The Location and Microenvironment of Dimerizing Cationic Dyes in Lipid Membranes as Studied by Means of Their Absorption Spectra

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The absorption spectra of three cationic dyes, Methylene Blue (MB), 3,3′-dipropyl-2,2′-thiadicarbocyanine (diS-C_3(5)), and 9-aminoacridine (9-AA), were measured in aqueous suspensions of a phospholipid membrane and a surfactant micelle. The dyes were intensely partitioned into the membrane phase and dimerized to exhibit a remarkably large reduction in absorbance. The increase in the amount of the membrane beyond the amount where all the dye molecules were dimerized in the membrane phase resulted in a gradual increase in the absorbance due to the increase in the available membrane volume and the dilution of dye in the membrane phase. The changes in the wave lengths of the absorption maxima of the dye monomers by the partitioning into membrane thus obtained were interpreted in terms of the effective polarities of the dye surroundings (microenvironments). It was found that the rather hydrophobic diS-C_3(5) penetrates deeply into the membranes and the micelles and is located in considerably nonpolar microenvironments, while MB remains at the polar-surface regions of the membranes and the micelles. On the basis of these results, the structures of the micelles and membranes are discussed.

Membranes, micelles, and microemulsions provide wide ranges of small environments and have great effects on chemical equilibria, kinetics, and structure organizations.[1] To estimate local effective polarities in micelles and microemulsions, various spectral methods have been employed. Charge-transfer bands of pyridinium iodide and 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)phenoxide exhibit considerably large shifts according to the nature of the dye surroundings.[2,3] Also, the vibronic structures of aromatic hydrocarbons, such as benzene, naphthalene, and pyrene, in absorption and fluorescence spectra show strong dependencies on the solvent, micellar, and microemulsion environments.[4,5]

With respect to lipid membranes, which offer promising prospects of understanding the complex natures of biological membranes and also for pharmaceutical and industrial uses, many fluorescent dyes have been employed.[1,6] However, the fluidity-dependent shift of the fluorescence maxima associated with solvent relaxation during the lifetime of the excited state introduces potential difficulties in the estimation of the local effective polarity of the membrane, which is regarded as a small heterogeneous fluid system.[7,8]

In this study, microenvironments in phospholipid membranes (liposomes), in addition to surfactant micells, were evaluated from measurements of the visible spectra of three cationic dyes, Methylene Blue, 3,3′-dipropyl-2,2′-thiadicarbocyanine, and 9-aminoacridine. These dyes partition into the membrane phase and dimerize to exhibit remarkably large reductions in absorbance and fluorescence. The increases in the amount of membrane over the amount at which all dye molecules are dimerized in the membrane phase give rise to dilutions of the dyes in the membrane and, thereby, to restorations of the absorbance. The absorbance maxima of the dye monomer accommodated in the membranes, which are shifted from those in aqueous media, were translated into the effective polarities of the dye surroundings.

Experimental

Materials. Egg yolk phosphatidylcholine (PC) was prepared by the method of Singleton *et al.*[9] Bovine brain phosphatidylserine (PS) and L-α-dipalmitoylphosphatidic acid (PA) were obtained from the Sigma Chemical Co. The heptaethylene glycol monododecyl ether (HED) and sodium tetradecyl sulfate (STS) were purchased from the Nikko Chemicals Co., Ltd., and ICN Pharmaceuticals, Inc., respectively. The disodium hexadecyl phosphate (SHP) was synthesized by the method of Nelson.[10] The 9-aminoacridine (9-AA) and Methylene Blue (MB) were obtained from the Nakarai Chemicals Co., Ltd., and the 3,3′-dipropyl-2,2′-thiadicarbocyanine (diS-C_3(5)) was purchased from the Japanese Research Institute for Photosensitizing Dye Co., Ltd. All the solvents were distilled before use.

