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Effects of lipid structure on energy transfer from carbazolyl to anthryl groups in a lipid bilayer

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Abstract

Two types of chromophoric amphiphiles were synthesized: one of them possesses a molecular structure of *N,N*-dialkyl aromatic amino acid (5*X*18 type, where *X* is A or Cz), and the other α,γ-dialkylglutamate connected to aromatic amino acid (*m*XG12 type, where *m* is an integer). 5-*N*-Ethylcarbazolyl and 9-anthryl groups were chosen as the chromophore, and introduced to each amino acid derivative. All the amphiphiles formed assembly showing gel–liquid crystalline phase transition. The phase-transition temperature of the assembly composed of *m*XG12-type amphiphile was higher than that of 5*X*18-type amphiphile. Absorption and CD spectra of 6-(trimethylammonium)hexanoyl-L-3-(5-*N*-ethylcarbazolyl)alanine *N,N*-dioctadecylamide bromide (5Cz18) in the assembly indicated the absence of strong ground-state interactions between the carbazolyl groups, while those of 6-(trimethylammonium)hexanoyl-L-3-(5-*N*-ethylcarbazolyl)alanyl-L-glutamic acid α,γ-didodecyl ester (5CzG12) or 11-(trimethylammonium)undecanoyl-L-3-(5-*N*-ethylcarbazolyl)alanyl-L-glutamic acid α,γ-didodecyl ester (10CzG12) indicated the ground-state interactions based on dimer or higher aggregates. Fluorescence spectra of 5Cz18 showed very weak excimer emission, while excimer and/or excited dimer or higher aggregates were observed in the assembly of 5CzG12 or 10CzG12. Similar results were obtained for amphiphiles (*m*AG12) with anthryl and hydroxyethyldimethylammonium groups in places respectively of carbazolyl and trimethylammonium groups of 5CzG12 and 10CzG12. Taking these results together into consideration, the molecular packing of *m*XG12 in the assembly should be tighter than that of 5*X*18. In the binary assembly of 6-(trimethylammonium)hexanoyl-L-3-(9-anthryl)alanine *N,N*-dioctadecylamide bromide (5A18)/5Cz18 (1/99 mol/mol), about 60% of photoenergy absorbed by the carbazolyl groups was transferred to the anthryl groups, indicating an efficient energy migration along the two-dimensional array of carbazolyl chromophores of 5Cz18. On the other hand, in the *m*CzG12/*m*AG12 binary assembly, the energy-transfer efficiency was much lower due to the formation of dimer or the higher aggregates acting as energy-dissipating sites.

Keywords: Anthryl/carbazolyl bilayer membrane; Energy transfer; Fluorescence spectroscopy

1. Introduction

Bilayer membranes composed of chromophoric amphiphiles have been studied as models for photon-harvesting systems [1–3]. The efficiency of photoenergy trapping by bilayer membranes should be influenced by energy migration between "antenna" chromophores and energy transfer to the final energy acceptor in two-dimensional (2D) array of chromophores. To realize an effective photoenergy-harvesting system, chromophore arrangement should be close enough to make photoenergy migration to neighbors possible, but distant enough to avoid formation of energy-dissipating sites.

Synthesis of amino acid amphiphiles carrying

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two octadecyl groups and a 1-naphthyl [3], 2-naphthyl [3], 9-anthryl [3], or 1-pyrenyl group [4] has been reported. However, in the vesicular assembly of these amphiphiles, excimer was formed, which might act as energy-dissipating site. On the other hand, in the assembly of amphiphilic phenanthrylalanine derivative which does not form excimer, the energy-transfer efficiency from the phenanthryl to anthryl group was about the same as those found in anthryl/naphthyl systems where a large amount of excimer is formed [5]. This result suggests that the energy transfer, which is a dynamic process, is faster than the excimer formation in the assembly. In order to obtain further information on photophysical properties of chromophoric assembly which does not accompany excimer formation, amphiphiles with 3-(5-*N*-ethylcarbazolyl)alanine were synthesized, since polymers carrying *N*-ethylcarbazolyl groups have been shown difficult to form excimers [6]. In addition, a new type of chromophoric amphiphile was synthesized in the present investigation, which is a dipeptide composed of α,γ-dialkyl glutamate [7] and a chromophoric amino acid. Connection of an additional chiral amino acid to an amino acid amphiphile should lead to tight molecular packing in the assembly due to the presence of two asymmetric carbon atoms in the molecular chain and increasing intermolecular hydrogen bondings. The different molecular packing in the assembly should result in different interactions between the chromophores and different photophysical properties of the assembly. The molecular structures of amino acid amphiphiles, 5*X*18 (*X* = Cz or A representing L-3-(5-*N*-ethylcarbazolyl)alanine or L-3-(9-anthryl)alanine, respectively), and dipeptide amphiphiles, m*X*G12, are shown in Fig. 1. Effects of the chromophore structure, the length of spacer arm between ammonium group and dipeptide unit, and the main-chain structure on the photophysical properties of the assembly were studied.

