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Fluorescence Behavior and Energy Transfer of Cyanine Dyes Bound to Bilayer Membranes of Double-Chain Ammonium Amphiphiles[1)]

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Negatively-charged cyanine dyes are bound specifically to aqueous bilayer membranes of double-chain ammonium amphiphiles, as reflected in their absorption spectra. The quantum yield of the fluorescence emission of a trimethine-thiacyanine dye is remarkably enhanced (up to 0.64) when the dye is bound to crystalline bilayer membranes of certain double-chain ammonium amphiphiles. The fluorescence intensity diminished drastically with the liquid crystalline bilayers. Efficient energy transfer is noted from an oxacyanine to the thiacyanine dye in the crystalline membrane matrix. The efficiency decreases by the membrane phase transition to the liquid crystalline state. These results are discussed in terms of specific dye binding and concentration of dyes at the membrane surface.

It has been shown by us and others that a large variety of synthetic amphiphiles produce bilayer aggregates spontaneously.[2-4)] Double-chain ammonium amphiphiles belong to a representative class of the bilayer-forming compounds. These synthetic bilayers possess the two-dimensional organization which is unique as sites for controlling aggregation and orientation of chromophores.

Chromophores can be introduced into bilayers as part of component molecules and as noncovalently adsorbed guest molecules. In the former case, unique spectral and related characteristics such as extraordinary enhancement of circular dichroism, large shifts in absorption spectra, and efficient energy transfer are observed for bilayers containing benzene,[5)] biphenyl,[6)] naphthalene,[7)] anthracene,[8)] azobenzene,[5,9-11)] carbazole,[12,13)] and viologen units[14,15)] as chromophores. The latter cases include membrane-bound dyes (Methyl Orange,[16)] cyanines and merocyanines,[17,18)] and phenylenediamine[19)]) of which spectra reflect specific modes of aggregation and orientation.

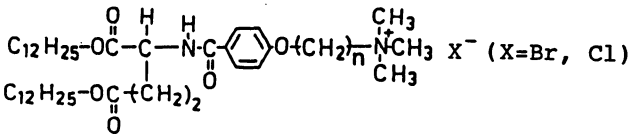
As an extention of these studies, we discuss in this paper the fluorescence behavior and the energy transfer of cyanine dyes bound to ammonium bilayer membranes. Cyanine dyes have attracted wide attention because of their characteristic photochemical and photophysical properties: as sensitizers in the photographic process,[20)] as spectroscopic probes for the physical state of the biological membrane,[21)] liposomes,[22,23)] and monolayers.[24,25)] The photophysical aspects of these dyes in highly organized bilayer assemblies pose an exciting topic, since it is known that specific aggregation and orientation of chlorophyll and carotenoid molecules play crucial roles in the efficient energy transfer in the biological photosynthetic machine.

The molecular structures of amphiphiles and dyes employed in this study are given in Scheme 1. We have reported preliminary results on the fluorescence characteristics of cyanine dye **5** bound to bilayer membranes of $2C_{12}$-L-Glu-phC_nN^+, (**1**, n=2 and 4) and $2C_{16}N^+2C_1$ (**4**).[18)] Amphiphiles **2** and **3** possess molecular structures closely related to **1**. The difference is in the connector portion which connects the double chain portion and the phenyl group. The glutamic acid unit is used as connector in **1**, while **2** and **3** contain aspartic acid and diethanolamine, respectively, as connector. These small structural differences lead to different binding modes of thiacyanine dye **5**. Oxacyanine dye **6** was employed to study the efficiency of energy transfer to **5** at the membrane surface.

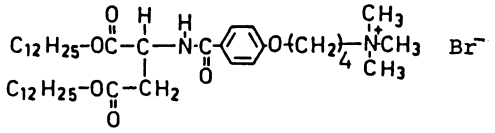
Experimental

Materials. Preparation of amphiphile **1** (n=4) was described before,[5)] and **1** (n=2 and 10), **2**, and **3** were obtained by similar procedures. The synthesis of the whole series will be discussed separately. The preparation of $2C_{16}N^+2C_1$ was given.[26)] Cyanine dyes **5** (NK 2012) and **6** (NK 1952) were purchased from Nippon Kanko Shikiso and used without further purification.

