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Association between Psoralens and Some Ionic Micelles

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The association between psoralens and some micelles is measured by the fluorescence quenching of psoralens by methylviologen (MV^{2+}) and bromide ion in some ionic micellar solutions. The association constants were estimated to be *ca.* 10^4 for all the psoralens studied even though they show different hydrophobicity.

Introduction

Psoralens(furocoumarins), naturally occurring coumarin derivatives in various plants, have been widely used in the photochemotherapy of various skin diseases[1,2]. The photosensitivity of psoralens is primarily correlated with their photoreactivity toward pyrimidine bases in DNA under the 320-380 nm UV light[3]. Psoralens also cause damage to ribonucleic acids[4], proteins[5], and membranes[6], and are used as molecular probe for nucleic acid structure[7].

The fluorescence of psoralens in some ionic micellar solutions have been studied in our laboratory as models for biomimetic system[8]. The psoralen molecules are located in the micelle-water interfacial region and show different fluorescence features from that in aqueous solution due to both the low polarity and large viscosity of the micellar interface. The fluorescent behavior of psoralens in micellar solutions, therefore, can be used to determine their partition between the micellar and aqueous phases[9].

In general, ionic quenchers localized in the aqueous phase fail to quench the fluorescence of substrates incorporated in the micellar phase when the ion and the micellar surface are like-charged and only the substrates in aqueous phase are quenchable. The quenching data, therefore, may be used to determine the association constants between substrates and micelles[9-11].

In this paper, we would like to report the fluorescence quenching of 8-methoxypsoralen(8MOP), 5-methoxypsoralen (5MOP), 4,5',8-trimethylpsoralen(TMP), and 5,7-dimethoxycoumarin(DMC) by ionic quenchers, Br^- and methylviologen(MV^{2+}) in some micellar solutions and the association constants between psoralens and micelles are calculated.

Experimental

Materials. 8MOP, 5MOP, and TMP were obtained from the Aldrich Chemical Company and used without further purification. DMC was purified by recrystallization from ethanol. Sodium dodecyl sulfate(SDS, $NaCH_3(CH_2)_{11}SO_4$, CMC = 8mM, Aldrich) was recrystallized three times from ethanol after washing with ether. Cetyltrimethylammonium bromide(CTAB, $CH_3(CH_2)_{15}N(CH_3)_3Br$, CMC = 0.94mM, Aldrich) was recrystallized twice from methanol and CTAC(cetyltrimethylammonium chloride, CMC = 1.4mM, Aldrich(25%)) was used as received. Methylviologen(1,1'-dimethyl-4,4'-bipyridinium chloride, MV^{2+}) was prepared by the method reported[12]. Sodium bromide(Junsei Chemical Co.) and sodium chloride(Kanto Chemical Co.) were used as received. Chromatographic and spectroscopic grade solvents were used for high performance liquid chromatography and emission spectroscopy, respectively. Deionized distilled water was used.

Methods. Fluorescence spectra were recorded on an Aminco-Bowman spectrofluorometer with an Amino XY recorder and/or a Perkin-Elmer LS 50 spectrofluorometer at room temperature. To compare the hydrophobicity of psoralens, high performance liquid chromatography was performed on a Waters Associates Model 244 Liquid Chromatograph equipped with Model 6000A solvent delivery system, Model 440 UV absorbance detector(254nm), and Model U6K universal injector. The Lichrosorb RP-18 column and 60% methanol eluent were used. Fluorescence quenching experiments were carried out with MV^{2+}(0-4 mM) and Br^-(0-0.1 M) keeping the ionic strength at 0.1 by addition of sodium chloride which does not quench fluorescence. The contribution of surfactants and MV^{2+} to the ionic strength can be neglected[13].

Results and Discussion

Fluorescence Quenching. The addition of MV^{2+} and sodium bromide to the aqueous solutions of psoralens decreased the fluorescence intensity. The quenching data were analyzed by Stern-Volmer equation,

Table 1. Stern-Volmer Constants of Psoralen Fluorescence Quenching by MV^{2+} and Br^- in Aqueous and Micellar Solutions.

