Photophysical Processes of Merocyanine 540 in Solutions and in Organized Media

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Photophysical processes of merocyanine 540 (MC540) are studied using picosecond time-resolved emission spectroscopy. The nonradiative rate of MC540 increases from 0.32×10^9 s^{-1} in dioxane to 8.73×10^9 s^{-1} in water, i.e., nearly 27 times. This is attributed to the decrease in the barrier of photoisomerization with increase in polarity and the consequent increase in the rate of photoisomerization for MC540. The polarity-dependent isomerization is also shown to be the cause for the observed increase in emission intensity and lifetime of MC540 in aerosol-OT microemulsions and aqueous bovine serum albumin solutions.

1. Introduction

Merocyanine 540 (MC540, **I**) is an anionic lipophilic polymethine dye which readily binds to biomembranes, micelles, and proteins. In aqueous solutions, the fluorescence intensity of MC540 increases by an order of magnitude on binding to these biological systems. Further, leukamia cells stained with MC540 are rapidly destroyed on irradiation by light. There have been several studies to explain the mechanism of the fluorescence sensitivity[1−7] and the light-induced cytotoxicity[8] of MC540. The fluorescence quantum yield and lifetime of MC540 in aqueous solutions are markedly lower than those in nonpolar solvents while its absorption maximum exhibits a blue shift in aqueous solution compared to that in a nonpolar solvent. In water an additional absorption peak arises at 500 nm which is assigned to the MC540 dimer. It is proposed that in aqueous solutions MC540 forms nonfluorescent dimers.[4] When MC540 binds to micelles or vesicles, the nonfluorescent dimers break to form the fluorescent monomers, and this is responsible for the emission enhancement of MC540.[4] In the resonating structure **Ib**, MC540 contains a negative and a positive end.

SCHEME 1:   Resonating Structures of Merocyanine 540 (I)

(a)   **(b)**
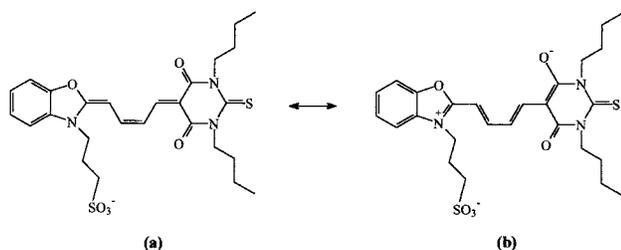
Thus, stacking of oppositely charged ends of two dye molecules on top of each other is quite plausible. However, if this mechanism is correct, the fluorescence lifetime of MC540 should have been the same in a nonpolar solvent, dioxane, and a polar solvent, water, since in both solvents the emission originates predominantly from the monomers. Again the fluorescence lifetime of MC540 should remain unchanged when in

aqueous solution MC540 binds to vesicles, micelles, etc. However, the experimental results indicate that the increase of fluorescence intensity of MC540 is invariably accompanied by an increase in the lifetime. This indicates that the fluorescence enhancement of MC540 is due to the suppression of nonradiative processes occurring in the excited state of the dye. For the cyanine dyes or in general for any polyenes the main nonradiative pathway is identified as the photoisomerization about the central double bond.[1−2,9−13] The rate of photoisomerization usually decreases with increase in viscosity.[9,10] However, the increase in the lifetime of MC540 from 110 ps in water (viscosity, $\eta = 0.89$ cP) to 456 ps in ethyl alcohol ($\eta = 1.14$ cP) and to 1490 ps in dioxane ($\eta = 1.17$ cP) cannot be explained in terms of the effect of viscosity as all the three solvents possess similar viscosity. In summary, the exact mechanism of fluorescence enhancement of MC540 on binding to biological systems is still not fully understood. In the present work we have attempted to throw new light on the photophysics of MC540.