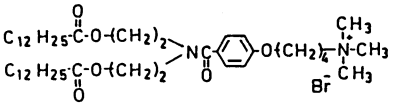
Measurement. Amphiphiles **1**—**4** suspended in distilled water were sonicated for 3 min (Branson Cell Disruptor 185, sonic power 40 W). The transparent dispersions obtained were incubated in ice water for 30 min. The dispersions were brought to given temperatures, aqueous dyes were added, and the mixtures were kept for 20 min. The 20-min aging at temperatures of the spectral measurement was required in order to secure reproducible spectra. This aging time may correspond to equilibration of dye molecules at the membrane surface. Absorption and emission spectra were measured with Hitachi 220 spectrophotometer and Hitachi 650-10S or 650-60 spectrofluorimeter, respectively. The 650-60 instrument possesses the correction function. The emission measurement was conducted under the aerated conditions, since no spectral difference was observed between the air saturated and N_2 saturated conditions. The excitation wavelength was set at the isosbestic point when the absorption spectrum showed temperature dependence. The quantum yield of emission of Rhodamin B (Wako Pure Chemical, recrystallized twice from ethanol, absorption maximum 542 nm, emission maximum 570 nm (corrected)) was assumed to be 0.5[27)] and used as the reference.



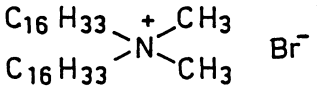
1, $2C_{12}$-L-Glu-ph-C_nN^+ (n=2, 4, 10)



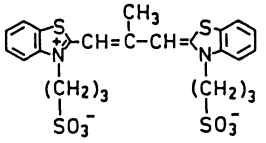
2, $2C_{12}$-L-Asp-ph-C_4N^+



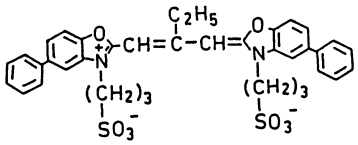
3, $2C_{12}$-Dea-ph-C_4N^+



4, $2C_{16}N^+2C_1$



5, thiacyanine dye



6, oxacyanine dye

Scheme 1.

Results and Discussion

Absorption and Fluorescence Spectra. Figure 1 displays temperature dependences of absorption and emission spectra of cyanine dye **5** bound to the bilayer matrix of $2C_{12}$-L-Glu-phC_4N^+ (**1**, n=4, X=Cl) at a molar ratio of 1/4000 (dye/bilayer). Both spectra show remarkable variations at a narrow temperature range of 27 to 29 °C. The spectral changes are small at lower temperatures (below 27 °C) and at higher temperatures (above 30 °C). The crystal-to-liquid crystal phase-transition temperature (T_c) of this bilayer is located at 31 °C (peak top temperature),[5] and the spectral variation is undoubtedly associated with the phase transition.

Similar results are found for other bilayers. The relative emission intensity (I_F) in different bilayers is plotted against temperature in Fig. 2. The dye concentration is very small (1/4000) relative to those of bilayers, and I_F is not affected by the dye concentration at this molar ratio (see below). Drastic I_F decreases are seen for the $2C_{12}$-L-Glu-phC_nN^+ (**1**) bilayers with different spacer lengths (n=2, 4, and 10) near the respective phase-transition temperatures: T_c=27 °C for n=2

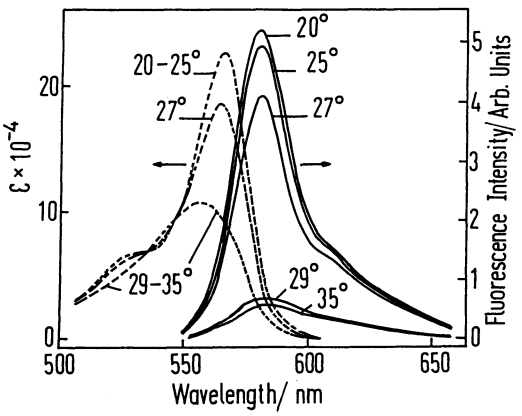


Fig. 1. Absorption (dotted line) and fluorescence (solid line, excitation at 545 nm) spectra of cyanine dye **5** (NK 2012) bound to the aqueous bilayer of $2C_{12}$-L-Glu-ph-C_4N^+ (**1**, n=4, X=Cl). [**5**]= 5.0×10^{-7} M.

