Interaction of Lucifer yellow with cetyltrimethyl ammonium bromide micelles and the consequent suppression of its non-radiative processes

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Abstract

The interaction of the fluorophore Lucifer yellow with micelles has been monitored using steady state and time resolved fluorescence techniques. Contrary to the popular belief that the fluorophore is too polar to associate with the stern layer or hydrophobic core of micelles, we have observed that it binds with the micelles of the positively charged surfactant cetyl trimethyl ammonium bromide and that such interaction causes a decrease in the rates of its non-radiative processes. This phenomenon cannot be explained solely in the light of a reduced polarity, as we have demonstrated that the photophysics of Lucifer yellow is complex and intramolecular charge transfer does not seem to be the only excited state process that is operative.

1. Introduction

The effect of restricted environments on the excited state properties has been widely studied in the past 15 years [1–8]. It has been demonstrated that the dynamics of solvation in restricted environments are much slower than in bulk liquid [1,2]. This retardation in the dynamics of the solvent molecules results in incomplete solvation of polar molecules and hence, affect the dynamics of any process which passes through polar transition states. Encapsulation effects are observed for various processes in restricted environments [4]. Very recently, the experimental observation of Marcus – inverted region for non-covalently bound electron donor acceptor systems has been reported using the restrictive properties of micelles [5,6]. The marked changes in fluorescence properties induced by restricted environments makes the fluorescence technique a useful tool in the study of local properties of microenvironments [7,8] as well as dynamics in macromolecular systems like protein folding and protein surfactant interaction [9,10]. Zewail and co-workers have elegantly used fluorescence techniques to follow the dynamics of drug–protein complexes [11,12]. Aggregation/deaggregation of porphyrins and other compounds by surfactants has been studied extensively [13–16]. Recently, we have demonstrated a dynamic quenching of fluorescence of chlorin p6 by surfactants with oppositely charged headgroups at low concentrations [16]. We have also studied an interesting salting-out effect on 6-methoxyquinoline in sodium dodecyl sulphate (SDS) micelles [17].

Lucifer yellow CH (LY, Scheme 1) is a polar tracer used in staining neurons [18]. There is no change in its absorption or fluorescence spectra in the pH range 2–9 [19]. It has been used to develop a highly sensitive sensor for Cu2+ ions based on the static quenching of its fluorescence by the Cu2+ ions [20]. The fluorescence resonance energy transfer of LY with one or two subunits of the chloroplast ATP synthase [21] and with anthroylauabain has been reported [22]. However, there has been no investigation of the basic photophysics of LY or its interaction with model systems like micelles. Our interest in Lucifer yellow stems from an attempt to identify
novel fluorescent probes for organized assemblies. LY exhibits a large Stokes shift in aqueous solution, indicating that some kind of excited state process is operative for LY in aqueous solutions. Its structural features include two negatively charged SO$_2$ groups on one hand and a fused ring system on the other hand which seemingly makes it a candidate for localization of interfaces of polar and apolar media, even though there has been no report of LY localizing in such interfaces till date. From the structure of LY, one may suspect that a number of intramolecular and intermolecular proton transfer and charge transfer processes may be operative. In a way it is somewhat analogous to hypericin in this matter where several kinds of proton transfer processes are possible [23]. Of course, Scheme 1 is really very speculative in nature. Ab initio calculations need to be performed before one can say with any degree of certainty whether the H and O atoms in question are really in close proximity to each other. This Letter reports the results of fluorescence studies on Lucifer yellow in cationic cetyl trimethyl ammonium bromide micelles and some supporting solvent variation studies. We discuss the change in the non-radiative rates of the fluorophore with surfactant concentration in the light of the general and specific solvent effects that may be important in this case. We should emphasize here that this is only a first report and a detailed experimental and theoretical investigation of the photophysics of Lucifer yellow is presently underway.

### 2. Materials and methods

Lucifer yellow CH (LY) was obtained from Molecular Probes and was used as received. Cetyl trimethyl ammonium bromide, sodium dodecyl sulphate and triton X-100 were from Sigma Chemicals. Triply distilled water was used for studies in micelles. Other solvents were of spectroscopic grade and were obtained from Spectrochem, Mumbai, India. These were distilled immediately prior to use. The steady state spectra are recorded on JASCO V570 spectrophotometer and Perkin-Elmer LS55 fluorimeter. Fluorescence quantum yields ($\phi_f$) were calculated using ZnTPP as the standard ($\phi_f = 0.11$) [24]. Time-resolved fluorescence measurements were performed at magic angle using a picosecond pulsed diode laser based TCSPC fluorescence spectrophotometer from IBH, UK.

