Modulation of the Excited-State Proton Transfer Rate of \( \text{D}-\)Luciferin in Mixed Reverse Micellar Systems

Arindam Das,* Sk Imadul Islam,‡ Dipak Kumar Das,* and Rajib Kumar Mitra*†

Department of Chemical, Biological and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences, Block-JD, Sector-III, Salt Lake, Kolkata 700106, India

Supporting Information

ABSTRACT: The excited-state proton transfer (ESPT) rate of photoacids in a confined medium depends on several physical parameters of the immediate environment. We introduce a new parameter in the form of charge type at the interface of reverse micellar (RM) systems to modulate the ESPT rate. We investigate the ESPT reaction of \( \text{D}-\)luciferin in mixed RM systems composed of nonionic polyoxyethylene(5)nonylphenylether (Igepal CO-520) with cationic didodecyldimethylammonium bromide (DDAB) and anionic sodium bis(2-ethylhexyl)sulfosuccinate (AOT) in cyclohexane (Cy) at different mole fractions of Ig (\(X_i\)) and fixed hydration. ESPT is feeble in AOT RM, whereas it is favorable in the other two RMs. Addition of Ig is observed to facilitate ESPT in AOT RM linearly, whereas in DDAB, it shows a synergistic effect. The various physical parameters of water in the mixed RM water pool have been investigated using dynamic light scattering, Fourier transform infrared, and time-resolved fluorescence spectroscopy measurements to underline the ESPT mechanism in these mixed RMs.

1. INTRODUCTION

The excited-state proton transfer (ESPT) reaction in confined media has emerged as a fascinating field of research in chemistry and biology.1,2 There have been several studies discussing the pathways of the ESPT reactions of different excited-state photoacids and roles of environment in proton transfer dynamics.3–10 ESPT is mostly governed by the local concentration of water molecules near the photo-acid and solvent reorganization.11 Following photo-acid excitation, a proton is transferred to the surrounding solvent. In case of a reversible process, proton diffusion recombines the two species, and in irreversible cases, recombination of RO*+ with the proton re-forms the ground-state ROH.12 Reverse micelles (RMs) often mimic the biological environment, and ESPT in RM can be modeled for specific activity of chromophores in real systems. ESPT offers valuable insights into the dynamical nature of water inside RM systems.3,5,7,13–15 The location of the probe and the corresponding ESPT dynamics inside RM have been found to be markedly dependent on the nature of the surfactant charge,3,16 pH and dielectric constant of water inside the RM environment,5 polarity of the environment, and screening effects of counter-ions present at the interface.6 An important aspect in this regard is the role of the interface in deciding the fate of the ESPT reaction, and accordingly the ESPT dynamics modulates with the charge type of the RM head groups. For example, in anionic sodium bis(2-ethylhexyl)sulfosuccinate (AOT) RMs, the rate of ESPT increases with an increase in the water content (\(w_0\)), whereas in cationic RMs, the interface-localized probe offers only subtle changes with \(w_0\). Lawler et al. reported that in AOT RM, the ESPT kinetics of 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) resembles that in bulk water, suggesting a diffusion-controlled power-law time-dependent process, whereas the ESPT rate in nonionic Ig-S20 RM shows a slow, two-component model, with one relatively bulk-water-like population and second surface-bound population with a slower lifetime.3 A few studies have also correlated the solvation dynamics around the ESPT probe and its intramolecular proton transfer dynamics.14,15 In this study, we introduce a new parameter in terms of the interfacial polarity of RMs by systematically varying the interfacial charge by mixing of surfactants of different charge types and to investigate its effect on the ESPT of \( \text{D}-\)luciferin.