2. Experimental

2.1 Materials

L-3-(5-*N*-Ethylcarbazolyl)alanine and L-3-(9-anthryl)alanine were prepared and optically re-

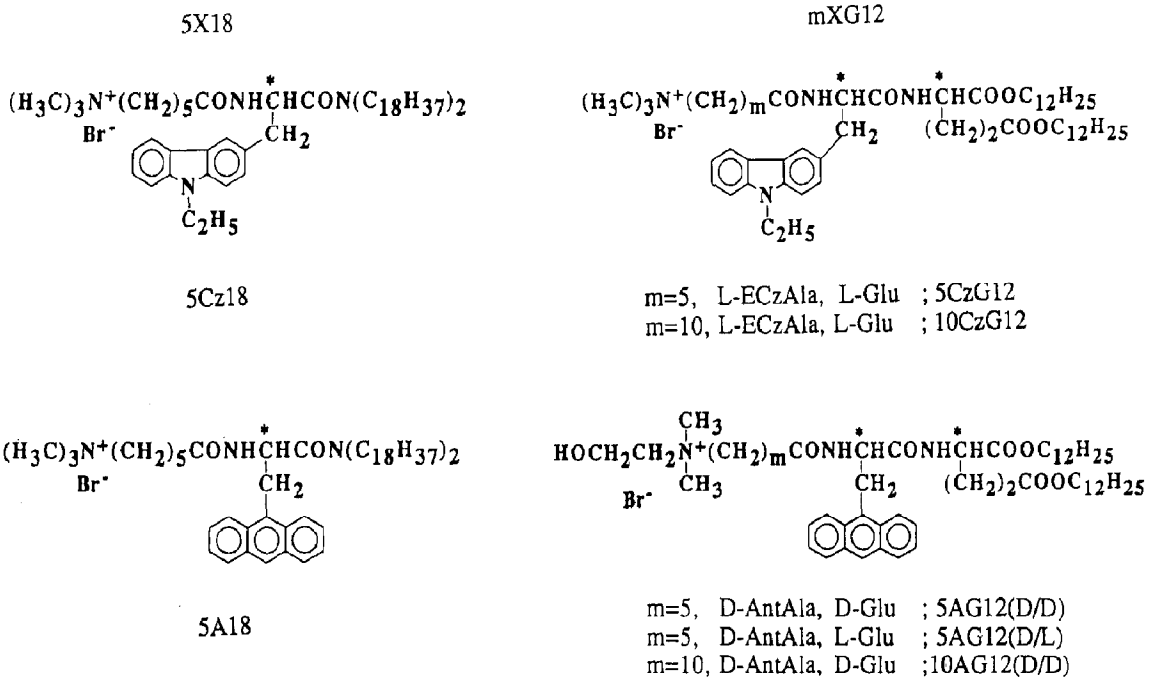


Fig. 1. Molecular structure of synthetic amphiphiles.

solved according to previous reports [8–10]. Synthesis of 5Cz18 has been reported previously [8]. Other amphiphiles including a glutamic acid unit were synthesized by the method similar to that reported for the synthesis of glutamic acid amphiphiles [7]. The synthesis of 5CzG12 is described below for a typical example (Fig. 2).

Boc-ECzAla-G12 α,γ-Didodecyl L-glutamate (195 mg), which was synthesized from L-glutamic acid and dodecanol in the presence of *p*-toluenesulfonic acid, was coupled with Boc-L-ECzAla (150 mg) in a chloroform solution by using dicyclohexylcarbodiimide (92 mg). The reaction product was extracted with ethyl acetate. The ethyl acetate solution was washed successively with aqueous solutions of citric acid (10%), $NaHCO_3$ (4%) and NaCl, and dried over $MgSO_4$. After the solvent was stripped off under reduced pressure, the residue was crystallized from acetone. Yield: 240 mg. Anal. Calcd. for $C_{51}H_{81}O_7N_3$: C, 72.22; H, 9.62; N, 4.95. Found: C, 71.97; H, 9.89; N, 5.05.