2. Experimental Section

MC540 (Sigma), aerosol-OT (AOT, dioctylsulfosuccinate, sodium salt, Aldrich), and bovine serum albumin (BSA, sigma) were used as received. Preparation of AOT microemulsion is described elsewhere.[17] For the determination of the fluorescence quantum yield of MC540 the sample was excited at 530 nm, and 3,3′-diethyloxadicarbocyanine iodide (DODCI) in water ($\phi_f = 0.3$)[16] was used as a standard. For lifetime measurements the samples were excited at 570 nm by the fundamental of a Rhodamine 6G dye laser (Coherent 702-1) synchronously pumped by a CW mode-locked Nd:YAG laser (Coherent Antares 76s). The emission is detected at magic angle polarization using a Hamamatsu MCP 2809U photomultiplier tube. The full width at the half-maximum for the instrument response at 570 nm is about 100 ps.

3. Results

3.1. Emission Properties of MC540 in Water−Dioxane Mixture. The emission spectra of MC540 in a series of mixtures of water and dioxane are given in Figure 1, and the corresponding emission decays are shown in Figure 2. The emission parameters of MC540 in different water−dioxane mixtures are

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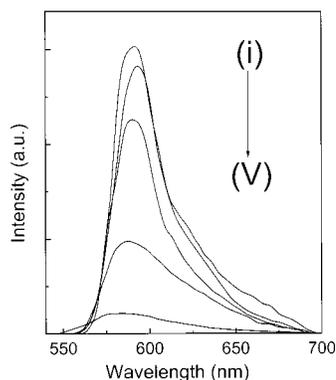
Figure 1. Emission spectrum of merocyanine 540 in water−dioxane mixture containing (i−v) 5, 10, 20, 40, and 60% water (v/v).
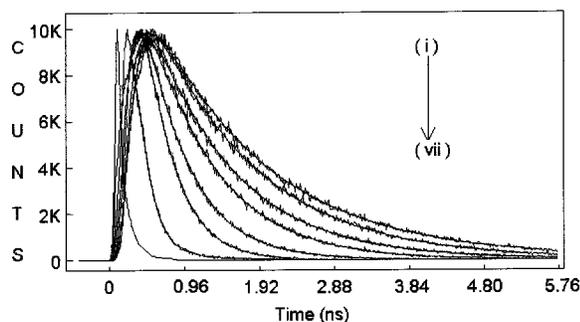
Figure 2. Fluorescence decays of merocyanine 540 in water−dioxane mixture containing (i−vii) 0, 5, 10, 20, 40, 60, and 100% water (v/v). The sharp peak near $t = 0$ denotes the system response.

TABLE 1: Emission Properties of MC540 in Dioxane−Water Mixture

| % of water (v/v)	$E_T(30)$	$\phi_f{}^a$	$\tau_f{}^a$ (ps)	$k_r \times 10^{-9}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
0	36.6	0.52	1490	0.35	0.32
5	43.8	0.52	1320	0.39	0.36
10	46.3	0.44	1040	0.42	0.54
20	49.2	0.34	850	0.40	0.78
40	52.1	0.26	540	0.48	1.37
60	55.8	0.20	350	0.57	2.29
100	63.6	0.04	110	0.36	8.73

$^a \pm 5\%$.

summarized in Table 1. It is readily seen that the emission quantum yield (ϕ_f) of MC540 decreases from 0.52 in dioxane to 0.04 in water while the lifetime (τ_f) decreases from 1490 ps in dioxane to 110 ps in water. The rate constants for the radiative (k_r) and the nonradiative (k_{nr}) processes of MC540 are calculated using the relations $k_r = \phi_f/\tau_f$ and $k_{nr} = (1 - \phi_f)/\tau_f$. The results indicate that the k_{nr} of MC540 increases quite dramatically by nearly 27 times from 0.32×10^9 s^{-1} in dioxane to 8.73×10^9 s^{-1} in water.

