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Influence of Dye Hydrophobicity on the Efficiency of Fluorescence Resonance Energy Transfer Between Dyes in Surfactant Micelles

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The fluorescence resonance energy transfer (FRET) between dyes $DiOC_n$ ($n = 2$, 6, 9, and 18) and DiI in aqueous sodium dodecyl sulfate solutions has been studied using the steady-state fluorescence spectroscopy technique. High values of the Stern–Volmer constant (K_{SV}) obtained indicate the long-range dipole-dipole interaction between an excited donor $DiOC_n$ and a ground-state acceptor DiI. The energy transfer efficiency (E) has been analyzed, by basing on both the donor luminescence quenching and the acceptor luminescence enhancement. An increase in E *with the donor hydrophobicity is explained in frames of the Förster theory due to shortening the distance between the donor and the acceptor in a SDS micelle.*

Keywords Fluorescence resonance energy transfer; fluorophore; hydrophobic forces; surfactant micelle

Introduction

Due to the high sensitivity, rapidity, simplicity, and non-invasiveness, the fluorescence technique is widely used to study a variety of biological processes [1,2]. Fluorescence resonance energy transfer (FRET) between energy donor and acceptor molecules is a fundamental phenomenon in the fluorescence spectroscopy [1,3–5]. FRET is a physical process, by which energy is transferred nonradiatively from an excited molecular fluorophore (a donor) to another fluorophore (an acceptor) by means of the intermolecular long-range dipole–dipole coupling [1,3]. FRET can be used for the accurate measurement of molecular proximity at 10–100 Å and is highly efficient if the donor and the acceptor are positioned within the Förster radius (the distance, at which half the excitation energy of the donor is transferred to the acceptor, is typically 3–6 nm) [1,3]. The efficiency of FRET depends on the inverse sixth power of the intermolecular separation [1,3], which makes it a sensitive technique for investigating a variety of biological phenomena that produce changes in the molecular proximity [1–5]. Fluorescent organic

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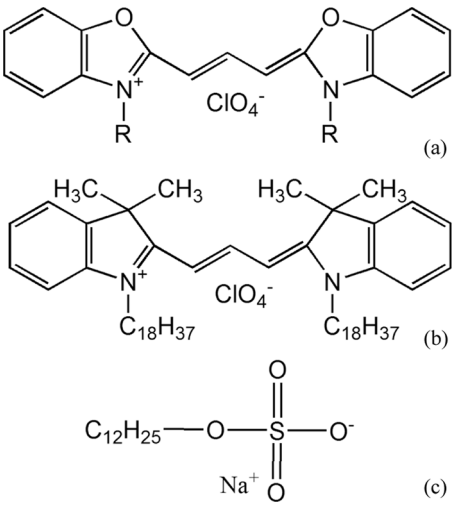

Figure 1. Structural formulas of the studied compounds: (a) $DiOC_n$ dyes. $R = C_2H_5$: 3,3′-diethyloxacarbocyanine perchlorate ($DiOC_2$), $R = C_6H_{13}$: 3,3′-dihexyloxacarbocyanine perchlorate ($DiOC_6$); $R = C_9H_{19}$: 3,3′-dinonyloxacarbocyanine perchlorate ($DiOC_9$); $R = C_{18}H_{37}$: 3,3′-dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}$); (b) 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; (c) sodium dodecyl sulfate.

molecules (fluorescence probes) have been widely used as energy donors and acceptors in a variety of FRET-based biological studies [2]. FRET between the fluorophores in a micelle solution has been extensively studied, because micelle systems are considered to be simple model membrane systems for biological applications [6–12].

Organic dyes can be introduced in biosystems either by covalent-labeling or by adsorption. However, due to the different strengths of electrostatic, hydrophobic, and van der Waals forces between the dye and the target, the adsorption interaction can be very specific and can affect the interpretation of the data obtained in biological researches. Thus, for a reliable interpretation of the data obtained by means of fluorescent spectroscopy including FRET-based applications, the contribution of physical parameters of a fluorescence probe should be taken into consideration.

In the present study, the effect of such a parameter as the dye hydrophobicity on the FRET efficiency between a pair of dyes used in a FRET-based application has been analyzed. For this purpose, a series of cationic dialkyloxacarbocyanine perchlorate ($DiOC_n$) dyes with the same chromophore part and different alkyl chain lengths ($n = 2$, 6, 9, and 18) was used as the energy donors (Fig. 1,a). The hydrophobic cationic dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was used as an energy acceptor (Fig. 1b). As a model system of cell membranes, micelles of anionic sodium dodecylsulphate (SDS) were used (Fig. 1c). The efficiency of FRET between the donor and acceptor molecules was analyzed by both the donor luminescence quenching and the acceptor luminescence enhancing [1].