### 3. Results and discussion

Three different surfactants were used in this study – the neutral triton X-100, anionic SDS and cationic CTAB. No change in fluorescence intensity or peak shift was observed in absorption or fluorescence spectra on addition of TX 100 or SDS, indicating the absence of any significant interaction between LY TX-100 and SDS. It is apparent that the hydrophobic effect was not sufficient to force it to leave the aqueous phase and enter the micellar phase in case of TX 100. The lack of interaction with SDS can be attributed to electrostatic repulsion between negative charge of SDS headgroup and two negatively charged sulfonate groups on LY. The same negative charge can be expected to cause a strong interaction with CTAB, which has positively charge heads groups. The lack of interaction with TX 100 and SDS are in line with the fact that LY is a polar tracer and does not prefer to enter the hydrophobic micellar phase.

The absorption spectrum underwent a slight red shift on addition of CTAB whereas the fluorescence spectrum underwent a slight blue shift (Fig. 1). The fluorescence quantum yield ($\phi_f$) in water was been observed to be about 0.21, which was in agreement with an earlier report [19]. Initially, fluorescence was quenched on addition of CTAB till it reached a minimum value of 0.08 at a concentration of 0.4 mM CTAB. On further addition of the surfactant, the $\phi_f$ gradually increased till a saturation value of ~0.30 at a concentration of 1.6 mM CTAB (Fig. 2). The initial decrease in fluorescence quantum yield can be ascribed to an aggregation of the fluorophore and the subsequent increase is due to

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**Scheme 1.** The structure of Lucifer yellow.
incorporation in surfactant assemblies. In a recent publication, we have reported a similar aggregation of chlorin \(p_6\) induced by surfactants with oppositely charged headgroups at concentrations comparable to those of the fluorophore [16]. Such aggregation is usually explained from electrostatic considerations. When the ratio of concentrations of the surfactant and the fluorophore is almost stoichiometric, then the oppositely charged surfactant molecules bring the fluorophore molecules close to each other and cause aggregation. Such electrostatically formed aggregates are disrupted at higher surfactant concentrations, when hydrophobic effect predominate, micelles or premicellar surfactant aggregates are formed and these aggregates solubilise the fluorophore molecules, thereby tearing them apart from one another [14]. Such aggregation/deaggregation usually affects the fluorescence properties remarkably, hence making fluorescence techniques very useful in monitoring such interaction [14,16]. In the present case, the relative magnitude of the initial decrease in \(\phi_f\) was smaller than in the case of chlorin \(p_6\). Similar trends were observed in the variation of the average lifetime (\(\langle \tau_i \rangle\)) with the concentration of CTAB (Fig. 2). Since there was an initial decrease in \(\langle \tau_i \rangle\), it is clear that there is some amount of dynamic quenching in the presence of CTAB in low concentrations. However, it should be noted that the relative decrease in \(\langle \tau_i \rangle\) was by a factor of about 0.2, as against a factor of 0.6 in \(\phi_f\), which indicates that a substantial amount of quenching was static in nature, as has been reported for other systems [14,16]. The increase in \(\phi_f\) and \(\langle \tau_i \rangle\) for concentrations of CTAB higher than 0.4 mM can be explained by the incorporation of LY in premicellar and micellar aggregates of CTAB. To the best of our knowledge, this is the first report of incorporation of LY in the interface of polar and apolar media. It is evident that the driving force behind this incorporation is electrostatic rather than hydrophobic. It has been reported in several studies that the increase in \(\phi_f\) and \(\langle \tau_i \rangle\) of fluorophores upon incorporation in micelles is mainly due to the fact that many non-radiative channels exist for these molecules in the aqueous environment, but in a micelle they are protected from these non-radiative channels [25,26]. On calculation of the non-radiative rates (\(k_{NR}\)) as a function of concentration of CTAB, it is indeed found to increase beyond a CTAB concentration of 0.4 to a saturation at \(\sim 1.6\) mM CTAB (Fig. 2). Thus, it is evident that the non-radiative processes in LY get suppressed on micellization. The issue that needs to be addressed at this point is that of the identification of a possible non-radiative channel for LY in water, which gets cut down upon encapsulation in surfactant aggregates (see Fig. 3).