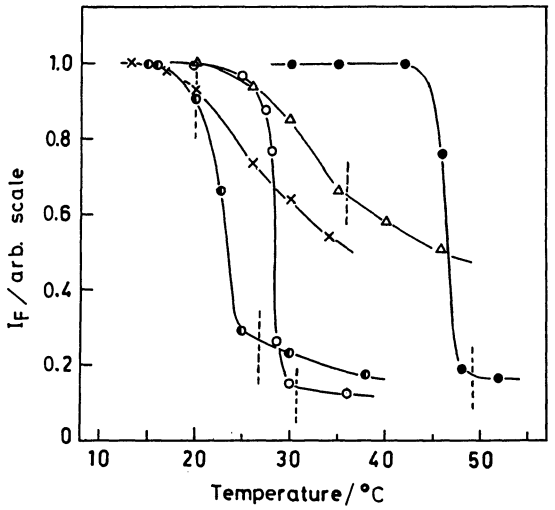


Fig. 2. Plots of I_F (arb. scale) of cyanine dye **5** against temperatures. [bilayer]=2.0×10^{-3} M, [**5**]=5.0×10^{-7} M. —◐—: 2C$_{12}$-L-Glu-phC$_2$N$^+$ (**1**, n=2), —○—: 2C$_{12}$-L-Glu-phC$_4$N$^+$ (**1**, n=4), —●—: 2C$_{12}$-L-Glu-phC$_{10}$N$^+$ (**1**, n=10), —△—: 2C$_{12}$-L-Asp-phC$_4$N$^+$ (**2**), —×—: 2C$_{12}$-Dea-phC$_4$N$^+$ (**3**). The emission intensity at 15 °C is taken as unity for the respective bilayer system. The dotted lines indicate the respective phase transition temperatures (peak top).

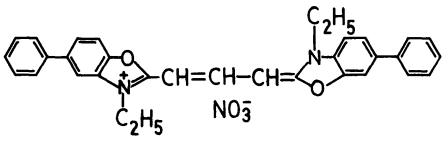
and 49 °C for n=10. The dependence on T_c is less drastic for bilayers **2** (T_c=36 °C) and **3** (T_c=20 °C). The temperature effect was also not remarkable for the bilayer of 2C$_{16}$N$^+$2C$_1$ (**4**) (data not shown). These different sensitivities clearly indicate that the mode of dye binding is strongly affected by the chemical structure of the bilayer component. In particular, the structures of 2C$_{12}$-L-Glu-phC$_4$N$^+$ (**1**, n=4) and 2C$_{12}$-L-Asp-phC$_4$N$^+$ (**2**) are different only by one CH$_2$ unit in the connector portion (glutamate vs. aspartate).

Klausner and Wolf[28] incorporated a series of long-chain (C$_{10}$ to C$_{22}$) cyanine dyes into lecithin vesicles and examined their fluorescence characteristics. However, the variation of I_F with the phase transition was very small.

Table 1. Quantum Yield for Cyanine Dye **5** in Various Media

Media	Φ_F		P	
	20 °C	35 °C	20 °C	35 °C
1 (n=2) (X=Br)	0.40	—	0.47	0.45
1 (n=4) (X=Cl)[a]	0.64	0.08	0.47	0.45
1 (n=6) (X=Br)	0.60	—	0.47	—
1 (n=10)(X=Br)	0.64	—	0.46	—
2	0.25	—	0.45	—
3	0.064	—	0.46	—
4	0.063	0.047	0.43	0.42
CTAC	0.035	0.019	0.39	0.39
Water	ca. 0.0025	ca. 0.0018	0.40	0.40
Methanol	ca. 0.0024	ca. 0.0017	0.39	0.38
Glycerol	0.24	—	0.47	—

a) Φ_F is almost the same when Cl is replaced by Br.



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Scheme 2.

Onuki et al.[29] recognized that the fluorescence intensity of double-chain cyanine dyes was modified by the phase transition of matrix liposomes, though a single-chain cyanine dye did not show such an effect.

The fluorescence quantum yields (Φ_F) of thiacyanine dye **5** in various media are summarized in Table 1. Φ_F is small in water and in methanol (ca. 0.0025), and is enhanced by a factor of 15 in the hexadecyltrimethylammonium chloride (CTAC) micelle. A similar Φ_F enhancement was reported by Grätzel and coworkers.[30] They found that the fluorescence intensity of cyanine dye **7** is enhanced 5 times in the sodium dodecyl sulfate micelle and 8 times in the cadmium lauryl sulfate micelle relative to that in methanol.