Preparation of Lipid Membrane (Liposome). Aliquots of phospholipid were removed from the stock solution in chloroform, after which the solvent was evaporated and dried under a vacuum for 15 h. The complete removal of the chloroform was essential. Trace amounts of the solvent remaining will introduce error in the spectroscopic measurement of dye in the membrane phase. The lipid was suspended in 1 mM (1 M = 1 mol dm^{-3}) Tris-HCl (pH 7.3) and dispersed ultrasonically for 20 min. The solvent was cooled in ice during this process. The ultrasonic device used was an UR-200P apparatus from the Tomy Seiko Co., Ltd. The ultrasonically dispersed lipid was then centrifuged for 30 min at 20000 g to remove the titanium dust from the ultrasonic tip and a small amount of untreated lipid. The lipid-membrane suspension was diluted with the 1 mM Tris-HCl buffer solution for measurement. The HED and STS micellar solutions were prepared in the 1 mM Tris-HCl buffer. For the SHP micellar solution, the pH was adjusted to 10.0 with a very dilute NaOH solution to prevent H^+ binding to the phosphate. The variation in the pH from 7.3 to 10.0 did not affect the absorption spectra of dyes in aqueous media.

Absorption Spectra. The spectra of dyes in an aqueous solution with phospholipid membranes (liposomes) or surfactant micelles were recorded with a Shimadzu UV-180 spectrophotometer. The amount of membrane composed

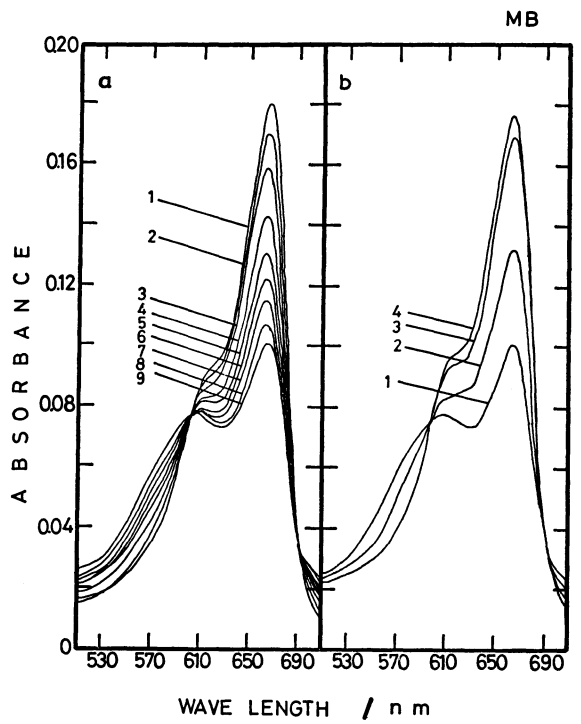

Fig. 1. (a) MB spectral change upon addition of PS membrane. The medium contains 1 mM Tris–HCl and 2 μM of MB at pH 7.3, 25 °C. The amount of (dried) lipid added (in μg/1 ml of medium): (1) 0; (2) 0.38; (3) 0.70; (4) 1.35; (5) 2.04; (6) 2.60; (7) 3.21; (8) 3.84; (9) 4.47. (1 μg PS/ml=1.4 μM PS). (b) MB spectral change upon addition of NaCl. 4.47 μg PS/ml (6.4 μM PS). The medium is the same as that on the (a) except for concentration of NaCl. [NaCl]: (1) 0; (2) 1; (3) 2; (4) 4 (in mM).

