Excimer Formation and Phase Separation of Hydrocarbon and Fluorocarbon Bilayer Membranes[1)]

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The benzene chromophore contained in double-chain(hydrocarbon) ammonium amphiphiles gives both of monomer and excimer fluorescence in the fluid(liquid crystalline) bilayer organization. The excimer emission disappears by phase transition to the rigid(crystalline) bilayer. In contrast, the corresponding fluorocarbon bilayer, in the absence of phase transition phenomena, shows excimer emission at all temperatures(15—50 °C). The excimer emission decreases when these probe amphiphiles are diluted in matrix bilayers. These results are applied to study the phase separation behavior of hydrocarbon and fluorocarbon bilayer membranes. The fluorocarbon bilayer is not extensively miscible with the hydrocarbon bilayer, and *vice versa*. The miscibility is influenced additionally by the chemical structure of amphiphiles. The rate of mixing is determined by the component miscibility as well as by the aggregate morphology. The DSC study supports these conclusions and further indicates that an unsymmetrical, double-chain(one hydrocarbon and one fluorocarbon chains) amphiphile can promote mixing of typical hydrocarbon and fluorocarbon bilayers.

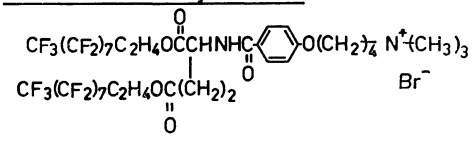
It was recently reported from these laboratories that bilayer membranes are spontaneously formed from single-chain and double-chain ammonium amphiphiles with perfluoroalkyl groups.[2)] These fluorocarbon membranes combine organized assemblage similar to that of the hydrocarbon bilayer membrane and peculiar physicochemical properties of fluorocarbon compounds. For example, the fluorocarbon bilayer vesicle was more stable and less permeable to some organic probes than the hydrocarbon counterpart.

One of the interesting characteristics of fluorocarbon micelles is their limited miscibility with the hydrocarbon micelle.[3-7)] It is therefore expected that phase separation is readily induced in mixed membranes of hydrocarbon and fluorocarbon amphiphiles. Phase separation plays important roles in regulation of the biomembrane function. In the case of the synthetic bilayer membrane, phase separation was used to detect the interaction of the surface receptor with added ions,[8)] and the rates of hydroxide- and imidazole-catalyzed cleavage of phenyl esters were affected by phase separation of reacting species in the bilayer matrix.[9,10)]

The excimer formation has been used to probe the distribution and orientation of component amphiphiles in bilayers. For example, phosphatidylcholine lipids which contain covalently-bound pyrene chromophore were prepared by Sunamoto and coworkers and the excimer formation in the liposomal matrix was discussed in terms of membrane organization.[11,12)] Nagamura *et al.* reported that dialkylammonium salts which contain the phenoxy moiety in the alkyl chain portion gave weak excimer emission in the bilayer state.[13)] We reported recently that anthracene-containing, single-chain amphiphiles form bilayer membranes and that strong excimer emission is observed when the amphiphile is buried in the dialkylammonium bilayer matrix.[14)]

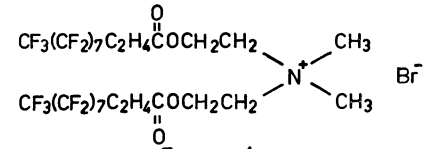
In the present paper, we describe our recent finding that double-chain ammonium amphiphiles which contain the benzene chromophore in the spacer portion

Fluorocarbon Bilayer Probe

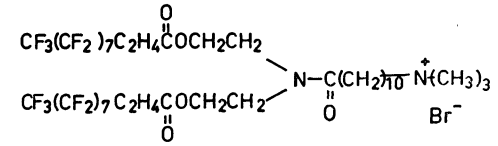


1, $2C_{10}^{F}$-L-Glu-ph-C_4-N^+

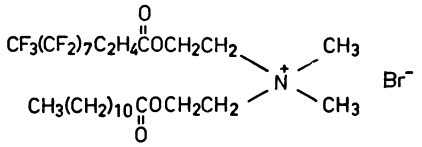
Fluorocarbon Bilayer Matrix



2, $2C_{11}^{F}$-de-N^+

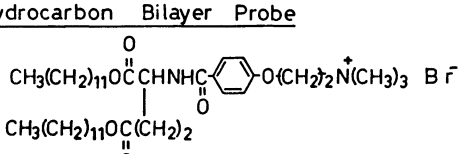


3, $2C_{11}^{F}$-de-C_{11}-N^+



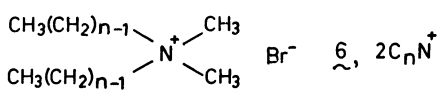
4, C_{11}^{F}, C_{12}-de-N^+

Hydrocarbon Bilayer Probe



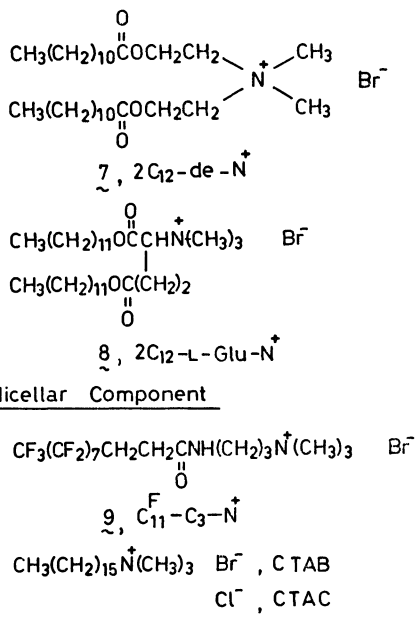
5, $2C_{12}$-L-Glu-ph-C_2-N^+

Hydrocarbon Bilayer Matrix



6, $2C_nN^+$

n=12, 14, 16



give strong excimer emission in the bilayer organization. Since the double-chain can be either hydrocarbon or fluorocarbon and since the excimer emission is highly dependent on the membrane physical state, these amphiphiles become very convenient probes for studying phase separation of hydrocarbon and fluorocarbon bilayers. The amphiphiles used are shown above together with their abbreviations.