Greater Φ_F enhancements are observed in the bilayer system at $T<T_c$. Φ_F values in bilayers of 2C$_{12}$-L-Glu-phC$_n$N$^+$ are ca. 20 times greater than that in the CTAC micelle and ca. 250 times greater than those in water and alcohol. Large changes in Φ_F among bilayers are noteworthy. In spite of the structural similarity, 2C$_{12}$-L-Glu-phC$_4$N$^+$ and 2C$_{12}$-L-Asp-phC$_4$N$^+$ give a 10 fold difference. These data are again indicative of the specific nature of the binding mode. In fact, Φ_F is drastically diminished by the gel-to-liquid crystal phase transition of the matrix bilayer. The binding specificity appears lost in the liquid crystalline bilayer.

The Φ_F enhancement may be explained in terms of suppression of the nonradiative decay due to "rigidization" of the dye specifically bound to the membrane surface. The specific binding would constrain the conformation of the dye in such ways that large absorption shifts and fluorescence enhancements result.

The influence of the dye concentration on the spectral characteristics was subsequently examined. Cyanine dye **5** bound to the rigid 2C$_{12}$-L-Glu-phC$_4$N$^+$ bilayer gives an absorption maximum at 565 nm and an emission maximum at 580 nm at a molar ratio of 1/4000 (see Fig. 1). When the molar ratio is raised to 1/20, these maxima undergo red shifts by ca. 10 nm each: λ_{max} 574 nm (absorption) and 590 nm (emission). Figure 3 demonstrates the effect of the relative dye concentration on the fluorescence intensity at 20 °C (below the phase transition) and at 35 °C (above the phase transition). The absolute emission intensity is much smaller at 35 °C as shown in Fig. 2. The dye concentration is fixed at 5.0×10^{-6} M (1 M=1 mol dm^{-3}), while the bilayer concentration is varied. The relative emission intensity is constant at molar ratios of 1/1000 and below. The intensity is lessened at higher ratios, prob-

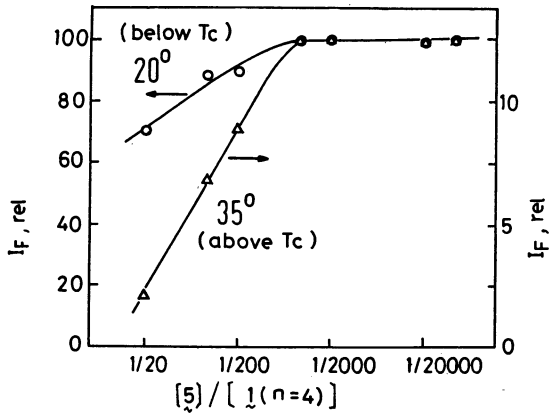

Fig. 3. Influence of the dye/bilayer molar ratio on the fluorescence intensity. [5]=5.0×10⁻⁶ M (constant). Bilayer, $2C_{12}$-L-Glu-phC_4N^+.

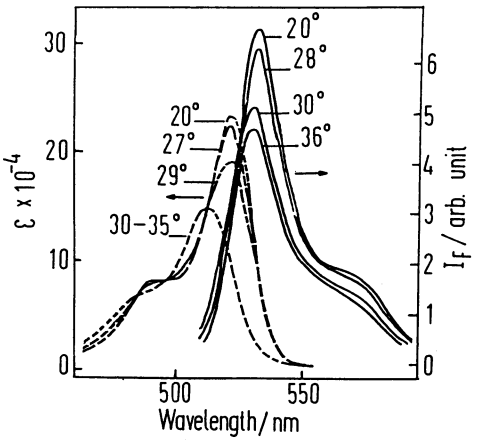

Fig. 4. Absorption (dotted line) and fluorescence (solid line, excitation at 500 nm) spectra of cyanine dye **6** bound to the aqueous bilayer of $2C_{12}$-L-Glu-phC_4N^+. [bilayer]=4.0×10⁻³ M, [dye]=1.0×10⁻⁶ M.

ably due to concentration quenching. The decrease is much larger in the crystalline bilayer, the intensity becoming ca. one third at the ratio of 1/20. This appears to be related to the enhanced energy transfer in the crystalline bilayer to be discussed later.

The emission intensity decreases drastically at T_c at the whole molar ratio range. The position of the descending line moves to higher temperatures with increasing dye concentrations. This is ascribed to the T_c rise of the dye-bound bilayer, as confirmed by the DSC measurement.[30] Similar trends were observed for absorption spectra and induced circular dichroism of this system.