Br-C_5-ECzAla-G12. To Boc-ECzAla-G12 (200 mg) a small amount of chloroform was added to 4 *N* HCl in dioxane (10 ml), and the solution was left standing for 45 min. The solvent was stripped off, and the residue was dissolved in chloroform. Triethylamine (63 ml) and bromohexanoylchloride (65 mg) was added dropwise to the chloroform solution. After stirring for 1 hour at 0°C, the solution was washed successively with an aqueous solution of $NaHCO_3$ and NaCl. The chloroform solution was dried over $MgSO_4$, and the solvent was stripped off. The residue was crystallized from a chloroform/methanol mixture. Anal. Calcd. for $C_{52}H_{82}O_6N_3Br$: C, 67.51; H, 8.93; N, 4.54; Br, 8.64. Found: C, 67.30; H, 9.15; N, 4.50; Br, 8.52.

5CzG12 Br-ECzAla-G12 (60 mg) was dissolved in ethanol/chloroform (1/1 v/v) mixture containing trimethylamine. After standing overnight quaternization, the reaction product was purified over a LH-20 column using chloroform as eluent. Anal. Calcd. for $C_{56}H_{93}O_7N_4Br \cdot H_2O$: C, 65.16; H, 9.28; N, 5.43; Br, 7.74. Found: C, 64.82; H, 9.29; N, 5.36; Br, 7.61.

Elemental analysis of 10CzG12 is as follows. Calcd. for $C_{61}H_{103}O_7N_4Br$: C, 67.56; H, 9.57; N, 5.17; Br, 7.34. Found: C, 67.48; H, 9.77; N, 5.18; Br, 7.24.

2.2 Membrane preparation

A small amount (a few mg) of amphiphile was dissolved in chloroform, and the solution was evaporated to form a thin film on the wall of flask. Distilled water (a few ml) was added and the mixture was warmed to obtain an opalescent dispersion. After a brief sonication (Tomy Seiko UR-200P, 20-40W), the dispersion became clear.

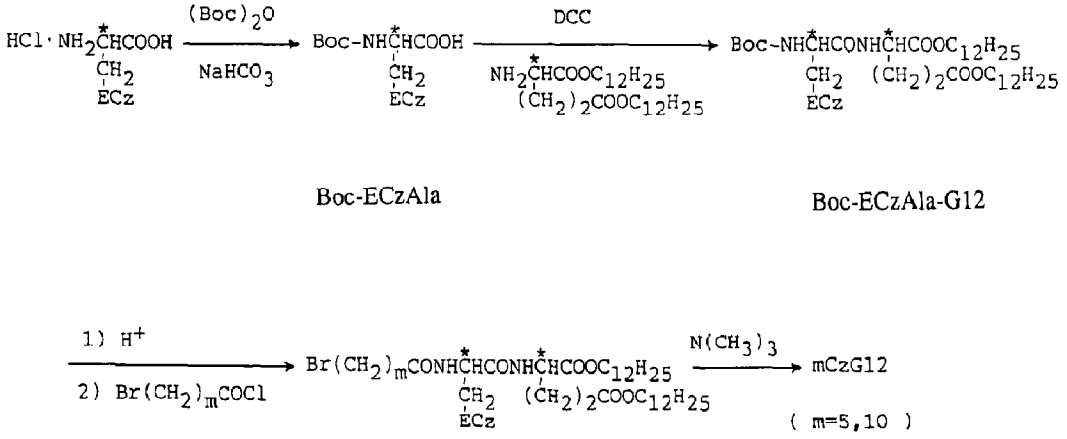


Fig. 2. Synthetic route of *m*CzG12 (*m* = 5,10).