3.2. MC540 in AOT Microemulsions. In a nonpolar solvent many surfactant molecules aggregate with their polar headgroups pointing inward to form a reverse micelle. The reverse micelles can entrap a considerable amount of water in the form of a water pool, and such a system is known as a microemulsion.[17] The water molecules confined by a layer of surfactant in the water pool of a microemulsion are interesting models of the biological water molecules. In *n*-heptane for the surfactant sodium dioctyl sulfosuccinate (aerosol-OT, AOT) the radius of the water pool, r_W (in angstroms), is nearly $2w_0$, where w_0 is the ratio of the number of water and AOT molecules present in the system.[17d] It has been demonstrated recently that the photoisomerization of a cyanine dye,[16b] intramolecular charge transfer,[17a,b] and
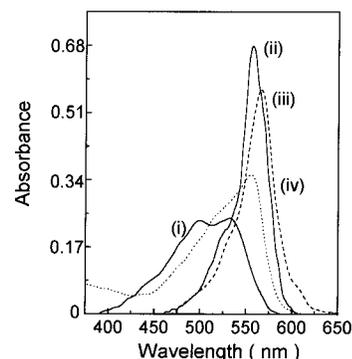
Figure 3. Absorption spectrum of merocyanine 540 in (i) water, (ii) 0.09 M AOT/*n*-heptane/H$_2$O, $w_0 = 32$, (iii) neat dioxane, and (iv) 360 μM buffered aqueous solution of BSA.
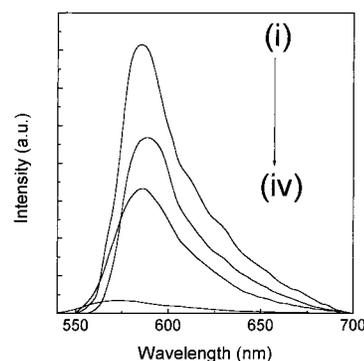
Figure 4. Emission spectrum of merocyanine 540 in (i) 0.09 M AOT/*n*-heptane/H$_2$O, $w_0 = 4$, (ii) 0.09 M AOT/*n*-heptane/H$_2$O, $w_0 = 32$, (iii) 360 μM buffered aqueous solution of BSA, and (iv) water.

TABLE 2: Emission Properties of MC540 in 0.09 M AOT Microemulsions

| w_0	$\phi_f{}^a$	$\tau_f{}^a$ (ps)	$k_r \times 10^{-9}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
4	0.66	2400	0.27	0.14
32	0.42	1500	0.28	0.39

$^a \pm 5\%$.

solvation dynamics[17c] are markedly retarded in the water pool of microemulsions. A recent compressibility study suggests that the first solvation shell of the AOT molecules becomes complete at $w_0 \approx 13$, and for $w_0 < 13$ the water structure in the water pool remains severely perturbed.[17e] In the present work, we have studied the photoisomerization of MC540 in a small ($w_0 = 4$) and a big ($w_0 = 32$) water pool of AOT microemulsion in *n*-heptane. While the absorption spectrum of MC540 in water exhibits a broad peak at 500 nm due to formation of dimers, in the water pool of AOT microemulsions the absorption spectrum of MC540 (Figure 3) does not exhibit such a broad peak around 500 nm. Instead, the absorption spectrum of MC540 in AOT microemulsions is very similar to that in dioxane with a sharp peak at 565 nm. This indicates MC540 experiences a relatively nonpolar microenvironment in the AOT microemulsion. In the AOT microemulsion for a small water pool ($w_0 = 4$, radius $r_w \approx 8$ Å), the fluorescence quantum yield of MC540 is 0.66 (Figure 4). The fluorescence decay of MC540 in the small water pool is single-exponential, and the corresponding lifetime (τ_f) is 2400 ps (Figure 5, Table 2) so that $k_{nr} = 0.14 \times 10^9$ s^{-1}. Evidently, the nonradiative decay of MC540 in the small water pool is nearly 62 times slower than that in ordinary water. For a large water pool ($w_0 = 32$, radius $r_w \approx 64$ Å), $\phi_f = 0.42$, and the fluorescence decay is single-exponential with $\tau_f = 1500$ ps
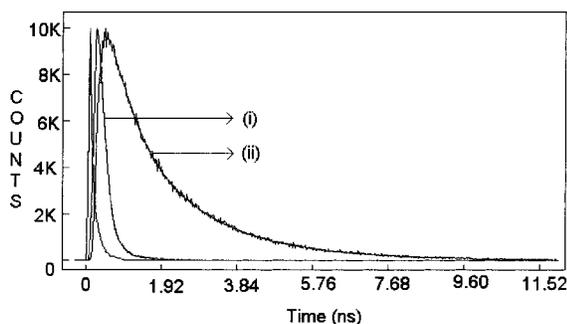
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TABLE 3: Emission Properties of MC540 in 360 μM BSA Solution

