will dominate the fluorescence profile of HM in acidic and neutral solutions (pH 5.5, 6.5 and 7.2), Fig. 1b. Whereas in alkaline solution (pH 10.0) the cationic emission is also accompanied with a small band feature in the wavelength regime 360–390 nm (Fig. 1b), characteristic of the emission from the neutral species [43–47,52–54]. However, in strongly alkaline solutions (pH 12.2 and 13.5), the neutral band intensity is found to be enhanced coupled with the appearance of a distinctly red-shifted fluorescence band having λ_{em} ~488 nm (Fig. 1b), which is attributed to the zwitterionic form of HM (Scheme 1), in view of the fact that fluorescence from the anionic structure would require an excessively alkaline solution (pH > 14) beyond the scope of the present study [43,53,54]. A