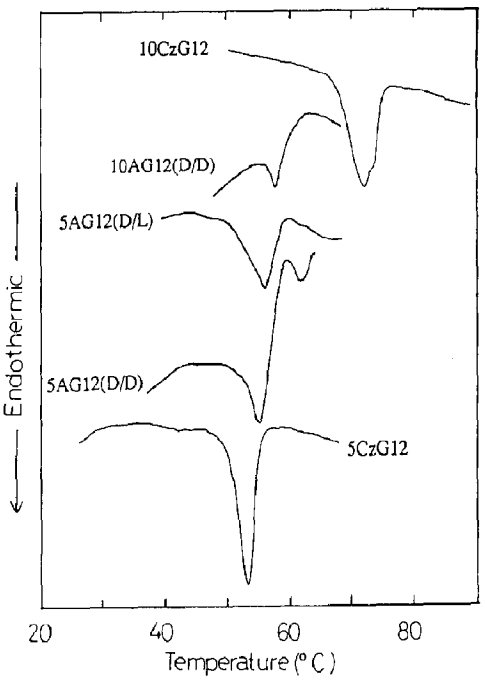


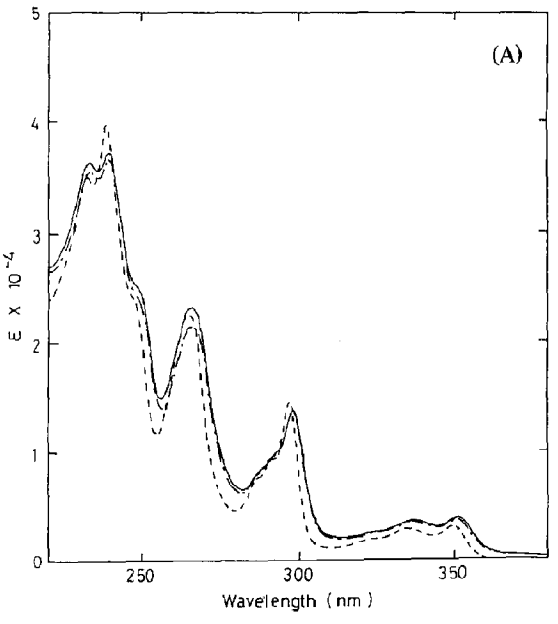
Fig. 3. DSC thermograms of the sonicated aqueous dispersion of *m*CZG12 an *m*AG12.

The pH of the dispersion was in the neutral range. Following this procedure, 5Cz18 and 5A18 have been shown to form a vesicular bilayer membrane by transmission electron microscopy measurements [8,9].

2.3 Measurements

Differential scanning calorimetry (DSC) thermogram was measured for mAG12 on a Daini-Seikosha SSC580 and for mCzG12 on a Seiko Instruments SSC5200. The amphiphile dispersion (20 m*M*, 50 ml) was sealed in a silver pan, and was heated with a rate of 2°C/min and 1°C/min, respectively. Spectroscopic measurements were carried out by using following instruments: UV absorption, Jasco Ubest-50; fluorescence, Hitachi MPF-4; CD, Jasco J600.

The energy-transfer efficiency was determined from the fluorescence spectra according to the method reported by Holden and Guillet [11].



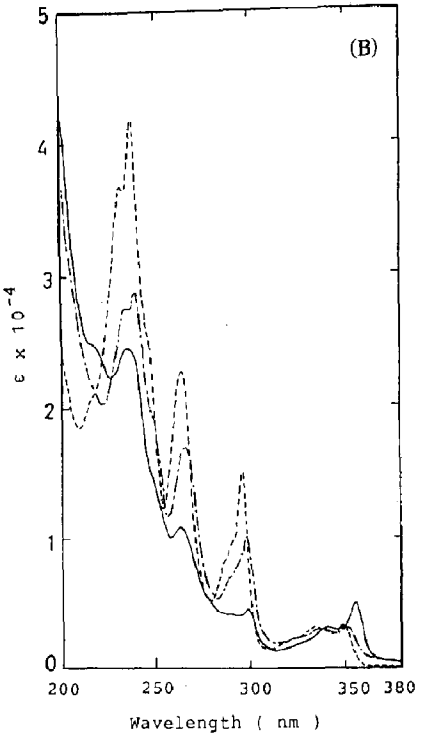


Fig. 4. Absorption spectra of (A) 5Cz18 at 10°C (—) and 40°C (-·-), and (B) 5CzG12 at 15°C (—) and 60°C (---), in the aqueous dispersion. The spectra in methanol solution (— — —) are also shown.

3. Results and discussion

3.1 Differential scanning calorimetry at phase transition

A phase change of the 5CzG12, 10CzG12, 5AG12(D/D), 5AG12(D/L) or 10AG12(D/D) assembly was followed by DSC (Fig. 3). An endothermic peak was observed in each case, which can be ascribed to a gel–liquid crystalline transition. 5A12 and 10CzG12 show respectively another small peak and a shoulder at higher temperature, which might be due to inhomogeneity in the vesicular size or the assembly type of the samples as reported in the case of 5A18 vesicles [12]. The phase-transition temperature of the assembly shifted to higher temperatures in the order of 5CzG12 < 5AG12(D/D) < 5AG12(D/L) < 10AG12(D/D) < 10CzG12, suggesting that the longer the spacer arm between ammonium group and dipeptide unit of the amphiphile, the more stable the assembly.