Experimental

Fluorocarbon Amphiphile. 2*H*,2*H*,3*H*,3*H*-Perfluorundecanoic acid(24.6 g, 0.05 mol, Ugine Kuhlmann) was suspended in 18 g(0.15 mol) of thionyl chloride and the mixture was refluxed for 2 h and distilled *in vacuo*: colorless oil, yield 22.4 g(88%), bp 102—104 °C/13—14 mmHg(1 mmHg ≈133.3 Pa). The acid chloride(12 g, 0.024 mol) in 50 cm³ of $CHCl_3$ was added dropwise with stirring to an ice-cooled mixture of 1 g(8.4×10^{-3} mol) of commercial bis(2-hydroxyethyl)methylamine and 2 g(0.02 mol) of triethylamine in 100 cm³ of $CHCl_3$. The mixture was stirred further at room temperature for 1 h and at 40—50 °C for 24 h, and washed with water and aqueous Na_2CO_3 and dried over Na_2SO_4. The solvent was removed and the residue was distilled: colorless wax, yield 6.4 g(71%), bp 140—190 °C/0.03 mmHg. The formation of the diester was confirmed by IR and NMR spectroscopies. A large excess of CH_3Br was introduced to the diester(1 g, 9.4×10^{-4} mol) in 100 cm³ of tetrahydrofuran (THF). The mixture was stirred for 120 h at room temperature, and the precipitates formed were recrystallized from methanol and benzene: colorless flakes, yield 0.7 g(64%), mp 165—169.5 °C. The spectroscopic data(NMR and IR) were consistent with **2**. Found: C, 28.88; H, 1.87; N, 1.25%. Calcd for $C_{28}H_{22}NO_4F_{34}$: C, 28.93; H, 1.91; N, 1.20%.

A mixture of 4.5 g(0.042 mol) of commercial bis(2-hydroxyethyl)methylamine and 1.0 g(0.01 mol) of triethylamine in 100 cm³ of $CHCl_3$ was added dropwise to 5.0 g(0.01 mol) of the acid chloride in 50 cm³ of $CHCl_3$ with ice cooling. The mixture was then stirred at room temperature for 72 h, washed with water and aqueous Na_2CO_3, and dried. The solvent was removed, and toluene was added to the residual yellow oil, which was then kept in a refregerator. The colorless precipitates were recovered by quick separation, but they turned to colorless oil at room temperature: yield 3.0 g(51%). IR and NMR spectra showed the product to be the monoester. Undecanoyl chloride(1.3 g, 6.1×10^{-3} mol) in 40 cm³ of $CHCl_3$ was added dropwise to a solution of 3.0 g(5.1×10^{-3} mol) of the monoester and 0.6 g(6×10^{-3} mol) of triethylamine in 60 cm³ of $CHCl_3$ with ice cooling. The mixture was further stirred at 40 °C for 12 h, washed with water and aqueous $NaHCO_3$, and dried. The solvent was removed and colorless oil(TLC-FID, single peak) was recovered. A large excess of CH_3Br was introduced to 3.6 g(4.6×10^{-3} mol) of the product dissolved in 100 cm³ of dry THF, and the mixture stirred at room temperature for 24 h. The colorless precipitates were recrystallized from benzene, separated from the $CHCl_3$-insoluble by-product, and recrystallized again from benzene: colorless wax, yield 0.9 g (22%), mp 100→193 °C(the arrow indicates the liquid crystalline range). The structure (**4**) was confirmed by NMR spectroscopy. Found: C, 39.58; H, 4.74; N, 1.65%. Calcd for $C_{29}H_{41}F_{17}O_4NBr\cdot0.5H_2O$: C, 39.60; H, 4.81; N, 1.59%.

Amphiphiles **1**, **3**, and **9** were provided by S. Yasunami[2,15]

Hydrocarbon Amphiphile. *N*,*N*-bis[2-dodecanoyloxy)-ethyl]methylamine hydrochloride[15] (colorless powder, mp 105→120 °C)(2.5 g, 4.4×10^{-3} mol) in benzene was treated with aqueous Na_2CO_3 and dried. A large excess of CH_3Br gas was introduced to the solution and the mixture stirred at room temperature for 70 h. The colorless precipitates formed were recrystallized from ethyl acetate to give colorless powder of **7**: yield 1.4 g(55%), mp 173—175 °C. Found: C, 62.06; H, 10.38; N, 2.24%. Calcd for $C_{30}H_{60}O_4NBr$: C, 62.26; H, 10.45; N, 2.42%. Amphiphile **5** was provided by H. Fukushima.[16] Amphiphile **8** was described elsewhere.[19] The preparations of a series of dialkylammonium salts **6** were described briefly.[17,18] CTAB and CTAC are recrystallized from ethanol.