\( \text{D}-\)Luciferin, found in the Lampyridae family of fireflies, is the substrate that is oxidized into oxyluciferin, responsible for the emission of yellow-green light.17,18 ESPT of \( \text{D}-\)luciferin has previously been studied in different solvents of varying polarities, pHs, temperatures, and hydrogen-bonding abilities.15,19–21 Such studies in a restricted water environment, like in RMs, are only sparse.7 The study by Kuchlyan et al. suggests that ESPT of \( \text{D}-\)luciferin in AOT RM is only feeble at \(w_0 \geq 12\) and gets favorable as the water content increases.7 ESPT of \( \text{D}-\)luciferin proceeds when sufficient water molecules stabilize the polar transition state and the products (protons and anions). The authors also found that ESPT changes as the...
polar protic core of RM is replaced with polar aprotic solvents like dimethylformamide and dimethyl sulfoxide. In light of all of these investigations, it is of interest whether the ESPT could be tuned by any other physical parameter, for example, the charge type at the interface, which could easily be achieved by mixing of surfactants. In the present contribution, we have studied the ESPT process of D-luciferin in two different mixed RMs: anionic–nonionic (AOT/Igepal-520) and cationic–nonionic didodecyldimethylammonium bromide (DDAB/Igepal-520). Mixed surfactant-based RMs could potentially be employed in a wide range of practical applications because of their enriched performance over the individual components and the unique advantage of tuning the interface vis-à-vis water properties by simply changing the composition.\textsuperscript{22–24} Double-tailed anionic sodium bis(2-ethylhexyl)sulfosuccinate (AOT), double-tailed cationic didodecyldimethylammonium bromide (DDAB), and nonionic polyoxyethylene(5)nonylphenylether (Igepal CO-520) (Scheme S1) are the most widely used and well-studied surfactants to form RMs in cyclohexane (Cy).\textsuperscript{25,26} In both AOT and DDAB RMs, Ig is gradually added with the assumption that all of the RMs exist as spherical interface stoichiometry.\textsuperscript{23,24} DDAB RMs are known to exhibit unusual physical properties compared to those of the conventional AOT and Ig RMs. They offer a unique phase behavior; with increasing hydration, their microstructure changes from cylindrical rodlike aggregated structures to discrete droplet-type structures at \( w_0 \geq 8.\textsuperscript{26} To exclude any effect arising out of the morphology of the concerned RM on the ESPT dynamics, we perform all of the studies fixing \( w_0 \) at 10 with the assumption that all of the RMs exist as spherical droplets. In both AOT and DDAB RMs, Ig is gradually added at different mixing ratios of \( X_{Ig} = 0, 0.2, 0.4, 0.6, 0.8, \text{ and } 1.0.\) We measure the micellar droplet sizes by the dynamic light scattering (DLS) technique. To understand the structure and dynamics of the entrapped water in these RM water pools, we have used Fourier transform infrared (FTIR) spectroscopy and time (sub-nanoseconds)-resolved fluorescence spectroscopy using coumarin-500 (C-500) as the fluorophore. We have investigated the ESPT reaction in these RM systems spectroscopically and tried to correlate if the process could indeed be modulated by the modification of the interface.

2. RESULTS AND DISCUSSION

2.1. Solubilization Capacity Measurements. Figure S1a depicts the maximum water solubilization capacity of the mixed DDAB/Ig/Cy RM systems. The corresponding values of AOT/Ig mixed systems\textsuperscript{25} have also been plotted for comparison. DDAB is soluble in Cy only beyond \( w_0 = 2, \) whereas Ig is soluble in Cy, and the Ig/Cy system can offer the maximum solubilization capacity (\( w_{0,max} \)) of 20–25.\textsuperscript{27} The highest solubilization capacity of the DDAB/Cy system has been observed to be \( \sim 13.\textsuperscript{27} DDAB/Ig mixed RM exhibits a noticeable synergism at \( X_{Ig} = 0.8, \) which in AOT/Ig was observed at \( X_{Ig} = 0.4 \) (Figure S1a). The observed synergism manifests an optimization between two opposing factors, namely, interdroplet interaction and surfactant monolayer elasticity.\textsuperscript{27,28} The major driving force of the solubilization limit of water in RM is the spontaneous curvature and the elasticity (or rigidity) of the interfacial film formed by the surfactant separating the water droplets from the oil continuum. It can be maximized by minimizing the interfacial bending stress of the rigid interface and increasing the attractive interdroplet interaction.\textsuperscript{29} As the interface is doped with a nonionic surfactant (herein Ig-520), it decreases the spontaneous curvature of the interfacial film, making the interface more fluid, and increases the interaction between the droplets. Solubilization increases up to the point where the spontaneous radius of curvature approaches the critical radius, \( R_c.\) The solubilization maximum is usually smaller in DDAB RM because of the low hydrophilicity as well as the bulkier quaternary ammonium head group size.\textsuperscript{26} Doping with Igepal increases \( w_{0,max} \) in DDAB RMs as hydrophilicity increases. It is evident that in the AOT/Ig system the decrease in the interfacial bending elasticity of the rigid AOT interface and the increase in the interdroplet interaction with the doping of the Igepal content are more effective to display higher synergism in comparison to that in DDAB/Ig systems.