	K_{sv}^a					
	Br^-				MV^{2+}	
	H_2O	SDS	CTAB	CTAC	H_2O	SDS
8MOP	0 (0.25)[b]	0	4.0	2.9	0	1100
5MOP	0 (0.34)[b]	0	18	31		
TMP	0.64(1.2)[b]	0	3.1	4.5	80(140)[b]	470
DMC	20 (24)[b]	4.0	30	63	64(87)[b]	2600

[a] Measured at $[Q] \rightarrow 0$ and ionic strength 0.1. [b] Measured without adjusting the ionic strength.

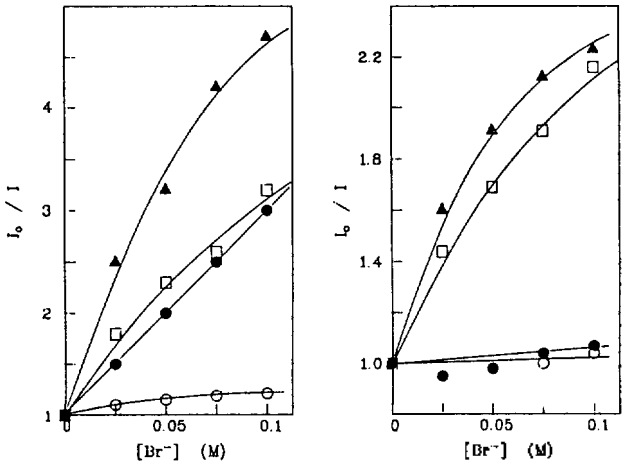

Figure 1. Stern-Volmer plot for fluorescence quenching of DMC (left) and 5MOP(right) by Br^- in water(●), 10 mM SDS(○), 10mM CTAC(▲) and 5mM CTAB(□) solutions. In all the cases, the ionic strength was 0.1.

$$I_o/I = 1 + K_{sv}[Q] \quad (1)$$

where I_o and I denote the fluorescence intensities in the absence and presence of a quencher, respectively. The Stern-Volmer constant K_{sv} is related to the second order rate constant (k_q) for the quenching and fluorescence lifetime (τ_F) of a fluorescing species by $K_{sv} = k_q \tau_F$.

The fluorescence quenching by MV^{2+} and Br^- showed linear Stern-Volmer relationships and K_{sv} values obtained from the slopes are summarized in Table 1. Since the lifetime of the 8MOP singlet state in aerated aqueous solution is 1.28ns[17], the quenching rate constant (k_q) of Br^- is determined to be 1.9×10^8 $M^{-1}s^{-1}$. From the lifetime of 7.2ns of DMC[17], the k_q values of Br^- and MV^{2+} can be calculated as 3.3×10^9 and 1.2×10^{10} $M^{-1}s^{-1}$, respectively. The fluorescence quenching of 8MOP and 5MOP by MV^{2+} was not observed under the experimental conditions ($[MV^{2+}] < 4$mM).

The fluorescence quenching of psoralens by ionic quenchers, Br^- and MV^{2+}, are diffusion-controlled and it is greatly affected by the variation of the local concentration in micelles. The Stern-Volmer plots for 5MOP and DMC in micellar solutions are shonw in Figure 1. The electrostatically attractive force between the cationic CTAB and CTAC micelle and Br^- enhances the fluorescence quenching of psoralens embedded in the micellar interior(Stern region),