Miscellaneous. Electron micrographs were obtained with a Hitachi H-600 instrument, and fluorescence spectra were measured with a Hitachi 650-10S spectrofluorimeter. The degree of fluorescence polarization, P was obtained by Eq. 1.

$$P=\frac{I_{//\,//}-I_{//\perp}(I_{\perp//}/I_{\perp\perp})}{I_{//\,//}+I_{//\perp}(I_{\perp//}/I_{\perp\perp})}, \quad (1)$$

where $I_{//\,//}$ and $I_{\perp\perp}$ are emission measured with parallel polarizers(vertical and horizontal) and $I_{//\perp}$ and $I_{\perp//}$ are those measured with crossed polarizers.[20] UV-visible spectra were obtained with Hitachi spectrophotometers 200 and 220 A. Differential scanning calorimetry (DSC) was conducted for 20-mM samples with a Daini-Seikosha SSC/560 instrument. The temperature was raised from 0 °C at a rate of 2 °C/min.[21]

Results and Discussion

Aggregation Behavior. Figure 1 shows electron micrographs of $2C_{11}^F$-de-N^+, C_{11}^F,C_{12}-de-N^+, and $2C_{12}$-de-N^+. The sample preparation has been described before.[22] However, satisfactory staining was not attained by the previous method in the case of fluorocarbon membranes. In an improved procedure, crystalline membrane compounds and uranyl acetate were suspended in water and sonicated together. The bilayer structure(vesicle and lamella) is clearly observed for $2C_{11}^F$-de-N^+. C_{11}^F,C_{12}-de-N^+ gives very

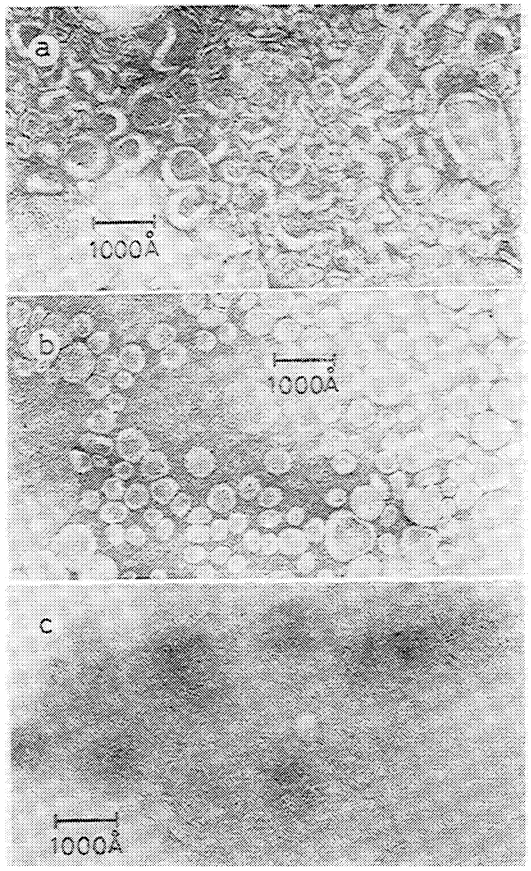


Fig. 1. Electron micrographs.
Sample 10 mM; initial magnification, ×30,000; stained by uranyl acetate, (a) $2C_{11}^{F}$-de-N^+ (b) C_{11}^{F}, C_{12}-de-N^+, (c) $2C_{12}$-de-N^+.

well dispersed, single-walled vesicles. The hydrocarbon counterpart, $2C_{12}$-de-N^+ gives multi-walled vesicles. These three compounds differ only in the long-chain portion(hydrocarbon or fluorocarbon). The bilayer organization is maintained when the hydrocarbon tail is replaced by the fluorocarbon tail of similar length, as mentioned previously.[2] However, it is interesting that the combination of hydrocarbon and fluorocarbon tails in an amphiphile gives single-walled vesicles that are much better dispersed than those of the bilayer aggregate of amphiphiles with two hydrocarbon(or fluorocarbon) tails.

All the other double-chain amphiphiles have been shown to form stable bilayer membranes.[2,15,16]

Excimer Emission of Hydrocarbon and Fluorocarbon Bilayers. Figure 2 describes fluorescence spectra of the aqueous bilayer of $2C_{12}$-L-Glu-ph-C_2N^+.[21] The excitation wavelength is set at 260 nm where the benzene ring absorbs. At 20 °C, the emission maximum appears at 320 nm, which is located close to that observed in methanol (312 nm). This peak is, therefore, attributed to the monomer emission. With rising temperature, this peak is lessened and a new, broader peak appears at 360 nm. The separation of the two peaks corresponds to *ca.* 4000 cm^{-1}, and this extent of energy difference is typical of the excimer formation.[24] The 360-nm peak shifts completely to 320 nm by addition of the CTAB micelle (2 mM), in consistence with excimer emission.