The sharp emission with a small Stokes shift and the red shift in the absorption spectrum strongly suggest the formation of the J-like aggregates[20] for cyanine dye **5** concentrated in the bilayer matrix of $2C_{12}$-L-Glu-phC_nN^+. We have shown separately that many other cyanine and merocyanine dyes form the J-like aggregates in this bilayer matrix.[31] The latter dye aggregates similarly give rise to enhanced fluorescence emission.

On the other hand, a blue shift is observed in the absorption spectrum (λ_{max} 498 nm), when dye **5** is bound to the bilayer of $2C_{12}$-L-Asp-phC_4N^+. The fluorescence emission is barely recognized in this system. These spectral data are indicative of the formation of the H-aggregates.[20] It is interesting that the mode of the dye binding — orientation and aggregation — is quite different between bilayers with a minor structural variation (glutamate vs. aspartate). The bilayer physical state also plays a crucial role. The J-aggregates act as better photosensitizers. Therefore, it is expected that the migration and transfer of the excitation energy among bilayer-bound dyes are similarly affected by the variation of the binding mode.

Fluorescence Quenching and Energy Transfer. The energy transfer from an oxacyanine dye to a thiacyanine dye in the multilayer system was pioneered by Kuhn and Möbius.[24] We similarly employed an oxacyanine dye (**6**, NK 1952) as an energy donor in this study. Figure 4 snows absorption and emission spectra of **6** in the bilayer matrix of $2C_{12}$-L-Glu-phC_4N^+. The absorption maximum is located at 531 nm at 20 °C. As the temperature is raised, the maximum shifts to shorter wavelengths (511 nm at 30 °C) and the intensity decreases. The change is most drastic at 27 to 30 °C and is negligible at 30 to 35 °C. The phase transition of the bilayer is responsible for the spectral change, as also observed for bilayer-bound **5** (see Fig. 1). The emission maximum is located at 530 nm, and the intensity decreases with temperature. A comparison of Figs. 1 and 4 indicates that the emission spectrum of **6** overlap with the absorption range of **5**. Therefore, the Förster-type energy transfer from the oxacyanine to the thiacyanine is anticipated.

Figure 5 compares emission spectra of membrane-bound oxacyanine **6** alone and in the presence of equimolar (1.0×10⁻⁶ M) thiacyanine **5**. The emission of oxacyanine **6** (excitation at 500 nm) decreases in the presence of thiacyanine **5** and the emission from the latter is enhanced. This clearly indicates the energy transfer from **6** to **5**. The transfer efficiency is largest at 20—25 °C, and decreases with increasing temperatures, as can be estimated by the relative emission intensity at 530 nm and at 584 nm.

The decrease in the oxacyanine emission becomes pronounced with increasing concentrations of the thiacyanine acceptor. The quenching process can be represented by the Stern-Volmer relation

$$I_F^\circ/I_F - 1 = K_{sv}[A] \quad (1)$$

where I_F° and I_F are emission intensities of a donor dye in the absence and presence of acceptor dye A, respectively. K_{sv} is the Stern-Volmer quenching constant.

Figure 6 gives Stern-Volmer plots in the bilayer and micelle systems. The quenching process obeys Eq. 1.

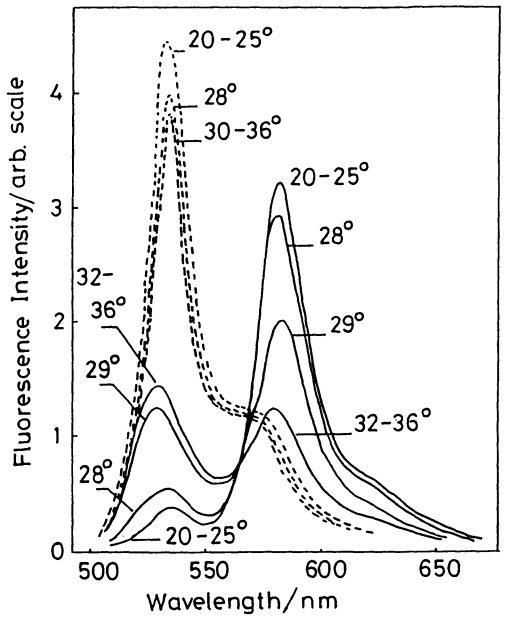

Fig. 5. Temperature dependence of fluorescence spectra of cyanine dyes (**5** or **6**) bound to the aqueous bilayer of $2C_{12}$-L-Glu-phC_4N^+. [bilayer]=1.0×10^{-3} M. Excitation at 500 nm. ----: Fluorescence spectra of **6** (1.0×10^{-6} M) alone. ——: Fluorescence spectra of **6** (1.0×10^{-6} M) plus **5** (1.0×10^{-6} M).