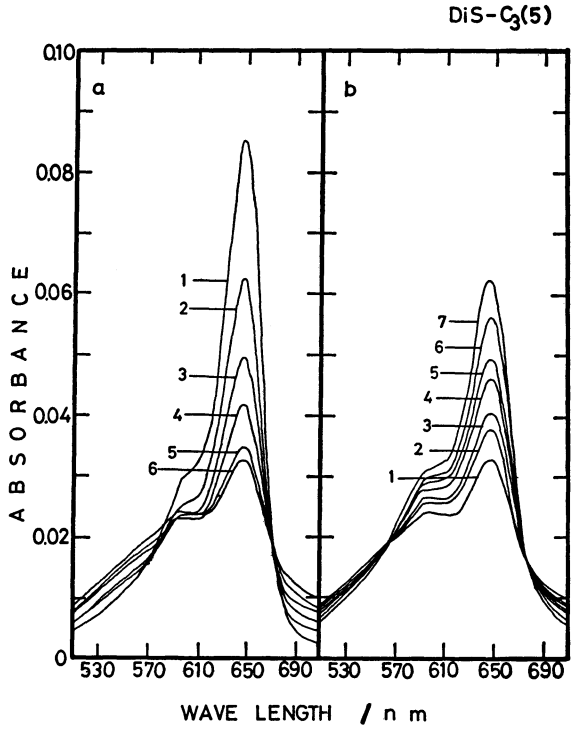

Fig. 2. (a) DiS-C_3(5) spectral change upon addition of PS membrane. The medium contains 1 mM Tris–HCl and 0.73 μM of diS-C_3(5) at pH 7.3, 25 °C. The amount of (dried) lipid added (in μg/1 ml of medium): (1) 0; (2) 0.06; (3) 0.126; (4) 0.190; (5) 0.252; (6) 0.315 (0.1 μg PS/ml=0.14 μM PS). (b) DiS-C_3(5) spectral change upon addition of NaCl. 0.315 μg PS/ml (0.45 μM PS). The medium is the same as that on the (a) except for the concentration of NaCl. [NaCl]: (1) 0; (2) 2; (3) 4; (4) 14; (5) 24; (6) 54; (7) 104 (in mM).

of an anionic phospholipid, PS or PA, in dye solutions was very small (less than 0.15 mg of the lipid in 1 ml of a medium). In the case of the PC membrane, the amout in solution went up to 8 mg in 1 ml of a medium, and the turbidity from the membrane gave rise to less accurate absorption spectra. Such data are marked in Table 1. The temperature was maintained constant at 25 °C by water circulation through the cuvet holder of the spectrophotometer. The dye concentrations of the MB, 9-AA, and diS-C_3(5) used were 2, 2, and 0.73 μM respectively, low enough for us to neglect the dimerizations in the aqueous phases.[11,12]

Results

Absorption Spectra of Cationic Dyes with Membranes and Micelles. In Figs. 1 and 2, the absorption spectra of MB (2 μM) and diS-C_3(5) (0.73 μM) in the buffer solutions with PS membranes are shown. It is shown that the monomer absorbance maxima of MB (664 nm) and diS-C_3(5) (648 nm) decrease with an increase in the amount of the lipid membrane. The dimer absorbance maxima of MB and diS-C_3(5) have been reported as 600 and 590 nm respectively.[11,12] Also, on an increase in the concentration of NaCl, the absorbances increased gradually. The 98% absorbance of MB is restored with 4 mM NaCl, while more than 100 mM NaCl recovered only 74% of the initial absorbance of diS-C_3(5). In the case of diS-C_3(5) with various amounts of PS (Fig. 2a), the small turbidity arising from the membrane (0.003 in absorption unit) disturbs the isosbestic point at 570 nm. Furthermore, the increases in the amount of the membrane beyond the amount at which the minimum absorbance was observed resulted in a gradual increase in the absorbance. The data for MB and diS-C_3(5) are shown in Fig. 3. As will be discussed later, the cationic dyes intensely bind to anionic lipid membranes or anionic surfactant micelles; the resulting enhancements in the concentrations of the dyes in the membrane phase prompt the formation of the dimer forms, which have lower extinction coefficients than the monomers. Therefore, an increase in the membrane amount beyond the amount at which almost all the dye molecules are in the membrane phase resulted in a gradual increase in the absorbance due to the decrease in the concentration of dyes in the membrane phase. The minimum absorbances of MB, diS-C_3(5), and 9-AA obtained with anionic membranes or micelles were 16, 25, and 36% of the initial absorbance values, which corresponded to the respective ratio of the extinction coefficients of a dimer to a monomeric dye and indicated that almost all the dye molecules formed dimers in the membrane or the