Experimental

The carbocyanine dyes 3,3′-diethyloxacarbocyanine perchlorate ($DiOC_2$), 3,3′-dihexyloxacarbocyanine perchlorate ($DiOC_6$), 3,3′-dinonyloxacarbocyanine

perchlorate ($DiOC_9$), and 3,3′-dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}$) were obtained from the dye collection of Dr. I. Borovoy (Institute for Scintillation Materials of the NAS of Ukraine). The purity of the dyes was controlled by thin-layer chromatography. Dye DiI and surfactant SDS were purchased from Sigma–Aldrich and used without purification. Organic solvent chloroform (Sigma–Aldrich) used to prepare stock solutions of the dyes was a spectroscopic grade product. The concentration of the surfactant in the solutions was 1×10^{-2} M. This value is higher than the critical micelle concentration (CMC) corresponding to 8×10^{-3} M for SDS to ensure the complete micellization [13]. The concentration of the donor dyes ($DiOC_n$) in the water-micellar solutions was 3×10^{-5} M and was kept constant for all experiments, whereas the concentration of acceptor dye (DiI) was varied within the 1×10^{-6}–4×10^{-5} M range. First, the stock solutions of each dye in chloroform of the 1×10^{-3} M concentration were prepared. To prepare solutions for measurements, the required amounts of the dye stock solutions and SDS were mixed in a flask. After the chloroform evaporation, the required amount of doubly distilled water was added. Visible absorption spectra were recorded using a microspectrometer USB4000 (Ocean Optics, USA) supplied with an incandescent lamp. Solutions were placed in a quartz cuvette with the 2-mm optical length. Luminescence spectra were taken with a spectrofluorimeter on the base of two grating monochromators MDR-23 and a xenon lamp. One of the monochromators was used to select a required wavelength (FWHM ~ 0.5 nm), another one was used for the luminescence collection.

Results and Discussion

Cationic dialkyloxacarbocyanine dyes $DiOC_n$, which were chosen as energy donor molecules, possess the same chromophore part and differ only in the lengths of hydrocarbon tails that imparts different hydrophobic properties to the dyes increasing with lengthening the tail (Fig. 1). The solubility of the dyes in water decreases as $DiOC_2 > DiOC_6 > DiOC_9 > DiOC_{18}$. Dyes $DiOC_9$ and $DiOC_{18}$ exhibit an extremely low water solubility [2]. The energy acceptor DiI is also a hydrophobic water insoluble dye [2]. In aqueous solutions, such hydrophobic dyes form non-fluorescent associates, which causes their fluorescence decay. However, in aqueous solutions containing surfactant SDS micelles, a sharp increase in the absorbance of $DiOC_6$, $DiOC_9$, $DiOC_{18}$, and DiI dyes, a red-shift of the absorption band maxima, and a drastic increase in the luminescence intensity were observed, which evidences the dye incorporation to surfactant micelles (data are not presented) [14,15]. For water-soluble $DiOC_2$ dye, the similar changes in absorption and luminescence spectra in a less polar medium (water-micellar solution) were revealed. The observed features indicate that all investigated dyes are solubilized by SDS micelles, which prevents their aggregation in water [14,15]. It is known that dyes from the polymethine family undergo the photo-induced *trans-cis* isomerization that plays a major role in the radiationless deactivation of the excited state due to the torsional motion of the polymethine chain [15]. However, the torsional motion in organized media (micelles, cell membranes) is blocked, which leads to a significant increase of the dye luminescence intensity. So, the increase of the luminescence intensity of $DiOC_n$ and DiI in aqueous solutions containing SDS micelles also points to the incorporation of the dyes to SDS micelles. Such a forced concentration of the pair of the dyes ($DiOC_n$ and DiI) in the nanoscale volume of SDS micelles (SDS micelle diameter

is 50 Å [11]) ensures a required distance between the dyes to observe FRET [1,3]. Luminescence spectra of $DiOC_2$/DiI, $DiOC_6$/DiI, $DiOC_9$/DiI and $DiOC_{18}$/DiI pairs in aqueous solutions containing SDS micelles are presented in Figure 2a–d. Luminescence was excited at 440 nm (short-wavelength edge of the $DiOC_n$ absorption band). At this wavelength, the DiI luminescence is not excited practically. Figure 2 shows that the presence of DiI in SDS micelles causes the $DiOC_n$ luminescence quenching which increases with the DiI concentration.