The phase-transition temperature has been reported to be 25.4°C for 5Cz18 [8] and 26.5°C for 5A18 [12]. Despite of the shorter alkyl-chain tails of 5*X*G12 (*X* = Cz or A) than 5*X*18, the 5*X*G12 assemblies possess higher phase-transition temperature than the 5*X*18 assemblies. Since the former amphiphiles have a dipeptide backbone, its assembly should be more stable than the 5*X*18 assembly, possibly due to more intermolecular hydrogen bondings in the 5*X*G12 assembly.

3.2 Ground-state interaction of N-ethylcarbazolyl groups

UV absorption spectra of 5Cz18, 5CzG12 and 10CzG12 in assembly and in methanol solution were measured and compared. The absorption wavelengths of 5Cz18 assembly are not largely shifted from those in methanol (Fig. 4A). On the other hand, absorption peaks were shifted and hypochromic effects were observed with 5CzG12 (Fig. 4B) and 10CzG12 in going from methanol solution to the assembly, suggesting the formation of ground-state dimers or higher aggregates in the latter assemblies. The striking difference between 5Cz18 and 5CzG12 or 10CzG12 should be explained in terms of tighter packing of the 5CzG12 and 10CzG12 assemblies with more in-

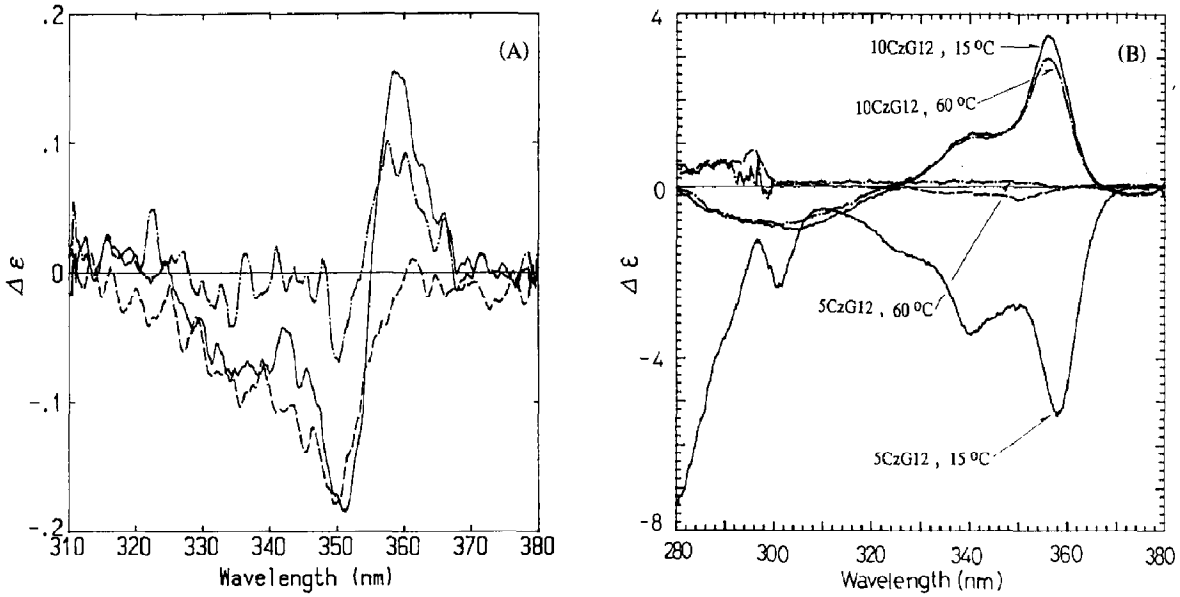


Fig. 5. CD spectra of (A) 5Cz18 at 15°C (—) and 60°C (– · –), and (B) 5CzG12 and 10CzG12, in the aqueous dispersion. The spectra in methanol solution (— — —) are also shown.

termolecular hydrogen bondings than the 5Cz18 assembly. This situation is consistent with the DSC results.