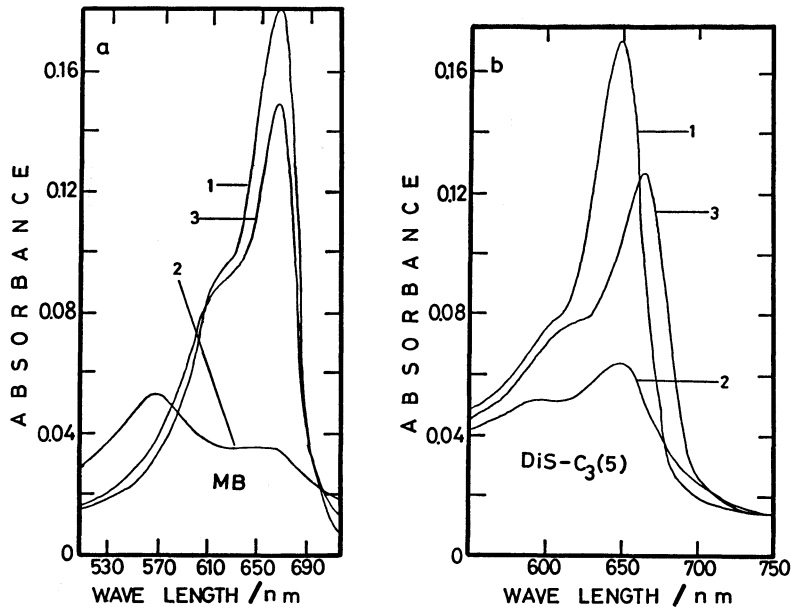

Fig. 3. Dye spectral change upon addition of PS membranes.
The medium contains 1 mM Tris–HCl at pH 7.3, 25 °C. (a) 2 μM MB with (1) 0; (2) 15; (3) 150 μg PS/ml. (b) 3 μM diS–C_3(5) with (1) 0; (2) 2.7; (3) 20 μg PS/ml. (1 μg PS/ml=1.4 μM PS).

micellar phases at the minimum points. Spectra 1 and 3 in Fig.3 are in the aqueous media and in the PS membranes respectively of MB and diS-C_3(5). In the case of MB, the wavelengths of the absorption maxima in an aqueous medium and in the membrane phase were very close, 664 and 662.8 nm. In contrast, diS-C_3(5) exhibited quite a large red shift of the absorption maximum in the membrane (648→664 nm).

In Table 1, the absorption maxima (in nm) of MB, diS-C_3(5), and 9-AA in anionic membranes and micelles (PS, PA, STS, and SHP) and in an electrically neutral membrane and micelle (PC and HED) are shown. Into the neutral membrane and micelle, the cationic dyes were not so intensely partitioned as to complete the dimerization. Therefore, we added amounts of the PC membrane and the HED micelle which were greater than the amounts at which the spectral shifts of dyes arose due to partitioning from aqueous media to the membrane or the micellar phases were complete. The accuracies of some absorption maximum values were judged to be less (±0.5 nm) than others (±0.2 nm), where appreciable turbidities from PC membranes were observed.

Solvent Effects on Absorption Spectra of the Cationic Dyes. The solvent effects on the visible spectra of MB, diS-C_3(5), and 9-AA were also studied. The absorption maxima (λ_{max}) of dyes obtained in various pure solvents and solvent mixtures are shown in Table 2. The polarities of solvents and solvent mixtures are characterized by their dielectric constants and also Reichardt-Dimorth's $E_T(30)$.[3b] The latter is empirically correlated with the solvatochromism of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)phenoxide at 25 °C. The dielectric constants of methanol-water mixtures have been known to be related to the weight fraction of methanol, w_{MeOH}, as $\varepsilon=78.5-(78.5-32.6)\,w_{MeOH}$.[13] DiS-$C_3$(5) and 9-AA showed monotonous increases in λ_{max} with a reduction of the solvent polarity, while MB has a minimum value around that of methanol. It was also found that the addition of concentrated NaCl (4—5 M) to the methanol–water