The efficiency of the donor luminescence quenching can be analyzed with the use of the Stern–Volmer equation [1,16]

$$\frac{I_0}{I} = 1 + K_{SV}[Q], \tag{1}$$

where I_0 and I are the luminescence intensities in the absence and the presence of a quencher, respectively; K_{SV} is the Stern–Volmer quenching constant; and $[Q]$ is the concentration of a quencher which is DiI dye in our case.

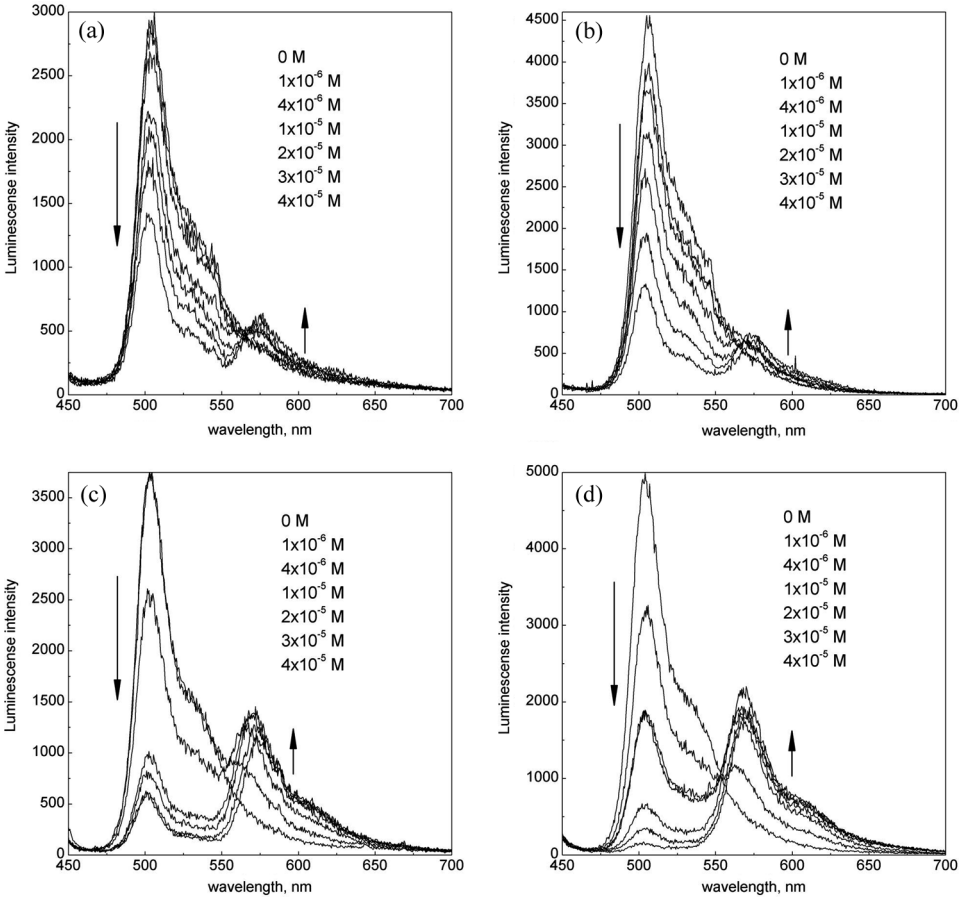

Figure 2. $DiOC_2$ (a), $DiOC_6$ (b), $DiOC_9$ (c), and $DiOC_{18}$ (d) luminescence intensity change as a function of the acceptor DiI concentration in a water-micellar solution.

Plots of I_0/I vs $[Q]$ for $DiOC_2$/DiI, $DiOC_6$/DiI, $DiOC_9$/DiI, and $DiOC_{18}$/DiI pairs are presented in Figure 3. Values of K_{SV} obtained as line slops are listed in Table 1. The result shows that the Stern–Volmer quenching constant K_{SV} increases with the hydrophobicity of donor dye $DiOC_n$. So, the luminescence of more hydrophobic dyes is quenched more effectively. The obtained values fall in the normal range reported for a similar type of FRET systems [6–10]. Absorption spectra of such solutions show no additional bands, which indicates the absence of a ground state complex formation between the donor and acceptor molecules. The luminescence spectra of the mixture of $DiOC_n$ and DiI did not show any additional band at longer wavelengths, which evidences the absence of the exciplex formation between the excited donor and ground state acceptor molecules (Fig. 2a–d) [16]. Thus, the more reasonable explanation for the donor luminescence quenching in surfactant micelles in the presence of DiI is the resonance energy transfer due to the long-range dipole–dipole interaction between the excited donor $DiOC_n$ and the ground state acceptor DiI [1,16]. Figure 2 shows that the efficiencies of the donor luminescence quenching and the acceptor luminescence enhancement differ in $DiOC_n$/DiI pairs.