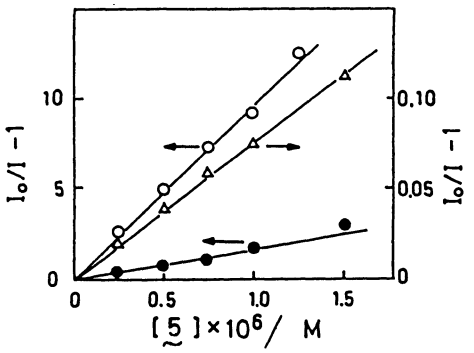

Fig. 6. Stern-Volmer plots for the emission quenching of cyanine dye **6** in the presence of dye **5**. [**6**]=1.0×10^{-6} M (constant). —○—: **1** (n=4), 1.0×10^{-3} M. —●—: **1** (n=4), 4.0×10^{-3} M. —△—: CTAB, 4.0×10^{-3} M.

The slopes in Fig. 6 give $K_{sv}=1.6\times10^6$ M^{-1} for the bilayer ($2C_{12}$-L-Glu-phC_4N^+) concentration of 4.0×10^{-3} M. The K_{sv} value is enhanced to 9.5×10^6 when the bilayer concentration is lowered to 1.0×10^{-3} M. In the CTAB micelle (4.0×10^{-3} M), $K_{sv}=7.5\times10^4$ M^{-1}, a value only 1/20 of the corresponding bilayer system. Thus the bilayer membrane provides a vehicle for energy transfer superior to the globular micelle. Concentration and specific binding of donor and acceptor dyes at the rigid bilayer surface must be responsible for the enhanced energy transfer.

Factors Affecting Fluorescence Quenching. It is apparent from Fig. 5 that the fluorescence quenching is strongly temperature-dependent. Figure 7 shows the

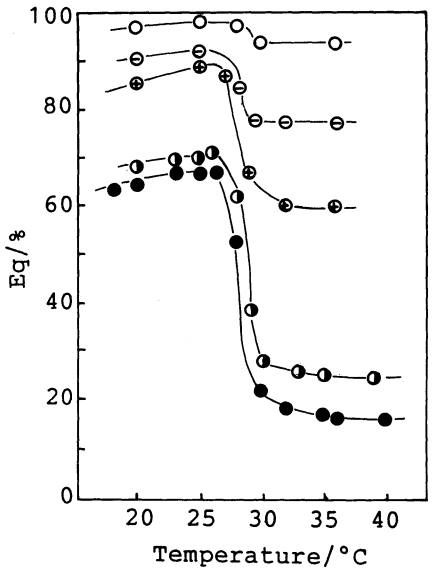

Fig. 7. Quenching efficiency (E_q) vs. temperature in the presence of the $2C_{12}$-L-Glu-phC_4N^+ bilayer. [**5**]=[**6**]=1.0×10^{-6} M. The bilayer concentration is as follows; —○—, 1.0×10^{-4} M; —⊖—, 5.0×10^{-4} M; —⊕—, 1.0×10^{-3} M; —◑—, 2.0×10^{-3} M; —●—, 4.0×10^{-3} M.

temperature dependence of the quenching efficiency

$$E_q = (1-I_F/I_F^\circ)\times 100\ (\%) \qquad (2)$$

at different concentrations of the $2C_{12}$-L-Glu-phC_4N^+ bilayer. E_q is reduced slightly at ca. 30 °C in the presence of 1×10^{-4} M of the bilayer, but remains almost unchanged at higher and lower temperature ranges. The reduction at 25—30 °C becomes drastic with increasing bilayer concentrations. In accordance with the spectral change in Fig. 5, the quenching is more efficient in the rigid bilayer matrix. The fluorescence quenching is well above 90% at all temperatures at the bilayer concentration of 1×10^{-4} M. A sonicated sample of $2C_{12}$-L-Glu-phC_4N^+ in water contains lamellar dispersions of which molecular weight cannot be less than ten millions.[31] We cannot at the moment distinguish the inter- and intra-aggregate processes. A crude estimate indicates that an average distance of the donor and acceptor dyes that are randomly distributed at the bilayer surface is 30—40 Å (the cross section of the bilayer component is 50—60 Å^2). The energy transfer via the Förster mechanism would proceed efficiently at this distance.