With temperature rise from 15 to 60°C, UV spectra of the 5CzG12 and 10CzG12 assemblies looked more like those in methanol, representing

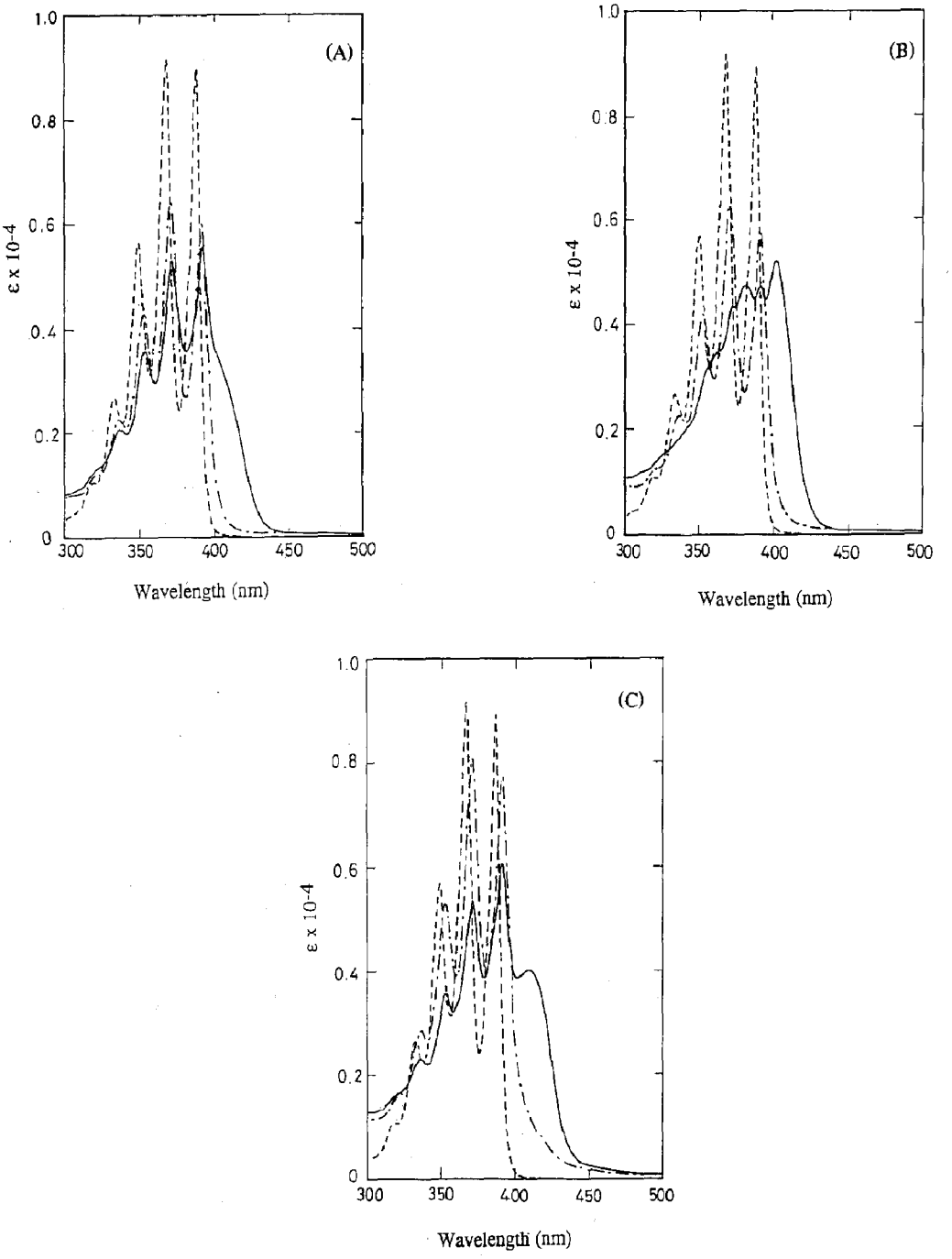


Fig. 6. Absorption spectra of (A) 5AG12(D/D), (B) 5AG12(D/L), and (C) 10AG12(D/D) at 5°C (—) and 60°C (–·–) in the aqueous dispersion. The spectra in methanol solution (— — —) are also shown.