a_1	τ_1 (ps)	a_2	τ_2 (ps)	$\langle\tau_f\rangle^{a,b}$ (ps)	$\phi_f{}^a$	$k_r \times 10^{-9}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
0.40	580	0.60	2000	1430	0.27	0.19	0.51

$^a \pm 5\%$. $^b \langle\tau_f\rangle = a_1\tau_1 + a_2\tau_2$.
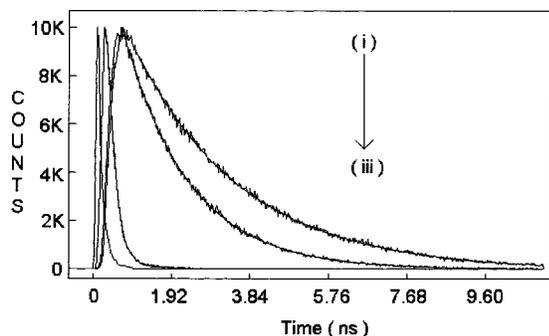
Figure 5. Fluorescence decays of merocyanine 540 in (i) 0.09 M AOT/n-heptane/H_2O, $w_0 = 4$, (ii) 0.09 M AOT/n-heptane/H_2O, $w_0 = 32$, and (iii) water.


Figure 6. Fluorescence decays of merocyanine 540 in (i) water and (ii) 360 μM buffered aqueous solution of BSA.

(Figure 5). Thus, at $w_0 = 32$, for MC 540, $k_{nr} = 0.39 \times 10^9$ s^{-1} (Table 2), which is 22 times slower than that in ordinary water.

3.3. MC540 in Aqueous Protein Solutions. On addition of the protein, bovine serum albumin (BSA), to a buffered aqueous solution of MC540 absorbance of the dimer peak at 500 nm decreases markedly, and the monomer peak exhibits a red shift to 560 nm which is very similar to the absorption peak of MC540 in dioxane and in AOT (Figure 3). The fluorescence quantum yield of MC540 in aqueous BSA solution saturates above 50 μM BSA. In 360 μM BSA the fluorescence quantum yield of MC540 is 0.27 (Figure 4). In BSA the fluorescence decay of MC540 was found to be multiexponential. The multiexponential decay is obviously due to the inherent inhomogeneity of the microenvironment of the probe MC540 molecules and the fact that the probe encounters different environments due to diffusion within its lifetime.[17a,18] Instead of giving too much importance to the individual components of the decay, we have fitted the decay to a biexponential, e.g., $a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$. The corresponding average lifetime $\langle\tau_f\rangle$ is given by $\langle\tau_f\rangle = a_1\tau_1 + a_2\tau_2$. In 360 μM BSA $\langle\tau_f\rangle$ of MC540 is 1430 ps (Figure 6). This leads to $k_{nr} = 0.51 \times 10^9$ s^{-1} (Table 3), which is nearly 17 times slower than that in neat water.