Reduction of the quenching efficiency with increasing bilayer concentrations is pronounced in the liquid crystalline region. For the crystalline bilayer, however, E_q is only reduced from 97 to 63% by the concentration change from 1×10^{-4} to 4×10^{-3} M. This reduction is too small to be accounted for by the concentration effect alone. It appears that E_q in the crystalline bilayer matrix remain high either due to the nonrandom distribution of dye molecules or due to the enhanced transfer rate. Absorption spectra of the dyes

bound to these bilayers display characteristic variations, implying that the modes (orientaion and aggregation) of dye binding are specific. This binding specificity may be responsible for these small concentration effects. The larger E_q reduction in the liquid crystalline bilayer matrix can be accounted for solely by the dilution effect.

The other bilayer matrices were similarly used for the quenching experiment, and the results are summarized in Fig. 8. The abrupt reduction in E_q is observed commonly for the bilayers of $2C_{12}$-L-Glu-phC_nN$^+$ (n=2, 4, and 10) at temperatures slightly below T_c (peak top temperature). However, the extent of the reduction is quite different. The difference is produced by an especially small quenching efficiency observed in the crystalline $2C_{12}$-L-Glu-phC_2N$^+$ bilayer. The binding mode in this bilayer appears not different from those in other bilayers of longer spacers, as the absorption spectrum of the bound cyanine is not sensitive to the spacer length. There could be changes in the mode of dye distribution that are not reflected in the absorption spectrum.

There are common trends in the T_c effect between the specificity of absorption spectra and the reduction of E_q. The $2C_{16}N^+2C_1$ bilayer which cannot bind dye molecules in specific manners,[31] gives almost constant E_q's at temperatures above and below T_c (28 °C). The fluid micelle of CTAB similarly gives temperature-independent E_q values. The smaller E_q value for CTAB (ca. 11%) relative to that of $2C_{16}N^+2C_1$ (ca. 21%) may reflect the smaller size of the CTAB micelle. E_q values in the fluid micelle and in the liquid crystalline bilayers are in the range of 10—20%. These data, together with the data shown in Fig. 7, unmistakably endorse the thesis that the specific nature of the dye binding enhance the efficiency of energy transfer.

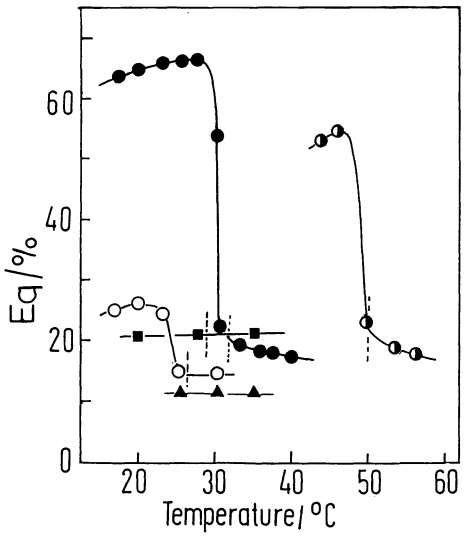

Fig. 8. Quenching efficiency (E_q) vs. temperatures in bilayers and micelles. [**5**]=[**6**]=1.0×10^{-6} M, [amphiphile]=4.0×10^{-3} M. —○—: $2C_{12}$-L-Glu-phC_2N$^+$. —●—: $2C_{12}$-L-Glu-phC_4N$^+$. —◑—: $2C_{12}$-L-Glu-phC_{10}N$^+$. —■—: $2C_{16}N^+2C_1$. —△—: CTAB. The dotted lines indicate the phase transition temperature (peak top) of the respective bilayer.

Conclusion

The present study leads to the following conclusions.

(1) The emission characteristics of anionic cyanine dyes bound to cationic bilayer membranes are strongly affected by the chemical structure and the physical state of the matrix membrane. The fluorescence emission is remarkably enhanced in crystalline bilayers of double-chain glutamate derivatives, and is characteristic of the J-like aggregate when the dye concentration is high. These unique spectral properties are related to binding of dyes to the specific space created at the membrane surface.

(2) Dye molecules concentrated at bilayer surfaces give rise to efficient energy transfer. The efficiency is affected by the nature of the matrix bilayer. The observed data imply that the energy flow in the bilayer can be regulated by appropriate disposition of dye molecules.