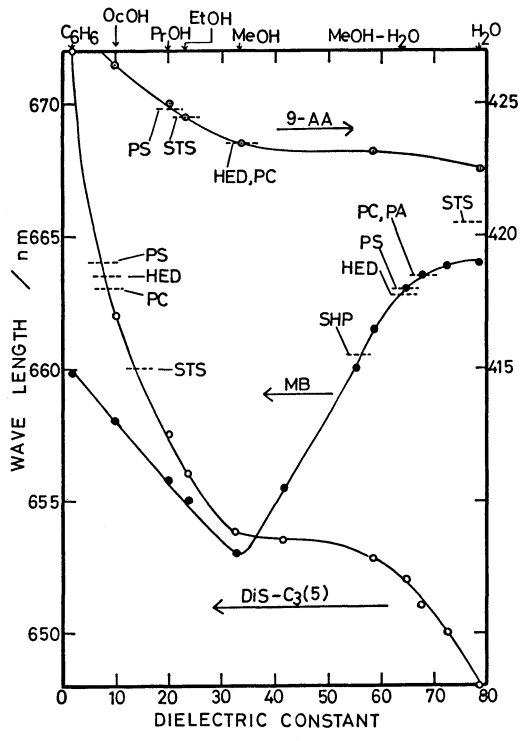

Fig. 4. Solvent effect on the wave length of absorption maximum of cationic dye monmer.
The values on the abscissa indicate dielectric constant of solvent. The wave lengths obtained in membranes and micelles are shown by the horizontal broken lines. The numerical values of effective dielectric constant estimated here are represented in Table 1.

TABLE 1. ABSORPTION MAXIMA, λ_{max}, OF CATIONIC DYE MONOMERS AND EFFECTIVE DIELECTRIC CONSTANTS, ε, IN MEMBRANES AND MICELLES

Membranes and Micelles	MB		DiS-C_3(5)		9-AA	
	λ_{max}/nm	ε	λ_{max}/nm	ε	λ_{max}/nm	ε
Phosphatidylcholine(PC) (11 mM)	663	68	663	8.5	423—424[a]	
Phosphatidylserine(PS) (0.03—0.21 mM)	662.8	65	664	7.5	424.8	20.5
Phosphatidicacid(PA) (0.03—0.21 mM)	663	68	663	8.5	424	27
Phosphatidicacid(PA) (0.21 mM, pH 10)	663	68				
Heptaethylene glycol monododecyl ether(HED) (20 mM)	663.5	63	663.5	8.0	423.6	33.5
Sodium tetradecyl sulfate(STS) (10 mM)	665.5[b]		660	13.5	424.5	23.0
Disodium hexadecyl Phosphate(SHP) (10 mM, pH 10)	661.5	56				

a) Less accurate because of appreciable turbidity. b) A close value of λ_{max} is obtained in an aqueous solution of 2 M NaCl. The concentrations of MB, diS-C_3(5), and 9-AA used are 2, 3, and 2×10^{-6} M respectively.

TABLE 2. SOLVENT EFFECTS ON THE ABSORPTION MAXIMA, λ_{max}, OF THE CATIONIC DYES

Solvent	Dielectric constant	$E_T(30)$ kcal mol^{-1} [d]	λ_{max}		
			MB	DiS-C_3(5)	9-AA
H_2O (1 mM Tris-HCl, pH 7.3)	78.5	63.1	664	648	422.5
Methanol	32.6	56.3	653	653.8	423.5
Ethanol	24.5	51.9	655	656	424.5
1-Propanol	20.1	50.7	655.8	657.5	425
1-Octanol	10.3		658	662	426.5
Benzene	2.3	34.5	659.8	672	428
Hexane	1.9	30.9	a)	675[b]	430[b]
Methanol-H_2O mixtures					
w_{MeOH}=0	78.5	63.1	664	648	422.5
0.13	72.6		663.8	650	
0.24	67.5		663.5	651	
0.30	64.8		663	652	
0.44	58.4		661.5	652.8	423.2
0.50	55.6		660		
0.56	52.8		658.5	653.3	
0.66	48.2		657.5	653.5	
0.79	41.8		655.5	653.5	
1	32.6	56.3	653	653.8	423.5
Methanol-aqueous 5 M NaCl mixtures					
w_{MeOH}=0	35		668	a)	423.5
0.13			669	653.5	424
0.24			668.5	654.5	424.2
0.30[c]			668	654.8	424.2
0.38[c]			665.5	655	424
0.44[c]			663.5	655	424

a) Insoluble. b) Rapid bleachings were observed. c) Methanol+aqueous 4 M NaCl. d) 1 cal=4.184 J.