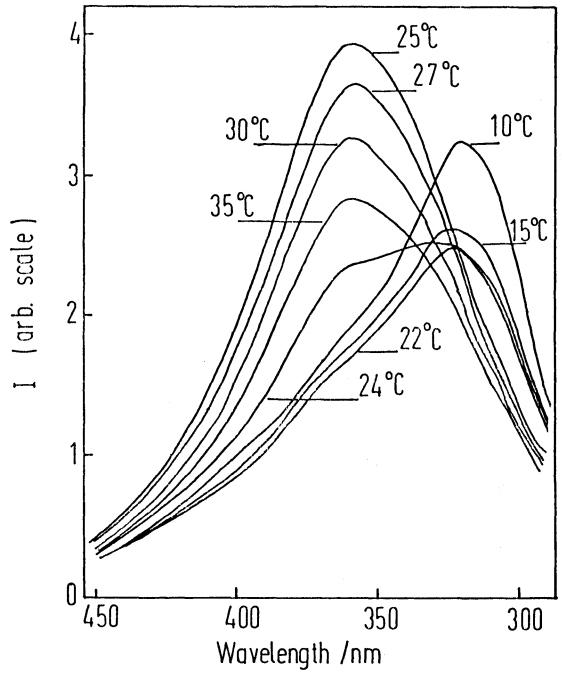


Fig. 2. Temperature dependence of fluorescence spectra of the aqueous $2C_{12}$-L-Glu-ph-C_2N^+ bilayer.
$2C_{12}$-L-Glu-ph-C_2N^+, 1.0×10^{-4} M, excitation at 260 nm.

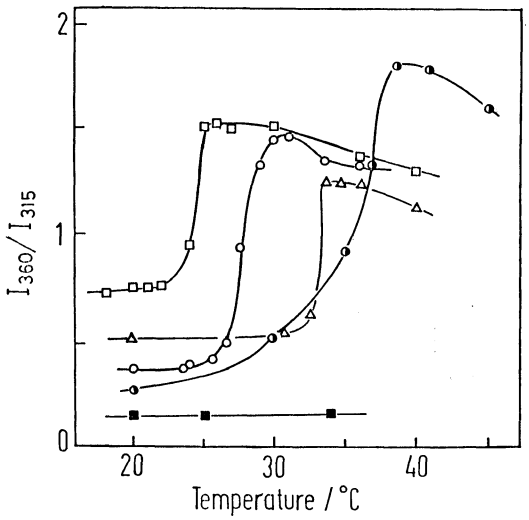


Fig. 3. Temperature dependence of I_{360}/I_{315} of hydrocarbon probes in water and in methanol.
[Amphiphiles] = 1.0×10^{-4} M.
—□—: $2C_{12}$-L-Glu-phC_2N^+ bilayer in water, —○—: $2C_{12}$-L-Glu-ph-C_4N^+ bilayer, in water, —◑—: $2C_{12}$-L-Glu-ph-C_6N^+ bilayer in water, —△—: $2C_{12}$-L-Asp-ph-C_4N^+ bilayer in water, —■—: $2C_{12}$-L-Glu-phC_2N^+ in methanol.

Figure 3 illustrates the temperature dependence of the ratio of fluorescence intensity at 360 and 315 nm. In the case of $2C_{12}$-L-Glu-ph-C_2N^+, this value increases rather abruptly in a narrow temperature range of 23 to 25 °C, and then gradually decreases with temperature. The maximum is located in the phase transition region: T_c (peak top temperature in the DSC thermogram)=27 °C. The I_{360}/I_{315} value in

methanol is smaller(0.15) and temperature-independent, as expected. Similar situations(excimer formation and temperature dependence) are found for other probe membranes in which the spacer length is different or the glutamate connector is replaced by the aspartate connector. The maxima exist more clearly in the case of the latter probes. It is interesting that the excimer emission is most enhanced with partially fluid membranes. Apparently the spatial arrangement of the neighboring benzene ring in the rigid bilayer is not suited for excimer formation.

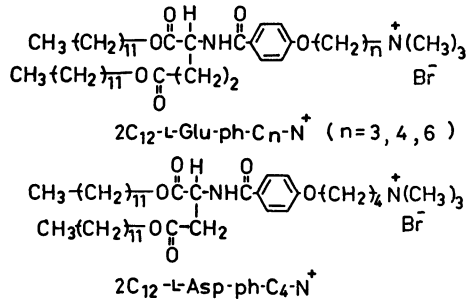


Figure 4 shows examples of the variation of fluorescence polarization(P) with temperature. In the case of a methanol solution in which $2C_{12}$–L-Glu–ph-C_4–N^+ is molecularly dispersed, P is 0.31 and temperature-independent. In the corresponding bilayer system, the emission at 310 nm gives a constant P value(0.43) in all temperature range, whereas P of the 360-nm emission changes in the phase transition region from 0.42 (low temperature) to 0.20(high temperature). The two P values (0.42 and 0.20) are mainly associated with the monomer and excimer emissions, respectively. The high P value observed for the monomer emission implies that this emission is derived from the benzene chromophore of fixed orientation. The lowered P value of the excimer emission is indicative of lessened orientation characteristic of the excimer. Similar results are found for $2C_{12}$–L-Glu–ph-C_2–N^+.