The suppression of non-radiative rates in micelles may be due to a decrease in polarity [26,27] or protection from specific solute-solvent interaction like hydrogen bonding [28]. An increased microviscosity may also suppress the rates of non-radiative processes like photoisomerization [29]. However, only non-polar fluorophores which bind to the hydrophobic core of the micelle can be expected to exhibit such an effect. A probe that is as polar as LY is expected to reside in the interfacial region where the microviscosity may not be sufficiently high so as to be an important factor. The \(\phi_f\) and \(\tau_f\) of LY in CTAB micelles were found to be similar to those in methanol (Table 1). Since the Stern layer of micelles have been reported to have polarities like methanol or ethanol, this observation may lead one to think that the suppression in radiative rates of LY in micelle is due to the decreased microolarity it experiences on binding to the Stern layer. Such a reduction in \(k_{NR}\) is usually because the more polar excited states of the fluorophores are solvated and stabilized in water, thereby causing a decrease in the energy gap with the ground state, whereas such a stabilization is absent in non-polar microenvisions. Understandably, such effects are more prominent in molecules which undergo excited state intramolecular charge transfer, causing a large increase in dipole moment on electronic excitation [26,27,30,31]. We have performed solvent

![Fig. 2. The variation of \(\phi_f\) (–•–), \(\langle \tau_i \rangle\) (–△–) and non-radiative \(k_{NR}\) (–○–) with concentration of CTAB. \(\lambda_{ex} = 406\) nm. The curves are merely aids to the eye.](image)

![Fig. 3. Fluorescence decay of LY in: (a) \(H_2O\), (b) 0.4 mM CTAB and (c) 5 mM CTAB solutions. \(\lambda_{ex} = 406\) nm. The solid lines denote the curves of best fit. The IRF is shown in dashed lines.](image)
variation studies to investigate the effect of solvent polarity and viscosity on the spectral and temporal properties of LY. The Stokes shift is found to follow an overall increasing trend in high polarity solvents. However, the linear correlation in the Lippert plot of LY is not at all strong, even in this region. In low polarity solvents, LY exhibits unusually large values of Stokes shift. This is an indication of the fact that polarity is surely the sole determinant in the excited state processes of LY, at least in the low polarity region. Such deviations from behavior from that expected from general solvent effect have been observed earlier in other molecules and have been ascribed to structural changes and other processes in the excited state [32–34]. To further investigate this phenomenon, we have calculated the radiative and non-radiative rates of LY in different solvents. The radiative and non-radiative rates vary significantly with change in solvents (Table 1). No systematic dependence of $k_R$ or $k_{NR}$ on the solvent viscosity is observed (data not shown). A plot of log $k_{NR}$ against $E_T(30)$ has been constructed in order to verify the applicability of the so-called ‘TICT’ model to LY (Fig. 4). Once again, the data in the low-polarity solvents stand out in being way greater in magnitude than expected. There is a weak linear correlation in solvents with higher polarities. Thus, it seems that even though a weak polarity dependence exists for the photophysics of LY in solvents of higher polarity, some kind of excited state process must be more important in governing the its excited state dynamics in media of low polarity. In the absence of a well defined dependence on $k_{NR}$ on the solvent polarity in media with low polarity, the decrease in the non-radiative rates on incorporation in micelles cannot be due to the opening up of processes excited state intramolecular proton transfer, which are often hindered in water, but are facilitated in micelles [35]. Thus, a preliminary model that emerges is that the excited state dynamics of LY cannot be solely be explained by the intramolecular charge transfer model. It is possible that such charge transfer is facilitated in media of higher polarity, but in media of lower polarity, some other mechanism seems to take over. At this point, we are unable to identify the additional non-radiative channel that seems to open up in low-polarity media, but a detailed fluorescence study involving temperature variation and solvent mixtures is presently under way in order to gain a better understanding of the excited state processes in Lucifer yellow.

### 4. Conclusion

The variation in the spectral and temporal fluorescence properties of Lucifer yellow on addition of the positively charged surfactant CTAB clearly indicates a binding of the fluorophore to the micelle of this surfactant. To the best of our knowledge, this is the first report of Lucifer yellow partitioning into the interface of water.