2.2. DLS Measurements. DDAB RMs in Cy are unique in the sense that at low hydration (2 \( \leq w_0 \leq 8), \) aggregated rodlike cylindrical structures predominate (length \( \sim 14–20 \text{ nm} \) and radius \( \sim 1.5,\textsuperscript{26,30} \) At a higher hydration (\( w_0 \sim 10, \) spherical aggregates of a smaller size are formed (Figure S1b)). The droplet size of DDAB/Ig RMs decreases drastically as Ig is mixed and does not change appreciably with a further addition of Ig. Interestingly, beyond \( X_{Ig} = 0.2, \) the droplet sizes are comparable to those of AOT/Ig mixed RMs and change only marginally with \( X_{Ig}.\) It is evident from Figure S1b that in DDAB/Ig mixed systems, with the increasing Ig content, the droplet sizes first decrease to show minima at \( X_{Ig} = 0.4 \) and 0.6 and then increase. The results indicate that at \( X_{Ig} = 0.4–0.6 \) smaller droplet formation prevails rather than the formation of less number of big droplets.

2.3. FTIR Measurements. The O–D stretching in the MIR region (2200–2800 cm\textsuperscript{-1}) is a potential tool to extract the structural information of water encapsulated in the RM nanopool.\textsuperscript{31–34} The overall spectrum of pure water in this frequency window produces a smooth curve peaking at \( \sim 2508 \text{ cm}^{-1}.\) In DDAB/Ig mixed RMs, however, the curves get broadened and could be deconvoluted into three Gaussian sub-peaks peaking at 2450, H-bonded water (HW); at 2545 ± 5, intermediated water (IW); and at 2640 ± 10 cm\textsuperscript{-1}, multimter water (MW).\textsuperscript{35} We calculate the relative area contribution (Figure S2) of each curve toward the total spectra and plot it as a function of \( X_{Ig} \) at different \( w_0 \) values of the mixed RMs (Figures S3 and S4). Progressive inclusion of Ig in the AOT interface increases the HW content, which is compensated by a concomitant decrease in IW and MW abundance, confirming a linear mixing behavior at the interface. We observe a somewhat unusual trend in DDAB/Ig mixed RMs in which abundance of HW and IW increases slightly as compensated by a decrease in the MW content. This study leads us to conclude that interaction of water molecules with the polar uncharged head group of Ig is weaker in comparison to that with the charged head group, whereas for DDAB, the bromine ion might play a role in interacting with water molecules. The lower droplet size and rigid interface of AOT decreases the HW contribution, whereas H-bond formation with water molecules through anionic charged AOT head groups increases the IW contribution in AOT RM compared to that in the Igepal RMs. However, the situation is contrasting in DDAB RM as the droplet size is big with high HW contribution and the large head group of DDAB inhibits interaction with water molecules, which decreases the IW contribution compared to that in the Igepal RMs, wherein feeble interaction between water and the poly(ethylene oxide) (PEO) head group of Igepal predominates.
2.4. Fluorescence Measurements. We use C-500 as the fluorophore as its excitation at 409 nm selectively excites the C-500 molecules at the interface only.25 The decay transients of C-500 are shown in Figure S5. C-500 in water produces an emission maximum (\(\lambda_{\text{max}}\)) at \(\sim 505 \text{ nm}\), and in DDAB/Ig RM, it suffers a progressive blue shift with decreasing \(w_0\); however, the change is only modest with \(X_{\text{Ig}}\). We construct the solvation correlation curves, and some representative \(C(t)\) plots for different \(X_{\text{Ig}}\) s at \(w_0 = 10\) are shown in Figure S6b. All of the \(C(t)\) curves are fitted bi-exponentially, and the time constants are presented in Table S1 (Supporting Information). The time constants are on the order of hundreds of picoseconds and a few nanoseconds.36 The average solvation time is calculated as \(\langle \tau_{\text{sov}} \rangle = \sum a_i \tau_i\), and the time constants are presented in Table S1 (Supporting Information). The \(\langle \tau_{\text{sov}} \rangle\) is faster in DDAB RM compared to that in Ig RM at \(w_0 = 10\). \(\langle \tau_{\text{sov}} \rangle\) shows a noticeable minimum at \(X_{\text{Ig}} \sim 0.6\) (Figure S6c), whereas it changes monotonically in AOT/Ig mixed RMs.23 A similar trend also follows in anisotropy measurements (Figure S6d).

2.5. Excited-State Proton Transfer (ESPT) Study. 2.5.1. Steady-State Measurements. The protonated and the deprotonated forms of \(\nu\)-luciferin show absorption maxima at \(\sim 330\) and \(\sim 390 \text{ nm}\), respectively.19 \(\nu\)-Luciferin in water exists in a protonated form, whereas in AOT RM, it exists in a protonated form mostly.19 In AOT RM, \(\nu\)-luciferin prefers to stay mostly in the protonated form as the pH inside AOT RM is lower compared to that in pure water.19 Taking into consideration its oil insolubility, one can expect \(\nu\)-luciferin to occupy the water-facing interfacial domain of the RMs. In neat water, the emission spectrum of \(\nu\)-luciferin is dominated by the emission from the deprotonated (\(\lambda_{\text{max}}^{\text{em}} = 535 \text{ nm}\)) excited state.