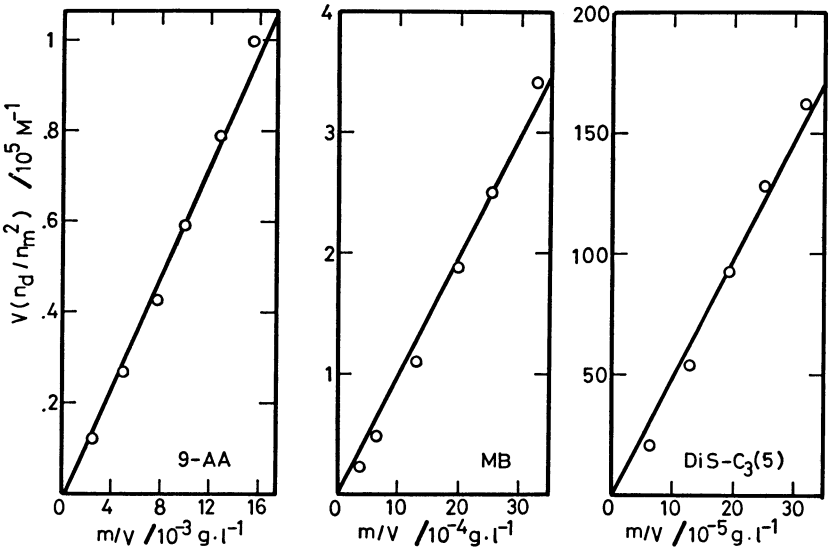

Fig. 5. Variations of $V(n_d/n_m^2)$ for 9-AA, MB, and diS-C_3(5) as a function of the concentration of PS membrane (m/V). See Eq. 9. (1×10^{-3} g PS/ml=1.4 mM PS).

mixtures gives rise to red shifts in the absorbance maxima of the cationic dyes used here. In Fig. 4, the λ_{max} values of dyes in membranes and micelles are compared with the values in solvents. The effective dielectric constants around dyes in micelles and membranes, ε, obtained in this figure are shown in Table 1.

Dimerization of Cationic Dyes in Membranes. The absorbance, A, of a cationic dye in an aqueous suspension of a lipid membrane is given by:

$$A = (\varepsilon_m n_m + 2\varepsilon_d n_d)/V, \quad (1)$$

where n_m and n_d are the moles of the dye monomer and the dimer, and where $n_m+2n_d=n_0$; here, n_0 is the total number of moles of the dye. V is the volume of the buffer solution containing the dye. The volume of the membrane phase, V_M, is very small and so can be neglected in Eq. 1. ε_m and ε_d are the extinction coefficients of the dye molecule in monomeric and dimeric forms. $\varepsilon_m/\varepsilon_d$ for MB at 664 nm has been established to be 0.157.[14] The $\varepsilon_m/\varepsilon_d$ values for diS-C_3(5) and 9-AA were estimated from the linear relations between the absorbance and fluorescence of the dyes in aqueous suspensions of anionic lipid membranes as 0.25 (at 648 nm) and 0.35 (at 422.5 nm) respectively (the dimers are nonfluorescent at the monomer fluorescence-maximum wavelength).[12] The absorbance of the same solution without a membrane, A_0, is given as:

$$A_0 = (\varepsilon_m n_0)/V. \quad (2)$$

From Eqs. 1 and 2, n_m and n_d are calculated as:

$$n_d = \frac{1}{2} n_0 (1-A/A_0)/(1-\varepsilon_d/\varepsilon_m), \quad (3)$$

and:

$$n_m = n_0 - 2n_d. \quad (3')$$

The equilibrium between monomeric and dimeric dyes in the membrane is represented by a dimerization constant, k_d, as:

$$C_{Md}/C_{Mm}^2 = k_d, \quad (4)$$

where C_{Md} and C_{Mm} are the concentrations of monomeric and dimeric dyes in the membrane phase. On the other hand, the partition equilibrium of a monomer between the membrane phase and the bulk phase can be written as:

$$C_{Mm}/C_{Bm} = P. \quad (5)$$

Here, P is a partition coefficient. From Eqs. 4 and 5, Eq. 6 is obtained:[15]

$$C_{Md}/C_{Bm}^2 = P^2 k_d. \quad (6)$$

Also:

$$C_{Md} = n_d/V_m = n_d/(vm). \quad (7)$$

Here, v is the partial specific volume of a phospholipid membrane, and m is the weight of a dried phospholipid in solution. Also,

$$C_{Bm} = (n_m - n_{Mm})/V \approx n_m/V. \quad (8)$$

n_{Mm} is the moles of the monomer in the membrane phase and is negligible when m is very small.[15] From Eqs. 6, 7, and 8, Eq. (9) is obtained:

$$V(n_d/n_m^2) = K(m/V), \quad \text{and } K = P^2 k_d v, \quad (9)$$

P involves the effects caused by both hydrophobic and electrostatic interactions between dye and membrane. When the pH and the ionic strength in an aqueous suspension of a PS membrane are maintained constant, P and, thereby, K can be regarded as constant for a dye.