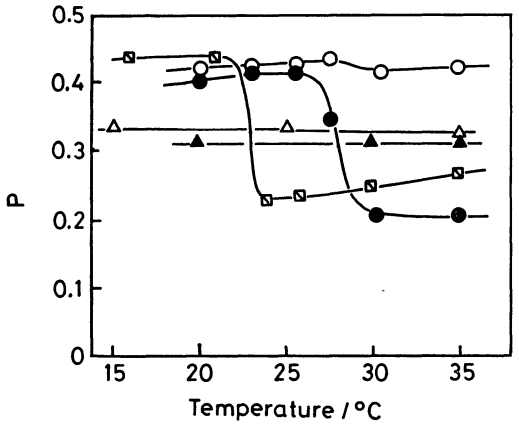


Fig. 4. Temperature dependence of the fluorescence polarization (P).
Sample, 1.0×10^{-4} M.
—□—: $2C_{12}$-L-Glu-ph-C_2-N bilayer (Ex 265 nm, Em 360 nm), —○—: $2C_{12}$-L-Glu-ph-C_4N^+ bilayer (Ex 265 nm, Em 310 nm) —●—: $2C_{12}$-L-Glu-ph-C_4N^+ bilayer (Ex 265 nm, Em 360 nm), —△—: $2C_{12}$-L-Glu-ph-C_2N^+ in methanol (Ex 265 nm, Em 310 nm), —▲—: $2C_{12}$-L-Glu-phC_4N^+ in methanol (Ex 265 nm, Em 310 nm).

The fluorescence behavior of the corresponding fluorocarbon bilayer is not the same. The spectrum of the $2C^F_{10}$–L-Glu–ph-C_4–N^+ bilayer possesses a maximum at 365 nm with a shoulder at *ca.* 320 nm. This spectrum does not change in the temperature range examined (15—50 °C), in consistence with the lack of major changes in the physical state(no DSC peak observed). The I_{365}/I_{320} value decreases linearly with temperature: 1.8 at 15 °C and 1.3 at 45 °C. Apparently the excimer emission is predominant at all temperatures. The excimer emission is suppressed when the fluorocarbon probe is diluted by a large excess of a fluorocarbon membrane matrix. The pure monomer peak (320 nm) is observed when $2C^F_{10}$–L-Glu–ph-C_4N^+ is imbedded in 50 times excess of $2C^F_{11}$–de-N^+. The degree of polarization of the excimer peak (365 nm) was 0.11—0.13 at 14 to 47 °C, and that of the monomer peak (320 nm) was 0.3 in the same range.

Comparison of Excimer Formation of Hydrocarbon and Fluorocarbon Bilayers. A comparison of the fluorescence behavior(excimer formation) between the hydrocarbon and fluorocarbon probes is interesting. The excimer formation is determined by the distance and orientation of the two chromophore. The X-ray diffraction data indicate that the interplanar distance is 3.2 Å for typical excimer-forming pairs of the chromophore.[24] The bilayer membrane of the hydrocarbon probe gives strong circular dichroism (CD) enhancement due to the exciton coupling, if in the crystalline state.[25] This implies that there exist a long-range interaction among the aromatic chromophores due to their fixed orientation. However, the excimer formation is not observed for these rigid membranes. Apparently, the spatial arrangement of the fixed chromophore is not suitable in this case for excimer formation. Instead, the excimer is formed much more readily for partially or fully liquid-crystalline bilayers. The CD enhancement is not observed in the same temperature ranges.

The excimer-formation behavior of the fluorocarbon probe resembles that of the partially fluid hydrocarbon probe at all temperatures. This result, together with the lack of phase transition, suggests that the fluorocarbon probe maintains fluidity required for excimer formation in the whole temperature range. This is consistent with the absence of the CD enhancement for this probe.[26] The excimer naturally disappears upon dilution with a nonfluorescent fluorocarbon bilayer. It is well known that the intermolecular interaction of fluorocarbons is much smaller than that of the corresponding hydrocarbon. This difference can explain the fluorescence behavior of the two kinds of the membrane.

Phase Separation as Estimated by Fluorescent Probe. It is clear from the above results that the excimer emission is related to the formation of the probe cluster. Phase separation of hydrocarbon and fluorocarbon membranes was subsequently examined by using fluo-

Table 1. Phase separation of fluorocarbon probe

	Hydrocarbon matrix			Fluorocarbon matrix		
	$2C_{16}N^+$	$2C_{12}$-L-Glu-N^+	$2C_{12}$-de-N^+	$2C^F_{11}$-de-N^+	C^F_{11},C_{12}-de-N^+	$2C^F_{11}$-de-C_{11}-N^+
I_{365}/I_{320}	1.09	0.98	0.90	0.46	0.64	0.71
Cluster(%)	86	73	63	∞	30	39

$(I_{365}/I_{320})_{monomer}$=0.40. $(I_{365}/I_{320})_{cluster}$=1.20, [matrix]=1.0 mM, [probe]=0.1 mM, 33 °C.

Table 2. Phase separation of hydrocarbon probe

	Hydrocarbon matrix	Fluorocarbon matrix				
	$2C_{12}$-de-N^+ 1 mM	$2C^F_{11}$-de-N^+ 1 mM	C^F_{11},C_{12}-de-N^+			
			0.1 mM	1 mM	5 mM	10 mM
I_{355}/I_{320}	0.52	0.84	1.20	0.71	0.66	0.61
Cluster(%)	2	36	74	22	17	12

[Probe]=0.1 mM, 33 °C. $(I_{355}/I_{320})_{monomer}$=0.50, $(I_{355}/I_{320})_{cluster}$=1.45.

rocarbon probe $2C^F_{10}$-L-Glu-ph-C_4-N^+. Matrix (1 mM) and probe (0.1 mM) were cosonicated for 1 min to give clear solutions. The I_{365}/I_{320} values of the mixed membrane thus obtained did not virtually change with time.