In Ig and DDAB RMs, it shows two distinct emission peaks, one at \(\sim 430 \text{ nm}\) (protonated or the neutral form, ROH) and the other at \(\sim 530 \text{ nm}\) (deprotonated or the anionic form, RO\(^-\)). Whereas in AOT RM, the peak of the deprotonated species is only feeble (Figure 1a). Figure 1b depicts the emission spectra of \(\nu\)-luciferin in DDAB/Ig mixed RMs at \(w_0 = 10\) and at different \(X_{\text{Ig}}\) s, and the corresponding figure for AOT/Ig mixed RMs is shown in the Supporting Information section (Figure S7). The fluorescence intensity of the protonated form increases along with a slight red shift as \(X_{\text{Ig}}\) increases. For a comprehensive understanding, we plot the ratio of apparent intensity of the protonated and the deprotonated form (\(I_{\text{RO}}/I_{\text{ROH}}\)) as a function of \(X_{\text{Ig}}\) (Figure 1c,d). We observe different trends in two different mixtures. In the AOT/Ig mixture, the relative abundance of RO\(^-\) increases monotonically with increasing \(X_{\text{Ig}}\); however, in DDAB/Ig, we found a maximum at \(X_{\text{Ig}} \sim 0.6\).

2.5.2. Time-Resolved Measurements. We measure the fluorescence transients at two selective wavelengths (420 and 590 nm) corresponding to the protonated and the deprotonated forms, respectively (Figure 2 and Table S2). We chose a slightly blue-shifted wavelength for the protonated form and a slightly red-shifted one for the deprotonated form to minimize the undesired mixing of the contributions of the two forms.4,5 The decay transients at 420 nm could be fitted with multiple decay components fixed at 80, 600, and \(\sim 1500\) ps, whereas those in the red end could be fitted only after considering rise component(s) (Table S2). The decay of \(\nu\)-luciferin at 450 nm in pure water is rather fast and is dominated by the \(\sim 80\) ps component (99%), whereas at 540 nm, it is slow with
The observed rise component for the deprotonated species unambiguously confirms the presence of an excited-state process in which emission is emanating from a previously formed excited state rather than from the species getting directly excited. We plot the contribution of the fast component (~80 ps), which is the fall time for the protonated as well as the rise time of the deprotonated species (Figure 3a). It is higher in DDAB RMs and decreases first modestly and then sharply beyond $X_{Ig} = 0.4$ (Figure 3a). The ~600 ps component is the lifetime of the excited state of D-luciferin, which undergoes proton transfer, forms a ground-state product, and is comparable to the recombination time of HPTS in different RMs.\textsuperscript{3} In AOT RM, the 600 ps component has contributions comparable to those of the faster one and does not change appreciably with $X_{Ig}$.

The multiple decay pattern of D-luciferin in the RMs can be rationalized assuming an approximate bimodal distribution in such a way that only a fraction of the probe undergoes ESPT (Scheme 1). It can be shown that the concentration of ROH at any time $t$ can be given as

$$[\text{ROH}^*] = [\text{ROH}^*]_{0,Y} e^{-\left(\frac{t}{\tau_f} + \frac{t}{\tau_{dp}}\right)} + [\text{ROH}^*]_{0,N} e^{-\frac{t}{\tau_{dp}}}$$

(1)

where $\tau_f$ and $\tau_{dp}$ stand for the time constants for fluorescence emission of the protonated and deprotonation forms, respectively. Subscripts Y and N stand for ROH molecules that “do” and “do not” undergo the ESPT reaction. We fit the

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**Figure 2.** Fluorescence transients of the protonated ($\lambda_{em} = 420$ nm) (a, c) and deprotonated ($\lambda_{em} = 590$ nm) (b, d) forms of D-luciferin in DDAB/Ig and AOT/Ig mixed RMs at $w_0 = 10$ ($\lambda_{ex} = 375$ nm).

**Figure 3.** (a) Fluorescence decay coefficient of the ~80 ps component of the protonated form of D-luciferin measured in mixed RM systems at $w_0 = 10$. (b) Fraction of D-luciferin molecules undergoing the ESPT reaction in mixed RM systems at $w_0 = 10$. 