4. Discussion

The most interesting finding of the present work is the nearly 27 times smaller value of k_{nr} in dioxane compared to that in water. Since the viscosities of dioxane and water are very
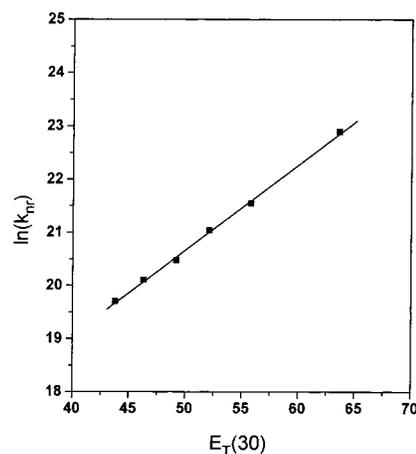
Figure 7. Plot of $\ln(k_{nr})$ of merocyanine 540 vs $E_T(30)$ of different water$-$dioxane mixtures.

similar, the nearly 27 times smaller value of k_{nr} in dioxane compared to that in water cannot be due to the differences in viscosity. Several authors examined the effect of polarity on the photoisomerization process of stilbene and polyenes.[9,14] Eisenthal et al.[14a] observed that the slope of the isoviscous plots of $\ln(k_{iso})$ against reciprocal of temperature decreases with increase in viscosity. Since for the alcohols higher viscosity is associated with lower polarity, they concluded that the barrier for isomerization decreases with decrease in polarity. It seems that the increase in the rate of the nonradiative process of MC540 with increase in water content, i.e., polarity, may similarly be due to the change in the barrier of isomerization of MC540 with polarity. Following Hicks et al.,[14b] we assume that the barrier for photoisomerization of MC540 decreases with increase in polarity $E_T(30)$[15] as

$$E_b = E_b{}^0 - Z[E_T(30) - 30]$$

where $E_T(30)$ is the empirical polarity parameter[15a] and $E_b{}^0$ is the barrier in a hydrocarbon of $E_T(30) = 30$. The $E_T(30)$ values for the water$-$dioxane mixtures are listed in the literature.[15b] For MC540 as well as other polyenes the main nonradiative pathway is the photoisomerization process.[1,2,9,11,13,14] According to the polarity-dependent barrier model, the rate constant of isomerization $[k_{iso} = A \exp(-E_b/RT)]$ contains a term independent of polarity $[A \exp\{-(E_b{}^0 + 30Z)/RT\}]$ and a polarity-dependent term $[A \exp\{ZE_T(30)/RT\}]$. Other nonradiative processes (e.g., internal conversion, etc.) also contribute to the polarity-independent nonradiative process. The polarity-dependent term increases rapidly with increase in polarity, i.e., $E_T(30)$ of the medium, and is responsible for the 27-fold increase in k_{nr} of MC540 in water compared to that in dioxane. At higher polarity for MC540, one may write, $k_{nr} \approx k_{iso}$. According to this model, the plot of $\ln(k_{nr})$ against $E_T(30)$ should be a straight line with positive slope. The actual results for MC540 in water$-$dioxane mixtures indicate that for $E_T(30) > 43$ $\ln(k_{nr})$ increases linearly with $E_T(30)$ (Figure 7). The linearity of the plot vindicates the assumptions of a polarity-dependent barrier and that $k_{nr} \approx k_{iso}$ for the isomerization of MC540 at higher polarity ($E_T(30) > 43$). At lower polarity, i.e., between $E_T(30) = 36$ (pure dioxane) and $E_T(30) = 43$, the nonradiative rate of MC540 remains almost constant (Table 1). This indicates that at lower polarity the contribution of the polarity-dependent term is very small, and the overall nonradiative rate is dominated by the polarity-independent term.