The amount of the probe cluster(*i.e.*, extent of phase separation) is estimated by

$$\text{cluster}(\%) = \frac{(I_{365}/I_{320})_{obsd} - (I_{365}/I_{320})_{monomer}}{(I_{365}/I_{320})_{cluster} - (I_{365}/I_{320})_{monomer}} \times 100, \quad (2)$$

where I_{365}/I_{320} for cluster and monomer are obtainable from Fig. 5.

Table 1 summarizes the phase separation data of the fluorocarbon probe. In the case of the hydrocarbon matrices, most of the probe molecule are in the cluster, $2C_{16}N^+$ being least miscible. The fluorocarbon matrix is more miscible with the fluorocarbon probe, as expected. $2C^F_{11}$-de-N^+ is best miscible, as also shown in Fig. 5. The I_{365}/I_{320} value decreases linearly with decreasing molar ratios of the probe and matrix, and reaches a constant value (cluster disappears) when more than 10 times matrix molecules are present. Unsymmetrical double-chain matrix C^F_{11},C_{12}-de-N^+ solubilizes the probe less efficiently. It is interesting that a considerable amount of the probe cluster remains in the double fluoroalkyl matrix of $2C^F_{11}$-de-C_{11}-N^+. Apparently, the presence of the hydrocarbon spacer, $(CH_2)_{10}$, is responsible for this result.

The influence of the micellar matrix is similar to that of the membrane matrix. Thus, the probe (0.1 mM) exists predominantly as monomer (90%) in the fluorocarbon micelle of C^F_{11}-C_3-N^+ (10 mM). The amount of the cluster increases in CTAB (56%).

Subsequently, phase separation was examined by using hydrocarbon probe, $2C_{12}$-L-Glu-ph-C_2-N^+. This probe was selected because of its low T_c. The experimental procedures were the same as in the case of the fluorocarbon probe. The temperature used (33 °C) is higher than T_c of the probe membrane, so

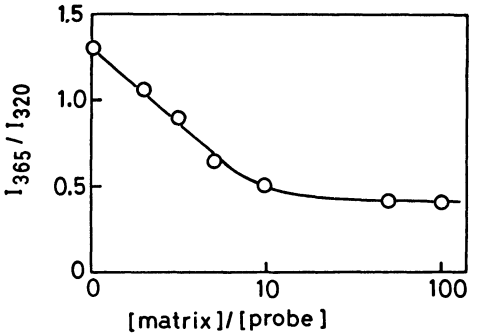


Fig. 5. Influence of the molar ratio ([matrix]/[probe]) on the relative excimer emission, I_{365}/I_{320}. 33 °C; $2C^F_{10}$-L-Glu-ph-C_4N^+ probe, 1.0×10^{-4} M; $2C^F_{11}$-de-N^+ matrix, 1×10^{-4}—1×10^{-2} M.

that the excimer formation is assured if the cluster is formed. The fluorescence intensities at 355 and 320 nm were compared, and the results are given in Table 2. The probe molecule exists almost completely as monomer in the hydrocarbon matrix (10 times excess) of $2C_{12}$-de-N^+. The cluster is formed more readily in the fluorocarbon matrix of $2C^F_{11}$-de-N^+ than in C^F_{11},C_{12}-de-N^+, and in the latter matrix, the cluster decreases with increasing matrix concentrations.

Membrane Mixing as Examined by Fluorescent Probe. Fusion and/or mixing processes of the bilayer aggregate can be followed by using the excimer emission. Sonicated solutions (0.9 cm^3) of the matrix membrane were aged in quartz cells for 30 min at 33 °C, and then sonicated solutions (0.1 cm^3) of the probe membrane were added. Figure 6 illustrates the decrease of I_{365}/I_{320} of the fluorocarbon probe with time in the fluorocarbon matrix. Mixing of the probe membrane with the C^F_{11},C_{12}-de-N^+ membrane proceeds more smoothly than with $2C^F_{11}$-de-N^+. This result is strange at first glance, since the extent of phase separation of the probe after sonication is larger with C^F_{11},C_{12}-de-N^+ than with $2C^F_{11}$-de-N^+ (Table 1). The mixing rate

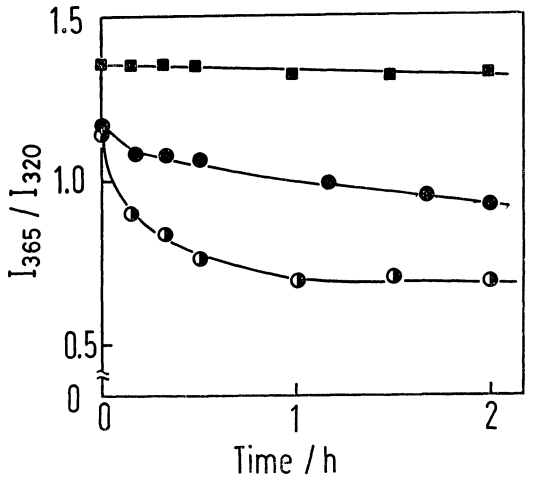


Fig. 6. Time course of I_{365}/I_{320} of the fluorocarbon bilayer probe upon mixing with fluorocarbon bilayer matrices.
33 °C; probe, 1.0×10^{-4} M; matrix, 1.0×10^{-3} M.
—■—: $2C_{11}^{F}$-de-$C_{11}N^{+}$ matrix, —●—: $2C_{11}^{F}$-de-N^{+} matrix, —◑—: C_{11}^{F},C_{12}-de-N^{+} matrix.