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3. Components 535 ps (40%) and 5.1 ns (60%). The observed rise component for the deprotonated species unambiguously confirms the presence of an excited-state process in which emission is emanating from a previously formed excited state rather than from the species getting directly excited. We plot the contribution of the fast component (~80 ps), which is the fall time for the protonated as well as the rise time of the deprotonated species (Figure 3a). It is higher in DDAB RMs and decreases first modestly and then sharply beyond $X_{Ig} = 0.4$ (Figure 3a). The ~600 ps component is the lifetime of the excited state of D-luciferin, which undergoes proton transfer, forms a ground-state product, and is comparable to the recombination time of HPTS in different RMs. In AOT RM, the 600 ps component has contributions comparable to those of the faster one and does not change appreciably with $X_{Ig}$.

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For DDAB RMs, however, the value is relatively high (possible recombination. In RM systems, the following deprotonation, protons di- that the power law dependency emanates from the fact that deprotonated species. For a better apprehension of the time-course of the complex process, specially considering the fact that there are multiple locations of the protonated species inside the RMs and that a fraction of them do not at all undergo any ESPT process (see Figure 3b); however, the trend in the estimated value of n is intriguing. Clearly, the bigger size of the DDAB

ROH decay transients using eq 1 for both AOT/Ig and DDAB/ Ig mixed RM systems. The τ f and τ fi values for the AOT, DDAB, and Ig RM systems are found to be (1.05, 0.12 ns), (0.75, 0.11 ns), and (0.83, 0.10 ns), respectively. The τ f values for the single RM systems are in good agreement with the average of the two slow components taken together (Table S2). We plot the relative population of n-luciferin undergoing ESPT reaction (Figure 3b) in both the mixed RM systems. The fraction is low (~0.4) and comparable in AOT and Ig RMs, whereas it is high (~0.9) in the DDAB RMs. The trend in DDAB/Ig mixed RMs is much similar to that of the relative contribution of the fast component and shows a dip at X Ig = 0.4.

ESPT is generally a fast process, yet we observe some slow components (on the order of nanoseconds) while fitting the emission decay transient of the ROH* species, which is due to the recombination of the dissociated proton with the RO* base. Such recombination is expected to be governed by a diffusion motion and thus by a t−3/2 power law.39,40 At a long time, the intensity of the protonated species thus can be assumed to follow a relation

\[
i \propto e^{-t/\tau f} t^{-n/2}
\]

with the ideal value of n being 3. We try to fit the ROH* decay transients beyond 600 ps using eq 2 fixing the value of τ f as the slowest component of the decay transient (see Table S2). Some representative fittings are provided in Figure 4a. The fittings are reasonably good except for a few systems,31 and the estimated values of n are plotted as a function of X Ig in Figure 4b. The analysis based on eq 2 should be considered after a note of caution that the long-time fitting is best-represented for bulk systems; however, being successfully employed in AOT RMs by Fayer et al.31 and for the purpose of comparison, it could possibly be extended in other RM systems also. For AOT RMs, the value of n is rather low (0.96) and is in good accordance with that obtained for the ESPT of HPTS in AOT/heptane RMs.3 In Ig RM, it is also comparable (n = 1.1); however, in the AOT/Ig mixed system, the value lies low (n ~ 0.4) (Figure 4b). For DDAB RMs, however, the value is relatively high (~1.63). With increasing X Ig, it shows a subtle increase up to X Ig = 0.4 (n = 1.81), beyond which it decreases sharply. It can be noted here that the power law dependency emanates from the fact that following deprotonation, protons diffuse away, prohibiting a possible recombination. In RM systems, the diffusion is prohibited by the confinement of water molecules; however, it eases with the increasing size of the water pool.3 Thus, a straightforward power law dependency is a mere simplification of the complex process, specially considering the fact that there are multiple locations of the protonated species inside the RMs and that a fraction of them do not at all undergo any ESPT process (see Figure 3b); however, the trend in the estimated value of n is intriguing. Clearly, the bigger size of the DDAB

RM is not the sole reason for the high n value as it does not decrease appreciably at X Ig = 0.2 RM, which has a comparable size as that of the AOT/Ig mixed system. RO* gets specifically stabilized at the DDAB interface, and the proton could be diffused with more ease as DDAB RM possesses more HW than that in AOT and Ig RMs, as concluded from the FTIR measurements (see Figures S3 and S4).