To analyze the efficiency of FRET in pairs $DiOC_2$/DiI, $DiOC_6$/DiI, $DiOC_9$/DiI, and $DiOC_{18}$/DiI (Fig. 2a–d), we took into consideration both the donor luminescence quenching and the acceptor luminescence enhancement [1].

In the first treatment, the energy transfer efficiency E_D is defined as [1]

$$E_D = 1 - \frac{I_{DA}}{I_D}, \tag{2}$$

where I_{DA} and I_D are the donor luminescence intensities in the presence and the absence of the acceptor, respectively.

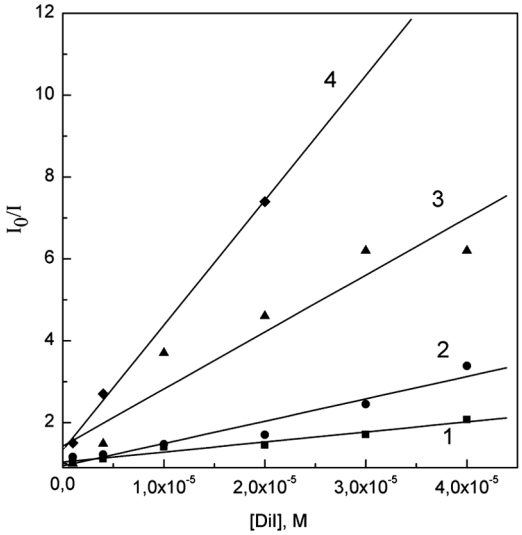

Figure 3. Stern–Volmer plots for the $DiOC_n$ luminescence quenching by DiI in a water-micellar solution: 1 – $DiOC_2$; 2 – $DiOC_6$; 3 – $DiOC_9$; 4 – $DiOC_{18}$.

Table 1. Energy transfer parameters of $DiOC_n$ in the presence of DiI in a micellar solution

Donor dyes	K_{SV}, M^{-1}	$\varepsilon_D(\lambda_D^{ex})$, $M^{-1}\,cm^{-1}$	E_D	E_A	E
$DiOC_2$	2.45×10^4	23600	0.63	0.92	0.72
$DiOC_6$	5.45×10^4	27400	0.65	1.74	0.83
$DiOC_9$	1.40×10^5	27400	0.91	0.78	0.90
$DiOC_{18}$	3.05×10^5	23430	0.93	3.23	0.98

The second analysis is based on the use of an enhanced acceptor luminescence signal [1]

$$E_A = \frac{\varepsilon_A(\lambda_D^{ex})}{\varepsilon_D(\lambda_D^{ex})} \cdot \left[\frac{I_{AD}(\lambda_A^{em})}{I_A(\lambda_A^{em})} - 1\right], \tag{3}$$

where $\varepsilon_A(\lambda_D^{ex})$ and $\varepsilon_D(\lambda_D^{ex})$ are the extinction coefficients of the acceptor and the donor at the donor excitation wavelength ($\lambda_D^{ex} = 440$ nm); and $I_{AD}(\lambda_A^{em})$ and $I_A(\lambda_A^{em})$are the acceptor intensities measured at the acceptor emission wavelength (λ_A^{em}) in the presence and the absence of donor, respectively.

Based on the two treatments, the real energy transfer efficiency can be calculated as [1]

$$E = \frac{E_A}{E_A - E_D + 1}. \tag{4}$$

The values of E_A and E_D were calculated using experimental data presented in Figure 2a–d. For the calculations, the spectra corresponding to the $DiOC_n$ and DiI concentrations equal to 3×10^{-5} M were chosen. At this concentration, the donor luminescence quenching and the acceptor luminescence enhancement are more pronounced for each pair. The concentration of micelles [M] in the solution can be estimated as [17]

$$[M] = \frac{[S] - CMC}{N_{agg}}, \tag{5}$$

were [S] is the total surfactant concentration, N_{agg} is the surfactant aggregation number ($N_{agg} = 63$ for SDS micelles [13]). So, we obtain [M] $= 3 \times 10^{-5}$ M.