The polarity-dependent isomerization rate seems to be the main reason for the increase in fluorescence intensity and

lifetime of MC540 on binding to proteins and microemulsions. The microenvironments of the proteins and microemulsions are much less polar than the bulk water. The observed retardation of the isomerization process of MC540 in microemulsions and proteins compared to that in water may be attributed to the lower local polarity. Comparing the k_{nr} of MC540 in BSA to that in the water−dioxane mixtures, one can infer that the polarity of the microenvironment of the MC540 molecules bound to BSA is similar to that of a mixture of 10% water and 90% dioxane (v/v) with $E_T(30) = 46.3$. In the case of the large ($w_0 = 32$) water pool of the AOT microemulsion, k_{nr} of MC540 is very similar to that of a mixture containing 5% water and 95% dioxane having $E_T(30) = 43.8$. Although one cannot rule out the effect of a high local microviscosity, polarity seems to be the major factor for the observed retardation of the isomerization process of MC540 in the big water pool of the microemulsion and in proteins.

In the case of the small ($w_0 = 4$) water pool of the AOT microemulsion, the k_{nr} of MC540 is nearly 62 times slower than that in water and is about twice as slow as that in dioxane. As noted earlier, the nonradiative rate of MC540 becomes constant at lower polarity, i.e., between $E_T(30) = 36$ (pure dioxane) and $E_T(30) = 43$. The fact that the nonradiative rate of MC540 in the small ($w_0 = 4$) water pool is twice as slow compared to that in dioxane cannot be explained by the polarity factor alone. In the small water pool ($w_0 = 4$, radius $r_w \approx 8$ Å), a portion of the large MC540 molecule is likely to get inserted among the AOT molecules. MC540 has been reported to form an association complex with anionic micelles[5] apart from the cationic and neutral micelles.[3] Thus, the dramatic retardation of isomerization of MC540, over and above that in dioxane, in the small water pool ($w_0 = 4$, radius $r_w \approx 8$ Å) of AOT microemulsions may be due at least partially to the aggregation of MC540 with AOT molecules and the consequent hindrance to the torsional motion.

In contrast to MC540, for another cyanine dye, 3,3′-diethyloxadicarbocyanine iodide (DODCI), the isomerization process is retarded only about 3 times in AOT microemulsions.[16b] The decay of DODCI in dioxane is biexponential with an average lifetime of 620 ps. This is actually very similar to the lifetime of DODCI in water at 25 °C (640 ps).[16a] Thus, in contrast to MC540 the rate of isomerization of DODCI does not vary with the polarity of the medium. Velsko and Fleming earlier showed that the slope of the isoviscous plots and hence the barrier for the isomerization process of DODCI do not depend on the polarity of the medium.[9a] The lack of polarity dependence of the isomerization process of DODCI seems to be responsible for the lower magnitude of its retardation in the microemulsions.

5. Conclusions

The present work indicates that the barrier for the isomerization process of MC540 decreases with increase in the polarity of the medium. This is responsible for the nearly 27-fold increase in the rate of isomerization of MC540 in water compared to that in dioxane, both of which possess similar viscosity. The polarity-dependent barrier is also responsible for the linearity of the plot of $\ln(k_{nr})$ against $E_T(30)$ of the medium at higher polarity ($E_T(30) > 43$). At lower polarity ($E_T(30) = 36−43$), the nonradiative rate of MC540 remains almost constant. This

suggests that at lower polarity the contribution of the polarity-dependent term to the rate of isomerization is very small, and the overall nonradiative decay is dominated by the polarity-independent term. The decrease in the rate of the isomerization, i.e., the main nonradiative process of MC540 with decrease in polarity, is considered to be responsible for the increase in emission intensity and lifetime of MC540 on binding to AOT microemulsions and to proteins in aqueous solutions. The polarity, i.e., $E_T(30)$, of the microenvironment of the MC540 molecules bound to BSA and that in the big water pool ($w_0 = 32$) of the AOT microemulsions are estimated be about 46.3 and 43.8, respectively. The nearly 2 times retardation of the nonradiative isomerization process of MC540 in the small ($w_0 = 4$) water pool of the AOT microemulsions, compared to that in dioxane, is ascribed to the aggregation of MC540 with AOT molecules and the consequent hindrance to the torsional motion.

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