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**Table 1**

<table>
<thead>
<tr>
<th>Medium</th>
<th>$E_T(30)$</th>
<th>$\phi_t$</th>
<th>$\langle \tau \rangle$ (ns)</th>
<th>$k_{NR} \times 10^8$ (s$^{-1}$)</th>
<th>$k_R \times 10^8$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Dioxane</td>
<td>36.0</td>
<td>0.06</td>
<td>3.4</td>
<td>2.76</td>
<td>0.18</td>
</tr>
<tr>
<td>Pyridine</td>
<td>40.5</td>
<td>0.26</td>
<td>6.3</td>
<td>1.17</td>
<td>0.41</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>41.1</td>
<td>0.03</td>
<td>2.5</td>
<td>3.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Acetone</td>
<td>42.2</td>
<td>0.29</td>
<td>4.6</td>
<td>1.54</td>
<td>0.63</td>
</tr>
<tr>
<td>DMSO</td>
<td>45.1</td>
<td>0.59</td>
<td>10.7</td>
<td>0.38</td>
<td>0.55</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>46.0</td>
<td>0.49</td>
<td>10.7</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>$n$-Octanol</td>
<td>48.1</td>
<td>0.75</td>
<td>8.8</td>
<td>0.28</td>
<td>0.85</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>48.4</td>
<td>0.62</td>
<td>9.6</td>
<td>0.40</td>
<td>0.65</td>
</tr>
<tr>
<td>Ethanol</td>
<td>51.9</td>
<td>0.54</td>
<td>9.2</td>
<td>0.50</td>
<td>0.59</td>
</tr>
<tr>
<td>Ethanediol</td>
<td>53.3</td>
<td>0.50</td>
<td>7.9</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Methanol</td>
<td>55.5</td>
<td>0.25</td>
<td>9.5</td>
<td>0.78</td>
<td>0.26</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>63.1</td>
<td>0.21</td>
<td>5.1</td>
<td>1.55</td>
<td>0.41</td>
</tr>
<tr>
<td>5 mM CTAB</td>
<td>–</td>
<td>0.32</td>
<td>9.2</td>
<td>0.76</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*The data were fit to the function $I(t) = I(0)\sum \phi_i \exp(-t/\tau_i)$, where $I(t)$ is the intensity at time $t$ ns after excitation. The decays in water, CTAB and methanol could be fitted with a single exponential function whereas in acetone, the fitting function was triexponential and the average lifetime was calculated from the relation $\langle \tau \rangle = \sum \phi_i \tau_i$. The non-radiative rates were calculated from the formula $k_{NR} = \frac{1}{\tau_{avg}}$. 

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Figure 4. Plot of log $k_{NR}$ with solvent polarity ($E_T(30)$, for 1,4-dioxane, pyridine, ethyl acetate, acetone, DMSO, acetonitrile, $n$-octanol, propan-2-ol, ethanol, methanol, ethylene glycol and water, denoted by numbers 1–12 in the same order.
and a micelle. The absence of a variation of these properties with the anionic SDS and neutral triton X 100 indicates that the binding of lucifer yellow to micelles cannot take place by hydrophobic effect alone, but assistance from electrostatic interactions is also required. The solvent variation study described above establishes that polarity dependent charge transfer processes alone cannot determine the non-radiative rates in Lucifer yellow, especially in media of low polarity. The photophysics of LY is likely to be complex, as several excited state intramolecular and intermolecular processes can occur in the molecule. It is possible that in higher polarity media, intramolecular charge transfer is the predominant excited state process, but in non-polar ones, where the highly polar charge transfer state is not stabilized, it loses out to some other process, possibly proton transfer which becomes the predominant non-radiative process. In the Stern layer of the micelles, the polarity is intermediate between water and non-polar solvents. Consequently, neither the charge transfer nor the other nonradiative process is very efficient in the Stern layer. We feel that this is why the fluorescence quantum yield as well as lifetime undergo an increase on incorporation of LY in CTAB micelles. Of course, at this point of time, the nature of this additional channel of radiationless depopulation of the excited state is not clear at all. Further detailed experiments are required to gain a sound understanding of the photophysics of Lucifer yellow.

Acknowledgement

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References