To interpret the decay components more analytically, we measure the fluorescence transients at several wavelengths across the full emission spectrum and construct the time-resolved area-normalized emission spectra (TRANES)32 (representative figures for DDAB/Ig and AOT/Ig mixed RMs with X Ig = 0.6 are provided in the Supporting Information section: Figure S8b,d). We observe a distinct iso-emissive point at ~500 nm, which unambiguously indicates the simultaneous presence of two distinct species. The TRANES profile also clearly manifests that initially the emission is dominated by the protonated species, which then diminishes with time with a concomitant increase in the emission intensity of the deprotonated species. For a better apprehension of the time evolution of the two species, we deconvolute each time-resolved emission spectrum (TRES) into two Gaussian curves corresponding to the two concerned species, and a representative deconvolution carried out in DDAB/Ig mixed RM at X Ig = 0.8 is shown in Figure 5. The yellow shaded curves refer to the protonated form, whereas the cyan ones represent the deprotonated form. From the time-dependent deconvoluted curves, we calculate the relative intensity (RI) of the RO* species compared with the maximum peak height; thus, RI approximately corresponds to the progress of the ESPT

Figure 4. (a) Representative time-dependent fluorescence intensity of protonated n-luciferin in different single and mixed RM systems. The solid lines represent the best fit to the \(e^{-t/\tau f} t^{-n/2}\) function. (b) Values of n obtained for different mixed RM systems as a function of X Ig.
process. We plot RI as a function of time for different mixed systems (Figure 6a,b). The curves are linear, however, with a noticeable change in the slope at a longer time as it approaches the equilibrium values. The slope of the linear fits (insets of Figure 6) is a manifestation of the time-evolved formation of the deprotonated form RO−*. In AOT/Ig mixed RMs, the slope is low and increases monotonically, indicating the ease of ESPT with the progressive addition of Ig. In DDAB/Ig mixed RMs, the ESPT process is fast as evident from the slope, however, it is not monotonic, showing a distinct maximum at $X_{\text{Ig}} \sim 0.4$. It could be noticed that at $X_{\text{Ig}} = 0.6$, the equilibrium value of RI, which is comparable to that at $X_{\text{Ig}} = 0.4$ (Figure 1c), is reached at a longer time.

It is evident from the representative TRES of $\nu$-luciferin (Figure S8a,c Supporting Information section) that the emission peak of the protonated species undergoes a noticeable red shift with time, which indicates its solvation. We plot the emission peak frequency as a function of time for both the mixed RMs (Figure 7a,b). We fit the curves with a single exponential decay function, and the corresponding time constants are plotted as a function of $X_{\text{Ig}}$ (Figure 7b). The Stokes shift observed in both the mixed systems is on the order of 500−1000 cm$^{-1}$ and is higher in DDAB RMs. Solvation is in general slower in DDAB RM and is faster in AOT RM, that of Ig RM being intermediate. The solvation in DDAB/Ig mixed RMs decreases sharply beyond $X_{\text{Ig}} = 0.4$, whereas in AOT/Ig mixed RMs, it shows a subtle maximum at $X_{\text{Ig}} = 0.6$.

We also measure time-resolved fluorescence anisotropy to understand the restriction imposed by the RM environment on the fluorophore, of both the protonated and deprotonated forms of $\nu$-luciferin in mixed RMs (a representative depiction is provided in Figure S9). We observe that anisotropy of both the forms of $\nu$-luciferin is slower in DDAB RMs compared to that in the AOT RMs, and the value is intermediate in the Ig RMs (Figure 8, Table S3). Fluorescence anisotropy of a fluorophore is intimately related to its location inside the RM. As the probe $\nu$-luciferin prefers to stay in the core in anionic RM, its rotational anisotropy is relatively faster compared to that of cationic DDAB in which the probe is more interface-bound and thus slow-rotating. In case of Ig, the poly(ethylene oxide) (PEO) head group of Ig inhibits the rotation of the probe molecule. The deprotonated species shows a noticeably slower rotational anisotropy compared with the protonated species in DDAB RMs, intuitively because of being more inclined to stay at the interface owing to charge neutralization. The charge repulsion, on the other hand, makes the deprotonated species...
stay at the water pool of AOT RMs and shows a faster rotational dynamics (Figure 8b). Addition of Ig in DDAB RM does not ease the rotation noticeably up to $X_{Ig} = 0.4$, beyond which it accelerates (Figure 8a). In the AOT/Ig RM system, the rotation gets slower with $X_{Ig}$ more or less linearly.