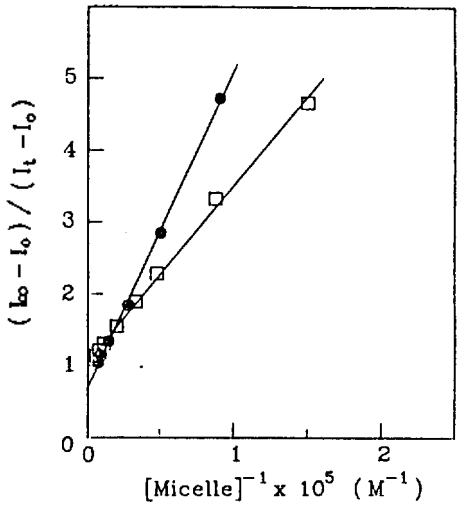

Figure 2. The variations of fluorescence intensity of 5MOP in CTAB(●) and CTAC(□) micellar solutions, plotted according to eq.(2).

whereas the repulsive interaction between the anionic micelle SDS and Br^- decreases the probability of quenching reaction. Unlike the plots of data taken in aqueous solution, the Stren-Volmer plots in micellar solutions exhibited a large deviation from the linearity. The curvature in Stern-Volmer plot represents, largely, the dependence of the apparent bimolecular quenching constant, $k_q = K_{sv}/\tau_F$, on the concentration of quenchers, and mainly arises from the competition of psoralens and quenchers for available sites in the micelles. When the concentration of quencher is low, both psoralens and quenchers condense in the Stern layer of the micelle and the fluorescence quenching is very efficient. However, further addition of quenchers at given concentration of the micelle may displace some psoralens from the micellar phase, resulting in less efficient quenching. Such quenching behavior is frequently observed in the polyelectrolyte[12,18] and micellar solutions.[19] The K_{sv} values extrapolated to [Q] = 0, i.e. slopes at [Q] = 0 are summarized in Table 1. For MV^{2+} quencher, the quenching reaction is greatly enhanced in SDS micellar solution.

Micelle-Psoralen Equilibrium Association Constants. The fluorescence intensity of psoralens in aqueous solution is quite different from that in the micellar solutions[8]. If the fluorescence quantum yields of psoralens in the micelle and aqueous phase are independent of the surfactant concentration, the equilibrium association constnat(K_{eq}) for psoralens in the micellar solution can be calculated from the relationship[9]:

$$(I_\infty - I_w^0)/(I_t - I_w^0) = 1 + (K_{eq}[M])^{-1} \quad (2)$$

I_∞, I_w^0, and I_t are relative emission intensity at 100% solubilization, without surfactant, and at intermediate surfactant concentration, respectively. The variation of fluorescence intensity of 5MOP in micellar solution reported earlier[8] are plotted according to eq.(2)(Figure 2) and the calculated K_{eq} are summarized in Table 2. The fluorescence intensity of psoralens in SDS solution varied very much in premicellar region and K_{eq} of psoralens in SDS solution could not be determined. By the same reason, that of TMP in CTAB and CTAC solutions could not be calculated.

Table 2. Association Constants Between Psoralen and Micelle(K_{eq}), and Distribution Coefficients Between the Micellar and Aqueous Phase(a/b)

Psoralen	Surfactant	eq. used	a/b	K_{eq}
8MOP	CTAB	eq.(2)	3.1	4.5×10^4
5MOP	CTAB	eq.(2)	3.1	4.5×10^4
	CTAC	eq.(2)	6.6	8.1×10^4
TMP	CTAB	eq.(5)	1.9*	
	CTAC	eq.(5)	2.9*	
DMC	SDS	eq.(3)	1.9-2.5*	$2.3\times10^{4*}$
		eq.(5)	2.2*	$2.3\times10^{4*}$
	CTAB	eq.(5)	1.6*	
		eq.(2)	2.8	4.2×10^4