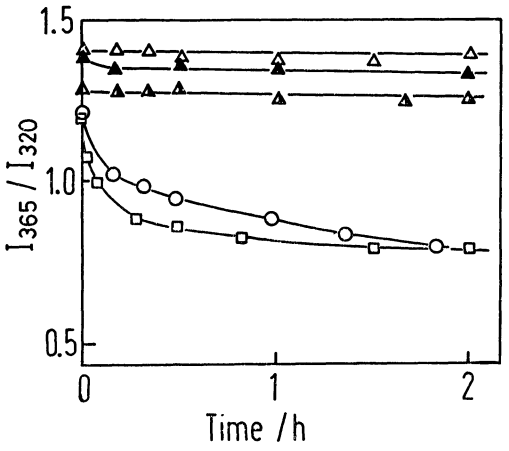


Fig. 7. Time course of I_{365}/I_{320} of the fluorocarbon bilayer probe upon mixing with hydrocarbon bilayer matrices.
33 °C; probe, 1.0×10^{-4} M; matrix, 1.0×10^{-3} M.
—△—, $2C_{12}N^{+}$; —▲—, $2C_{14}N^{+}$; —◭—, $2C_{16}N^{+}$; —○—, $2C_{12}$-L-Glu-N^{+}; —□—, $2C_{12}$-de-N^{+}.

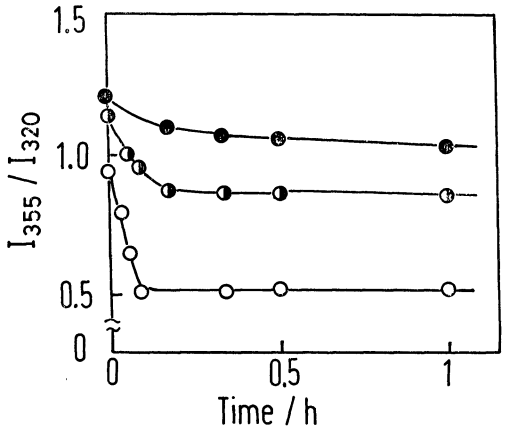


Fig. 8. Time course of I_{355}/I_{320} of the hydrocarbon bilayer probe upon mixing with hydrocarbon bilayer matrices.
33 °C; probe, 1.0×10^{-4} M; matrix, 1.0×10^{-4} M.
—●—: $2C_{11}^{F}$-de-N^{+} matrix. —◐—: C_{11}^{F},C_{12}-de-N^{+} matrix. —○—: $2C_{12}$-de-N^{+} matrix.

is determined by the kinetic and thermodynamic factors. Since the thermodynamic factor can be inferred from the extent of phase separation after sonication, the discrepancy may be associated with the kinetic factor. Electron microscopy indicates that the aqueous aggregate of C_{11}^{F},C_{12}–de-N^{+} is much better dispersed (single-walled vesicle) than that of $2C_{11}^{F}$–de-N^{+} (lamella and vesicle). Therefore, the initial mixing with the probe membrane would be more efficient in the case of C_{11}^{F},C_{12}–de-N^{+}.

Mixing of the fluorocarbon probe with $2C_{11}^{F}$–de-C_{11}–N^{+} is very slow in spite of the fact that the two compounds possess the same fluoroalkyl chain in common. This result reflects the fact that $2C_{11}^{F}$–de-C_{11}–N^{+} does not mix well with the fluorocarbon probe probably because of the hydrocarbon spacer (Table 1). The morphological characteristics of $2C_{11}^{F}$–de-C_{11}–N^{+} (lamella)[15] may make the mixing even more sluggish.

Figure 7 displays the mixing process of the fluorocarbon probe and hydrocarbon matrices. The mixing with the dialkylammonium membranes is very slow, as anticipated from their extensive phase separation (Table 1). The mixing with the hydrocarbon membranes of $2C_{12}$–L-Glu–N^{+} and $2C_{12}$–de-N^{+} is unexpectedly facile, and comes close to the equilibrated phase separation of Table 1 within 1—2 h. The contact of probe and matrix must be efficient because of the morphologies of the latters.

Similar mixing experiments were carried out for the hydrocarbon probe (Fig. 8). As expected, this probe is readily mixed with the hydrocarbon matrix of $2C_{12}$–de-N^{+}. The miscibility is lessened with increasing fluoroalkyl contents. The I_{355}/I_{320} values become virtually constant in 15 min. The final values in the fluorocarbon matrix are larger than those obtained upon sonication. Therefore, complete mixing of the hydrocarbon probe and the fluorocarbon matrices cannot be attained without sonication.

DSC Study of Membrane Mixing. Synthetic bilayer membranes of dialkyl amphiphiles usually give DSC peaks due to the crystal-to-liquid crystal phase transition.[18] Therefore, the DSC technique can be conveniently applied to the study of the phase separation behavior of multi-component membranes when the spectroscopic probe is absent.