It is demonstrated that bilayer membranes provide superior media for organizing chromophores in specific manners.

References

1) Contribution No. 839 from Department of Organic Synthesis.

2) For our recent article; T. Kunitake, N. Kimizuka, N. Higashi, and N. Nakashima, *J. Am. Chem. Soc.*, **106**, 1978 (1984).

3) J. H. Fendler, "Membrane Mimetic Chemistry," Wiley-Interscience Pub., New York (1982).

4) J. H. Fuhrhop and J. Mathieu, *Angew. Chem., Int. Ed. Engl.*, **23**, 100 (1984).

5) T. Kunitake, N. Nakashima, M. Shimomura, Y. Okahata, K. Kano, and T. Ogawa, *J. Am. Chem. Soc.*, **102**, 6642 (1980).

6) T. Kunitake, N. Nakashima, and T. Morimitsu, *Chem. Lett.*, **1980**, 1347.

7) N. Nakashima, N. Kimizuka, and T. Kunitake, *Chem. Lett.*, **1985**, 1817.

8) M. Shimomura, H. Hashimoto, and T. Kunitake, *Chem. Lett.*, **1982**, 1285.

9) M. Shimomura, R. Ando, and T. Kuntiake, *Ber. Bunsenges. Phys. Chem.*, **87**, 1134 (1983).

10) N. Nakashima, K. Morimitsu, and T. Kunitake, *Bull. Chem. Soc. Jpn.*, **57**, 3253 (1984).

11) T. Kunitake, *Colloid and Surfaces*, **19**, 225 (1986).

12) T. Kunitake, M. Shimomura, Y. Hashiguchi, and T. Kawanaka, *J. Chem. Soc., Chem. Commun.*, **1985**, 833.

13) N. Takeyama, S. Sakaguchi, Y. Hashiguchi, M. Shimomura, T. Kunitake, and T. Matsuo, *Chem. Lett.*, **1985**, 1735.

14) E. Baumgartner and J. H. Fuhrhop, *Angew, Chem., Int. Ed. Engl.*, **19**, 550 (1980).

15) T. Nagamura, K. Tanaka, N. Takeyama, and T. Matsuo, *Ber. Bunsenges. Phys. Chem.*, **87**, 1129 (1983).

16) N. Nakashima, H. Fukushima, and T. Kunitake, *Chem. Lett.*, **1981**, 1555.
17) N. Nakashima, R. Ando, H. Fukushima, and T. Kunitake, *J. Chem. Soc., Chem. Commun.*, **1982**, 707.
18) N. Nakashima and T. Kunitake, *J. Am. Chem. Soc.*, **104**, 4261 (1982).
19) J. H. Fuhrhop and D. Fritsch, *J. Am. Chem. Soc.*, **106**, 4287 (1984).
20) e.g., A. H. Herz, *Adv. Colloid Interface Sci.*, **8**, 237 (1977).
21) A. S. Waggoner and A. Grinvald, *Ann. N. Y. Acad. Sci.*, **303**, 217 (1977).
22) K. Kurihara, Y. Toyoshima, and M. Sukigara, *J. Phys. Chem.*, **81**, 1833 (1977).
23) T, Handa, C. Ichibashi, I. Yamamoto, and M. Nakagaki, *Bull. Chem. Soc. Jpn.*, **56**, 2548 (1983).
24) H. Kuhn, D. Möbius, and H. Bücher, "Techniques of Chemistry," ed by A. Weissberger and B. W. Rossiter, Wiley (1972), Vol. 1. Part IIIB, p. 577.
25) D. Möbius, *Acc. Chem. Res.*, **14**, 63 (1981).
26) Y. Okahata, R. Ando, and T. Kunitake, *Ber. Bunsenges. Phys. Chem.*, **85**, 789 (1981).
27) T. Karsten and K. Kobs, *J. Phys. Chem.*, **84**, 1871 (1980).
28) R. D. Klausner and D. E. Wolf. *Biochem.*, **19**, 6199 (1980).
29) K. Onuki, K. Kurihara, Y. Toyoshima, and M. Sukigara, *Bull. Chem. Soc. Jpn.*, **53**, 1914 (1980).
30) R. Humphrey-Baker, M. Grätzel, and R. Steiger, *J. Am. Chem. Soc.*, **102**, 847 (1980).
31) N. Nakashima, T. Kunitake, et al. manuscript in preparation.