In Fig. 5, the values of $V(n_d/n_m^2)$ calculated by means of Eq. 3 for an aqueous PS membrane suspension are plotted against the concentration of the membrane, m/V. The linear relations obtained for 9-AA, MB, and diS-C_3(5) are considered to be additional evidence for the formation of dye dimers in the membrane. Similar results were found for PA membrane and for STS, and SHP micelles.

Discussion

Microenvironments Provided by Membranes. To estimate the local effective polarities of membrane and

micelle on the basis of spectroscopic measurements with probing dyes, the data obtained for the membrane and the micelle should be interpreted in terms of some numerical indices representing the effective polarities of dye surroundings. Mainly, two factors can be proposed to account for the effective polarities in membranes and micelles: (a) the presence of a hydrocarbon environment in an aqueous medium, and (b) the presence of a high local concentration of charges arising from the head groups and the counter ions.[16] To estimate (a), alcohols and methanol–water mixtures have been considered to be better for the calibration solvents because of their hydrogen-bonding abilities with dyes.[16,22] Dipolar aprotic solvents have been found to be unsuitable for use with 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)phenoxide as the probing dye.[3a]

As is shown in Table 2 and Fig. 4, the simple rule for solvatochromism predicted from the change in the permanent dipole moment of the electronic transition, $\pi \rightarrow \pi^*$, cannot explain the experimental results. Alternatively, the interaction between the rapid-fluctuating transition moment of the dye molecule and the solvent is taken into account. The symmetric polymethin dyes, *e.g.*, diS-C_3(5), have been known to show very little defference in the permanent dipole moments of the ground and excited (Frank-Condon) states: therefore, the dispersive effect on the transition moment due to the dye-solvent interaction seems to exert the predominant effect on the solvatochromism of diS-C_3(5).[17] Thus, diS-C_3(5) has a blue shift with an increase in the solvent polarity. While the solvent effects on MB and 9-AA are substantially described by similar explanations, an anomalous minimum in the λ_{max} of MB around methanol must be understood in terms of the specific solvation of water molecules, *e.g.*, hydrogen bonding with MB or solvent-cage effects on the ground-state dipole moment of MB. The deviation in the absorption maximum of MB from linearity in methanol–water mixtures also suggests the preferential solvation of water molecules.

From the results shown in Tables 1, 2, and Fig. 4, it may be found that the local effective polarities sensed by diS-C_3(5) and MB are represented by those in 1-octanol and in a water–methanol mixture respectively. Therefore, diS-C_3(5) penetrates deeply into membranes and micelles and is located in quite nonpolar environments. This is compatible with the fact that the dye is able to permeate through cell and lipid membranes.[18] On the other hand, MB is thought to be located at polar environments, that is, the surface regions of membranes and micelles. It is noteworthy that the λ_{max} of MB in STS is largely red-shifted and not covered by the calibration solvents used here. In the outermost regions of membranes and micelles, in addition to water molecules, electrical charges arising from the head groups and the counter ions have important effects on the microenvironments. As may be seen in Table 2, the addition of concentrated NaCl to methanol–water mixtures resulted in red-shifts of the MB adsorptions, covering the MB spectrum in the STS micelle. This indicates that the MB molecules located at the surface region, apart from the effect of alkyl chains, really experience the effect from the (b) factor. The locations of the MB molecules evaluated here are also consistent with the fact that they cannot penetrate cell and lipid membranes.[14,15]

It is also noteworthy that, even when the bulk dielectric constants are close, the dyes used here exhibit reversed spectral shifts in a methanol (dielectric constant=32.6) and in a 5 M NaCl aqueous solution (dielectric constant=35[19]). The dielectric constant reflects only the average properties of the medium, while the microenvironment evaluated spectroscopically is to be understood as the effective polarity in the close vicinity of the probing dye molecule.[16] Very often, it has been found that there is no correlation between the dielectric constant and the rate of the equilibrium constant of a solvent-dependent chemical reaction[3b]

According to the oldest chemical rule that "like dissolves like," it is concluded that the rather hydrophobic dye, diS-C_3(5), and the hydrophilic dye, MB, prefer nonpolar and polar hydrophilic environments respectively, even in the same membrane or micelle.