Three double-chain amphiphiles of closely related structures were selected for this purpose. Multi-component samples were prepared by sonication or simple mixing. Clear aqueous solutions were obtained by sonication of multi-component suspensions (20 mM each) in the sonication procedure, and the separately-prepared(by sonication) solutions were mixed without sonication in the simple-mixing procedure. The former procedure gives information on the final state of mixing, and the latter data are related to the mixing process.

Figure 9 illustrates the variation of the DSC peak of $2C_{12}$–de-N^{+}. The single-component sample gives a

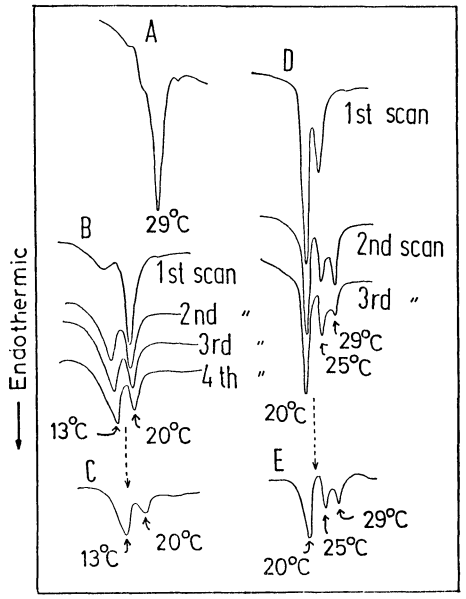


Fig. 9. DSC thermograms of the $2C_{12}$-de-N^+ bilayer alone and in combinations with related bilayers.
A, $2C_{12}$-de-N^+ alone (20 mM). B, an equimolar mixture with C_{11}^F,C_{12}-de-N^+. The sample was prepared by mixing of the two solutions without sonication. C, the same sample as B after sonication. D, an equimolar mixture with $2C_{11}^F$-de-N^+. The sample was prepared by mixing of the two solution without sonication. E, the same sample as D after sonication.

sharp peak at 29 °C with ΔH=38.5 kJ/mol, but those of $2C_{11}^F$-de-N^+ and C_{11}^F,C_{12}-de-N^+ do not give any DSC peak at 0 to 70 °C. An equimolar mixture of solutions of $2C_{12}$-de-N^+ and $2C_{11}^F$-de-N^+ (prepared without sonication) gives endothermic peaks at 20 °C (major peak) and 25 °C, in the first scan. In the second and third scans, three peaks appear at 20 (major peak), 25 and 29 °C. The DSC behavior observed upon repeated scans agrees with that of the sonicated sample. The ΔH value of the triplet peak is 15.9 kJ/mol, which is much smaller than 38.5 kJ/mol of $2C_{12}$-de-N^+ alone. An equimolar mixture (without sonication) of $2C_{12}$-de-N^+ with C_{11}^F,C_{12}-de-N^+ give peaks at 13 and 20 °C, and the latter peak becomes smaller upon repeated scans. In fact, a sonicated sample of the mixture gives peaks at 13(major) and 20 °C with reduced intensity(ΔH=10.0 kJ/mol).

It is suggested from these data that the fluorocarbon bilayer of $2C_{11}^F$-de-N^+ and the hydrocarbon bilayer of $2C_{12}$-de-N^+ are partially miscible. The decreased ΔH value implies the presence of rather small domains of $2C_{12}$-de-N^+. The miscibility is apparently improved in the mixture of $2C_{12}$-de-N^+ and $2C_{11}^F$-de-N^+, because the peaks appear at lower temperatures with smaller intensity.

Sturtevant and co-workers[27] prepared phospholipids labeled by fluorine at several positions of the acyl chain, and examined their mixing behavior with unlabeled phospholipids. The DSC data showed that the fluorine-labeled phosphocholines are not ideally distributed. These results are consistent with our data on a mixed membrane of $2C_{12}$-de-N^+ and $2C_{11}^F$-de-N^+ in that T_c shifts to lower temperatures and that ΔH decreases considerably.

The miscibility of the two-component membranes is improved in an equimolar, three-component mixture of $2C_{12}$-de-N^+, $2C_{11}^F$-de-N^+, and C_{11}^F,C_{12}-de-N^+. Very small DSC peaks appear at 16.5 and 22.5 °C in the first scan of this mixture(without sonication), but they disappear completely in the second scan. The peaks are totally absent in a sonicated mixture. This indicates that, although $2C_{12}$-de-N^+ is only partially miscible with either of $2C_{11}^F$-de-N^+ and C_{11}^F, C_{12}-de-N^+ it is completely miscible in a mixed membrane of $2C_{11}^F$-de-N^+ and C_{11}^F,C_{12}-de-N^+.

Conclusion

It is established that the excimer formation of the benzene unit is unusually facilitated due to the bilayer assemblage. This novel phenomenon is very useful for examining the phase separation behavior of hydrocarbon and fluorocarbon bilayers. We showed elsewhere that the anthracene chromophore included in the bilayer assemblage give strong excimer emission when it is partially buried in a bilayer matrix.[14] The intensity of the excimer emission depended on the mole ratio of the anthracene amphiphile and the dialkylammonium matrix. Thus, the excimer formation may be used widely as a means for elucidation of the phase separation.

The limited miscibility of hydrocarbon and fluorocarbon bilayers may also be used for developing novel catalytic functions. We already showed in the micellar system that high catalytic efficiency and selectivity are attainable by combinations of hydrocarbon and fluorocarbon reagents.[28]

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