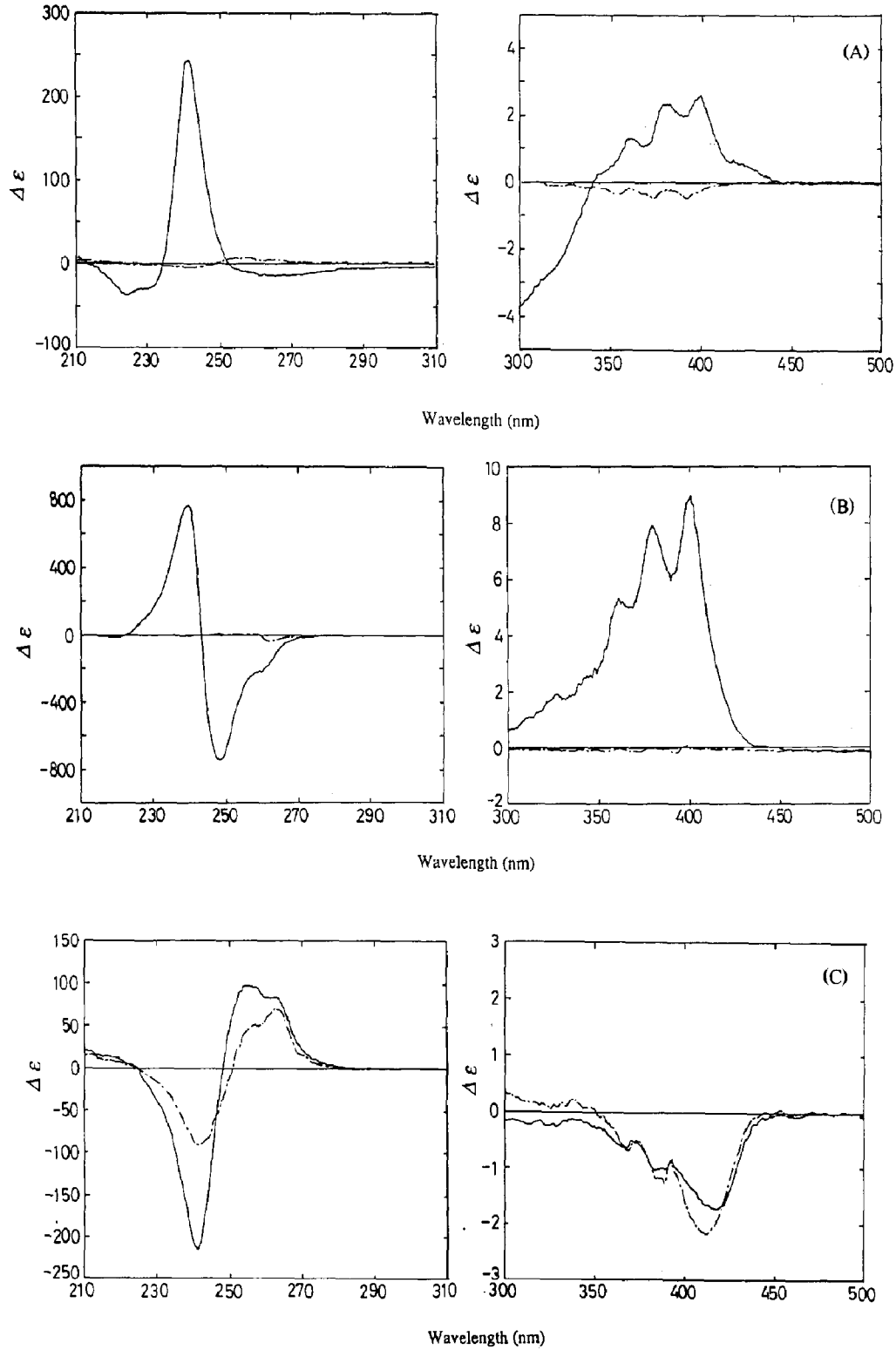


Fig. 7. CD spectra of (A) 5AG12(D/D) (B) 5AG12(D/L), and (C) 10AG12(D/D) at 5°C (—) and 65°C (-·-) in the aqueous dispersion.

weak interactions of chromophores caused by disorder in the membrane structure at higher temperatures.

Circular dichroism (CD) spectra of the same samples are shown in Fig. 5. The 5Cz18 bilayer assembly shows a pair of negative and positive Cotton effects around 355 nm which is ascribed to exciton coupling. 5CzG12 and 10CzG12 show a negative and a positive Cotton effect, respectively, around 350 nm, which is not detected in methanol. These results indicate the ground-state interaction between chromophores in the bilayer assembly. The opposite sign of the Cotton effect between 5CzG12 and 10CzG12 indicates that the orientation of chromophores in the bilayer assembly differs depending on the length of spacer arm between ammonium group and dipeptide unit.

The interaction between ground-state carbazolyl groups diminishes with temperature rise. In other words, the chromophore arrangement is rigid in the gel-state assembly.

3.3 Ground-state interaction of anthryl groups

The absence of ground-state dimer of 5A18 in the bilayer assembly has been reported [12]. On the other hand, UV absorption spectra of 5AG12(D/D), 5AG12(D/L), and 10AG12(D/D) show peak shifts as well as hypochromicity in going from methanol solution to bilayer assembly (Fig. 6). Furthermore, new peaks appeared at 242 nm with 5AG12 (D/L) and at 250 nm with 10AG12 (D/D), which are assigned to the ^1B_b absorption band, at 410 nm (shoulder) with 5AG12 (D/D), at 402 nm with 5AG12(D/L), and at 410 nm with 10AG12 (D/D), which are assigned to the ^1L_a absorption band. Especially, in the case of 5AG12(D/L), new peaks are recognized distinctly between the ordinary peaks (Fig. 6B). These new absorptions indicate the occurrence of the ground-state dimer or higher aggregates in the bilayer assembly, and their structure differs one by one.