*: Calculated at ionic strength 0.1

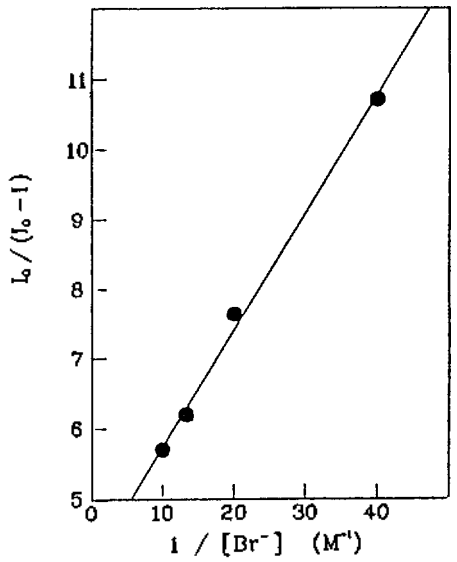

Figure 3. DMC fluorescence quenching by Br^- in 10mM SDS solution, plotted in the form given by eq. (3). The ionic strength was 0.1.

When a quencher and micellar system have the same sign of charges, the quencher impinging on the micelle surface is repelled instantaneously by the like-charged micelle head groups as a result of electrostatic repulsion and the quenching reaction with the solubilized psoralens in micellar phase rarely occurs. Hence, the quenching effects were attributed solely to the psoralens in the aqueous phase. From the selective quenching of the fluorescence arising from the aqueous phase, Quina and Toscano[10] evaluated the probe distribution between the micelle and aqueous phase by the equation (3)

$$\frac{\Phi_F^o}{\Phi_F^o-\Phi_F}=\left\{\frac{a}{b}\frac{\Phi_{Fm}^o}{\Phi_{Fw}^o}+1\right\}\left\{1+\frac{1}{k_q\tau_{Fw}^o[Q]}\right\} \quad (3)$$

Φ_F is relative fluorescence quantum yield in the presence of the bulk solution quencher Q. Φ_F^0 is the total fluorescence yield in the absence of a quencher, Φ_{Fm}^0 and Φ_{Fw}^0 are the fluorescence quantum yields in the micelle and aqueous phase, respectively. a and b are the fraction of probe in the micelle and aqueous phase. The ratio of a/b is related to the equilibrium constant, K_{eq}, for the association of the probe with the micelle by eq. (4).

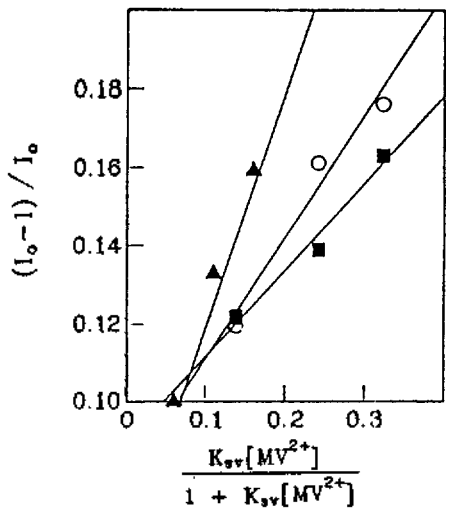

Figure 4. Fluorescence quenching of DMC in 5 mM CTAB(▲), TMP in 5mM CTAB(○) and 10mM CTAC(■) solutions by MV^{2+}, plotted in the form given by eq.(5). In all the cases, 0.1M NaCl was added.

$$K_{eq}=\frac{a}{b}\left\{\frac{[D]_t-CMC}{\bar{N}}-a[Ps]_t\right\}^{-1}\simeq\frac{a}{b}\left\{\frac{[D]_t-CMC}{\bar{N}}\right\}^{-1} \quad (4)$$

The intercept and slope of the plot of $\Phi_F^0/(\Phi_F^0-\Phi_F)$ *vs.* $[Q]^{-1}$ can be used to calculate a/b. The plot of the experimental data according to eq.(3) for the fluorescence quenching of DMC by Br^- is shown in Figure 3. The aggregation number, $\bar{N}$, for SDS in the presence of 0.1 M NaCl is 88[20] and the ration of I_{Fm}^0/I_{Fw}^0 for DMC was measured to be 1.5. The equilibrium constant K_{eq} for DMC in SDS based on eq.(3) and eq.(4) is $2\text{-}2.8\times10^4$ M^{-1}. The ratio of intercept/slope (K_{sv}) is in reasonable agreement with the value obtained from the Stern-Volmer plot in aqueous solution. Abuin and Lissi[11] have proposed a modified equation(5):