The approximate number of dye molecules (n) incorporated into a single micelle can be estimated by the equation [17]

$$n = \frac{[dye]}{[M]}, \tag{6}$$

where [dye] is the dye concentration in the solution.

As [$DiOC_n$] = [DiI] $= 3 \times 10^{-5}$ M, in such a case one SDS micelle contains one donor molecule $DiOC_n$ and one acceptor DiI.

The value of $\varepsilon_A(\lambda_D^{ex})$ is 8400 $M^{-1}\,cm^{-1}$, and values of $\varepsilon_D(\lambda_D^{ex})$, E_D, E_A, and E are presented in Table 1.

Table 1 shows that the calculated FRET efficiency E in the pairs $DiOC_2$/DiI, $DiOC_6$/DiI, $DiOC_9$/DiI, and $DiOC_{18}$/DiI dyes in SDS micelles increases with the donor dye hydrophobicity and is equal to 72%, 83%, 90%, and 98%, respectively. According to the Förster theory, the energy transfer efficiency is governed by the distance between the donor and acceptor r and the Förster distance R_0 as [1,16]

$$E = R_0^6/(R_0^6 + r^6), \tag{7}$$

R_0 (in Å) is expressed as [1,16]

$$R_0^6 = 8,79 \times 10^{23}[k^2 n^{-4} \Phi_D J(\lambda)], \tag{8}$$

where $J(\lambda)$ represents the overlap integral, Φ_D is a quantum yield of the donor in the absence of the acceptor, n is the refractive index of the medium, and k^2 is the factor expressing the relative orientation of the donor and the acceptor.

Thus, the main parameters that can affect R_0 are Φ_D and $J(\lambda)$. However, since the acceptor was the same in all pairs, and the donor dyes possess the same chromophore parts, all pairs show the same overlap between the donor fluorescence and acceptor absorption spectra. The values of Φ_D for $DiOC_n$ molecules differ not significantly. Moreover, since Φ_D is taken to a power of $1/6$ in the calculation of R_0, a small error of Φ_D does not have a large effect on R_0 [see Eq. (8)]. So, R_0 is the same for all dye pairs. Thus, we can conclude that the increase in the FRET efficiency in pairs $DiOC_2$/DiI, $DiOC_6$/DiI, $DiOC_9$/DiI, and $DiOC_{18}$/DiI is associated mainly with the shortening of the donor–acceptor distance with increase in the donor hydrophobicity [see Eq. (7)]. As mentioned above, $DiOC_n$ dyes differ in the lengths of hydrocarbon tails, which imparts different hydrophobic properties to the dyes (Fig. 1a). The tails act as ''anchors'' holding the dyes in SDS micelles in such a way that the hydrocarbon tails anchor in the micelle hydrocarbon core, while the chromophore group is localized in the micelle headgroup region [2]. The shorter the tail, the weaker the hydrophobic forces holding the dye within the micelles, and the electrostatic repulsion between the positively charged donor and acceptor dye molecules can result in increasing the distance between the dyes [18]. However, the absence of a shift of the absorption and luminescence maxima indicates that the dye molecule is localized in the micellar region (Fig. 2a–d). Our conclusions are in a good agreement with the values of binding constants of the $DiOC_n$ dyes to SDS micelles determined in our previous work using the Benesi-Hildebrand method. There, we showed that the efficiency of the $DiOC_n$ dye binding to SDS micelles increases with the dye hydrophobicity [19].

Conclusions

We have studied the fluorescence resonance energy transfer between the donors of different hydrophobicity $DiOC_n$ ($n = 2$, 6, 9, and 18) and acceptor DiI incorporated in SDS micelles. The donor luminescence quenching has been analyzed by the Stern–Volmer equation. High values of the quenching constant K_{SV} indicate that the dominant mechanism of fluorescence quenching is the resonance energy transfer due to the long-range dipole–dipole interaction between the excited donor $DiOC_n$ and the ground state acceptor DiI molecules. We have studied the FRET efficiency

E, by basing on both the donor luminescence quenching and the acceptor luminescence enhancement. The E values obtained show that the FRET efficiency increases in the row $DiOC_2/DiI < DiOC_6/DiI < DiOC_9/DiI < DiOC_{18}/DiI$. Based on the Förster theory, it has been concluded that an increase in the FRET efficiency in the dye pairs is associated with the shortening of the donor–acceptor distance with increase in the donor hydrophobicity.

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