3. SUMMARY

The proton transfer rate of D-luciferin depends on various parameters, such as the size of the RMs, water content ($w_0$), pH of the interface in RM, etc. In this article, we introduce a new parameter in the form of mixing of surfactant(s) of different charge types. DLS measurements conclude that pure DDAB RMs are bigger in size compared with AOT RMs. Addition of Ig decreases the size of the former, whereas it increases the size of the latter. However, at and beyond $X_{Ig} = 0.2$, the sizes of both the mixed RMs are more or less comparable (Figure S1b). FTIR measurements indicate abundance of intermediate water (IW) inside RMs, which is highest in AOT RM followed by Ig and DDAB RMs. In AOT/Ig mixed RMs, IW passes through a relatively low value at $X_{Ig} \sim 0.4$, while in DDAB/Ig mixture the change is more or less monotonic (Figures S3b and S4b). ESPT is mostly prohibited in the AOT RMs even at $w_0 = 10$ (Figure 1), and this result is in accordance with a previous finding by Kuchlyan et al. On the other hand, ESPT is relatively favorable in both Ig and DDAB RMs. A gradual addition of Ig in AOT RMs induces the formation of the deprotonated species. It can be noted that Ig RM is only slightly bigger in size than the AOT RMs (Figure S1b); however, the ESPT process is 90% less in the latter one, which confirms the predominant role of charge stability of the deprotonated species at the RM interface. The amount of HW is relatively less in AOT (Figure S3), which could also inhibit the formation and stability of deprotonated species. ESPT is more efficient in Ig rather than in DDAB RMs, which has a positively charged interface and can presumably stabilize the negatively charged deprotonated species. Rotational anisotropy results also suggest that the deprotonated species is highly restricted in the DDAB interface, indicating its preferred location therein. Interestingly, the most efficient ESPT is observed at $X_{Ig} = 0.6$, wherein the emission peaks of the protonated and the deprotonated species have comparable intensities (Figure 1b). Notably, the solvation dynamics and anisotropy of C-500 show its minimum in this mixing ratio of DDAB/Ig mixed RMs, indicating the system approaching more bulklike behavior. It seems that ESPT and solvation usually follow each other. It has recently been shown that when solvation gets faster, so does the ESPT in 4′-N,N-dimethylamino-3-hydroxyflavone in AOT RM. Datta et al. have correlated the slowing down of the ESPT process of 2-(2′-pyridyl)benzimidazole with the slow solvation of the more polar excited state of the tautomeric form of the probe in the restricted environment of RMs. This leads us to infer that the Ig-induced promotion of ESPT of D-luciferin could be due mostly to its solvatochromic behavior. The AOT interface is expected to be more acidic compared with the core as the H$_3$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4). In neutral Ig RMs, the H$_2$O$^+$ species balances the surfactant charge by diffusing to the interfacial domain, as also confirmed from the long-time fitting (see Figure 4).
concluded that the bufferlike action of RM is more prominent in AOT RM than in positively charged CTAB RMs.\(^{5,46}\) This makes the neutral species preferentially get stabilized at the AOT interface. Addition of Ig reduces the effective charge at the AOT interface, which favors the formation of the deprotonated species. Similarly, the apparent increase in ROH\(^*\) with \(X_{Ig}\) above 0.6 in the DDAB/Ig system is due to the decrease of \(pH\) at the interface by dilution of charge of DDAB with addition of Ig.

From the fluorescence decay transients, we obtain the fraction of D-luciferin undergoing ESPT, and we observe contrasting behaviors in the two mixed RMs (Figure 3b). Surprisingly, in pure AOT and Ig, the decay patterns of the protonated form are quite comparable and thus also the fraction of the protonated forms (Figure 3), which predicts comparable ESPT in these two systems. However, the strong charge repulsion and possible recombination probability eventually inhibit the ESPT process in AOT RMs. In DDAB, the fraction of protonated D-luciferin undergoing ESPT is high and it decreases rapidly at and beyond \(X_{Ig} = 0.6\). The time-resolved evolution of the deprotonated species, as manifested by the value of the change in the slope of RI, is an approximate yet useful parameter to a priori understand the progress of the ESPT process. In AOT, the slope is rather low, corroborating the less ease to form the charged ROH\(^*\) species, whereas in DDAB, it is presumably high, which is a manifestation of the fact that in this a larger number of probe molecules are undergoing ESPT than in the former (Figure 3b). Addition of Ig increases the slope in AOT/Ig mixed RMs almost linearly. In DDAB/Ig mixed RMs, it initially increases with the increasing \(X_{Ig}\) and passes through a maximum. ESPT is favored if the deprotonation step speeds up by the stabilization of H\(_3\)O\(^+\) and/ or RO\(^*\)\(^*\). Initially, a larger droplet size, comparatively high HW content, diffusion of H\(_2\)O\(^+\) to the core, and RO\(^*\)\(^*\) stabilization by electrostatic interaction in DDAB RM collectively assist the progress of ESPT to a greater extent compared to that in Ig. Additionally, interaction of the PEO head group at the Ig interface could initially hinder the complete exposure of D-luciferin toward water molecules. However, as H\(_2\)O\(^+\) and RO\(^*\)\(^*\) are formed, they get stabilized by the PEO head group and the forward deprotonation step gets accelerated. The high proton diffusion rate and high fraction of ESPT undergoing ROH\(^*\) are expected to show the maximum rate at an intermediate mixing ratio. Our results show that the ESPT rate inside RM water pool can be modulated (both increase and decrease) by only mixing surfactants of different charge types and keeping all other parameters unchanged. This finding could find its applicability in its broader implications, specially in situations where a proton is generated in restricted environments and at an interface exposed to water.