Partition and Dimerization Equilibrium of Cationic Dye in Membrane. The cationic dyes employed in this study are intensely concentrated to dimerize in anionic membranes and micelles. Although the stacking interaction generally gives not only the dimer, but also *n*-meric forms, the isosbestic points observed in Figs. 1 and 2 suggest that the two major species, monomer and dimer, are in equilibrium and that the formation of higher aggregates is remarkably reduced in lipid membranes. Aggregations of dye ions have been known to occur much more favorably in aqueous environments than in nonaqueous environments. These facts have been interpreted in terms of water-structure promotion around the dye ions and consequent hydrophobic effects, and also in terms of the effect of the higher dielectric constant of water in reducing the repulsive force between the similarly charged dye ions in the aggregate.[11,20] These effects are more remarkable for the higher polymeric dye aggregates.

The effects of increasing amounts of NaCl on the absorbance of dyes shown in Figs. 1 and 2 suggest that the partitioning of an MB to a PS membrane is mainly attributable to electrostatic force, whereas, in the partitioning of diS-C_3(5), effects other than the electrostatic one seem to play an important role. Assuming that the intrinsic partition coefficients of the dyes to membranes are independent of the lipid composition, the surface potentials of the membranes composed of PC and PA or of PC and PS were estimated from the partition equilibria of the dyes and discussed in relation to the Göuy-Chapman double-layer theory.[15] The potential obtained with MB substantially agreed with the theory, while the potential change with the NaCl concentration, as estimated with diS-C_3(5), deviated greatly from the theory.[21] Also, Table 1 and Fig. 4 show that the surrounding microenvironments of MB in PC, PA, and PS membranes are similarly polar (ε=65—68). For diS-C_3(5), the microenvironments in PC, PA, and PS are found to be nonpolar (ε=7.5—8.5). These results indicate that

the hydrophobic interaction may be an important factor in the partitioning of diS-C_3(5) into the lipid membranes.

Structures of Micelle and Membrane. In many other studies, the effective polarities for ionic surfactant micelles have been evaluated to be in the range between 30 and 40 in terms of the dielectric constant, ε.[1-6,22,23] These results have been interpreted on the basis of Stigter's two-state model (a modification of the Hartley model)[22,24] or explained by the use of Menger's porous model, which is inconsistent with the former model.[23] The effective polarities estimated for micelles in this study show a remarkably great variation; $\varepsilon=11\pm3$ by diS-C_3(5), 28 ± 5 by 9-AA, and 60 ± 4 by MB. These facts suggest that, in surfactant micelles, considerably nonpolar environments exist; this is in conflict with the porous model. For a nonionic micelle (Triton X-100), a micropolarity with $\varepsilon=5$—8 has been reported.[25] Table 1 and Fig. 4 also indicate that STS, SHP, and HED micelles contain various polar environments, from quite polar ($\varepsilon=60$) to moderately polar ($\varepsilon=28$), along the radial direction and, again, in contradistinction to the two-state model on which a micelle is represented as a combination of a nonpolar interior ($\varepsilon<10$) and a thin polar surface layer ($\varepsilon=30$—40).[22,24] Therefore, the following model for micelles will be proposed in this study: that micelles contain both nonpolar and polar regions, and, that the polar regions have a finite thickness with a polarity gradient.

With respect to phospholipid membranes, fairly thick polar regions have been clarified by X-ray-diffraction experiments.[26] The results on the membranes in this study show that the lipid bilayer membranes also supply a wide variety of microenvironments, from polar to nonpolar, along the normal direction to the membrane surface.

These wide varieties in environment are considered to be an important effect provided by membranes and micelles for understanding chemical equilibria, kinetics, and structure organizations in these systems. We have also found that photoinduced charge separations mediated by thiacarbocyanine dyes in lipid membranes and micelles are remarkably affected by the natures of the microenvironments around the dye.[27]

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