$$(I_o-I)/I_o=a\,K_{sv}[Q]/(1+K_{sv}[Q]) \quad (5)$$

where a is the fraction of light emitted from the aqueous phase at $[Q]=0$. The fraction of probe in the aquous phase can be obtained by eq. (6),

$$\%\text{ in the water phase}=100\,a\,I_{Fm}^0/I_{Fw}^0 \quad (6)$$

The calculated K_{eq} for DMC in SDS micelle solution from eq.(5) and eq.(6) (2.3×10^4) agreed well with the value from eq. (4). The quenching data of TMP and DMC by MV^{2+} in CTAB and CTAC micellar solutions are plotted according to eq.(5) and are shown in Figure 4.

The HPLC experiments were performed(Figure 5) to measure the ralative hydrophobicity of psoralens. The solubility of 8MOP, 5MOP, and TMP in water is 23, 5, and 1 μg/m*l*, respectively. From the solubility and retention time of HPLC, the order of relative hydrophobicity are expected to be DMC < 8MOP < 5MOP < TMP. The binding abilities of psoralens to the micelles with hydrophobic interaction are little different in spite of the differences in hydrophobicity and are affected little by the addition of salts.

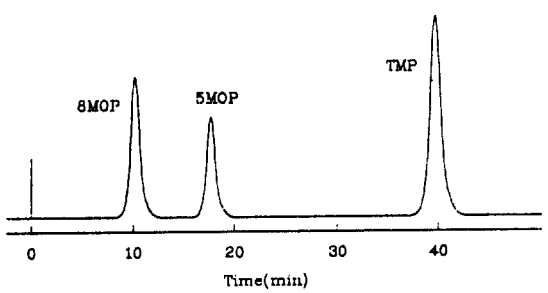

Figure 5. HPLC profile of 8MOP, 5MOP, and TMP.

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13. Taking the CMC of SDS to be 1.4 mM[14] and the degree of micellar ionization to be about 0.2[15] at the ionic strength 0.1, the concentration of the free sodium ion due to the detergent is 3 mM which is considerably smaller than 0.1. Because the CMC of CTAB and CTAC at the ionic strength 0.1 is smaller than 0.95 and 1.4 mM reported in the absence of salts[16], respectively, the contribution of these surfactants to the ionic strength can be neglected.
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New Crown Compounds Derived from 1,2-Bis(2-hydroxybenzyl)benzene (II): Bisaryl Crowns

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New bisaryl corands (crown ethers) bearing 1,2-dibenzyl- and 1,2-dibenzoylbenzene subunits have been synthesized: The reaction of 1,2-bis(2-hydroxybenzyl)benzene in base with mono-tetrahydropyranyl oligoethylene glycol tosylate, deprotection of the bis-condensation product to give a corresponding diol, tosylation of the free hydroxyls of the diol, and condensation of the ditosylate in base with 1,2-bis(2-hydroxybenzyl)benzene afforded a new type of bisaryl corand(I) of 1,2-dibenzylbenzene system. Oxidation of the benzylic positions of the corands(I) furnished novel aromatic corands(II) containing partly carbonyl functions.

Introduction

Various bisaryl crown ethers, such as **1**, **2**, **3** and **4**, have been synthesized by many workers, in which two aromatic hydrocarbon subunits are linked symmetrically or unsymmetrically by two ethyleneoxy chains to form a macrocycle. Although the crowns **1-4** are structurally analogous, their complexing properties vary markedly with the structures of the aromatic subunits and the polyether ring sizes. Dibenzo-18-crown-6(**1**), synthesized by C. J. Pederson in his early investigations for crown ether synthesis,[1,2] and reasonably selective toward the complexation of potassium ions, is the first