4. EXPERIMENTAL SECTION

Sodium bis(2-ethylhexyl) sulfosuccinate (AOT), dodecylmethylammonium bromide (DDAB), polyoxyethylene(5)-nonylphenylether (Igepal-520), cyclohexane (Cy), coumarin-500 (C-500), and D-luciferin (Scheme S1) were products of Sigma-Aldrich. All of the chemicals were used without further purification. AOT, DDAB, and Ig were individually dissolved in Cy keeping the surfactant concentration fixed at 0.1 (M) to prepare two stock solutions and then mixed in the desired proportions to vary the mole fraction of Igepal, \(X_{Ig} = [\text{Igepal}] / ([\text{AOT}] + [\text{DDAB}] + [\text{Igepal}])\), from 0 to 1. Reverse micelles (RM) are produced by adding calculated amounts of water into it so as to fix \(w_0 = ([\text{water}] / [\text{surfactant}]) 10\). All of the measurements were carried out at 298 K.

We determine the water solubilization capacity of these mixed RM systems by titration followed by visual inspection, as described in our earlier studies.\(^{23}\) A Nano-S Malvern instrument employing a 4 mW He–Ne laser (\(λ = 632.8\) nm) equipped with a thermostated sample chamber was used for DLS measurements.\(^{27}\) FTIR spectra with 4% D\(_2\)O in H\(_2\)O were recorded in a JASCO FTIR-6300 spectrometer (transmission mode) using CaF\(_2\) window in the 2200–2800 cm\(^{-1}\) frequency window. Cy shows negligible absorbance in this frequency range. We measured the absorbance of the wet RM (i.e., surfactant (s)/Cy/water mixture) following a baseline correction with the stock solutions (i.e., dry RM, \(w_0 = 0\)), which takes care of the absorbance (if any) of the surfactant(s).

The steady-state absorption and emission were measured with a Shimadzu UV-2450 spectrophotometer and Jobin Yvon Horiba Fluorolog fluorimeter, respectively. Fluorescence transients were measured and fitted using a commercially available spectrophotometer (Life Spec-ps) from Edinburgh Instrument, U.K. (80 ps instrument response function).\(^{48}\) We measure wavelength-dependent transients of C-500 emission, and from the time-dependent fluorescence Stokes shifts, as estimated from time-resolved emission spectra (TRES), we construct the solvent correlation function, \(C(t)\).\(^{43}\)

\[
C(t) = \frac{\tilde{v}(t) - \tilde{v}(\infty)}{\tilde{v}(0) - \tilde{v}(\infty)}
\]

where \(\tilde{v}(0)\), \(\tilde{v}(t)\), and \(\tilde{v}(\infty)\) represent the frequency (wavenumber) maximum at time zero, at time \(t\), and at infinity, respectively. We take the \(\tilde{v}(\infty)\) value to be the peak maximum, beyond which the spectral shift is insignificant. The \(C(t)\) function represents the temporal response of the solvent relaxation process, as occurs around the probe following its photo excitation and the associated change in the dipole moment. Anisotropy, \(r(t)\), defined as

\[
r(t) = \frac{I_{\text{par}}(t) - G I_{\text{perp}}(t)}{I_{\text{par}}(t) + 2GI_{\text{perp}}(t)}
\]

was measured by adjusting the emission polarization to be parallel or perpendicular to that of the excitation. \(G\) is the grating factor, which was determined following the long time tail matching technique.\(^{49}\) All of the anisotropies were measured at the corresponding emission maxima.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00800.

Molecular structure of the chemicals used (Scheme S1); DLS, FTIR, and fluorescence data (Figures S1–S9); fluorescence decay fitting parameters (Tables S1–S3) (PDF)

AUTHOR INFORMATION

Corresponding Authors

*E-mail: arindam.das47@gmail.com. Tel: 91-33-23355706. Fax: +91-33-23353477 (A.D.).
*E-mail: chem2007dip@gmail.com (D.K.D.).
*E-mail: rajib@bose.res.in (R.K.M.).
A.D. acknowledges the S.N. Bose Centre for an e-SRF fellowship. S.I.I. acknowledges the University Grant Commission, India, for fellowship.

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