At higher temperatures, UV spectra of mAG12 in the bilayer assembly become similar to those in methanol, suggesting that the formation of dimer and higher aggregates in the assembly is suppressed in the liquid-crystalline state due to disorder in the molecular arrangement.

The results of CD measurements are shown in Fig. 7. With respect to ^1L_a absorption band, 5AG12(D/D) and 5AG12(D/L) show several positive Cotton effects, while 10AG12(D/D) neg-

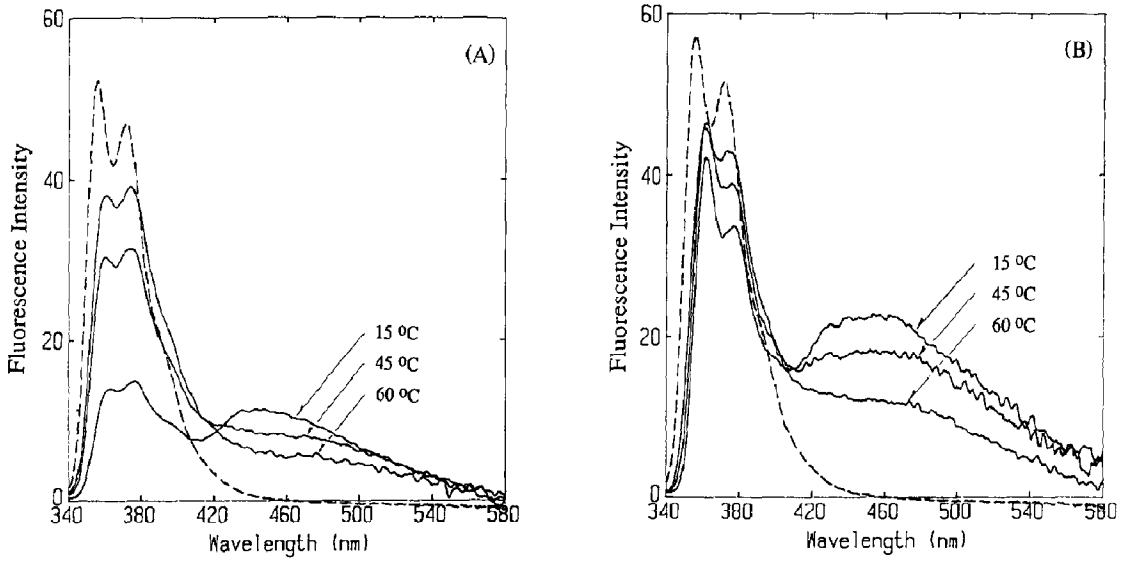


Fig. 8. Fluorescence spectra of (A) 5CzG12 and (B) 10CzG12 in aqueous dispersion ([*m*CzG12] = 1×10^{-5} *M*) at three different temperatures. The excitation wavelength is 298 nm. The spectra in methanol (— — —) are also shown.

ative Cotton effects. These situations suggest that the orientation of anthryl groups in the assembly is more sensitive to the length of spacer arm than to the chirality of glutamic acid unit. The same conclusion can be derived from the ^1B_b absorption band, where a positive Cotton effect around 240 nm was observed with 5AG12(D/D) or 5AG12(D/L) (negative chirality) and a negative Cotton effect with 10AG12(D/D) (positive chirality).

Taking these observations together into consideration, the following conclusion is drawn irrespective of the nature of chromophore. The amphiphiles $5X18$ and $mXG12$ form bilayer assembly showing gel–liquid crystalline phase transition. The packing of $mXG12$ molecules in the assembly is more tight than $5X18$ as shown by higher phase-transition temperature and formation of dimer or higher aggregates in the gel state. It is notable that the ground-state interaction in the $5X18$ assembly is so weak that it is not detectable by UV measurement. It is detectable only by CD measurement. Furthermore, the effect of the phase transition of the $5X18$ assembly on the ground-state interaction is so subtle that CD spectra were not susceptible to the phase transition.

3.4 Fluorescence spectroscopy on the excited-state interactions

The excited-state interaction is sensitive to the type of chromophore. For example, it has been

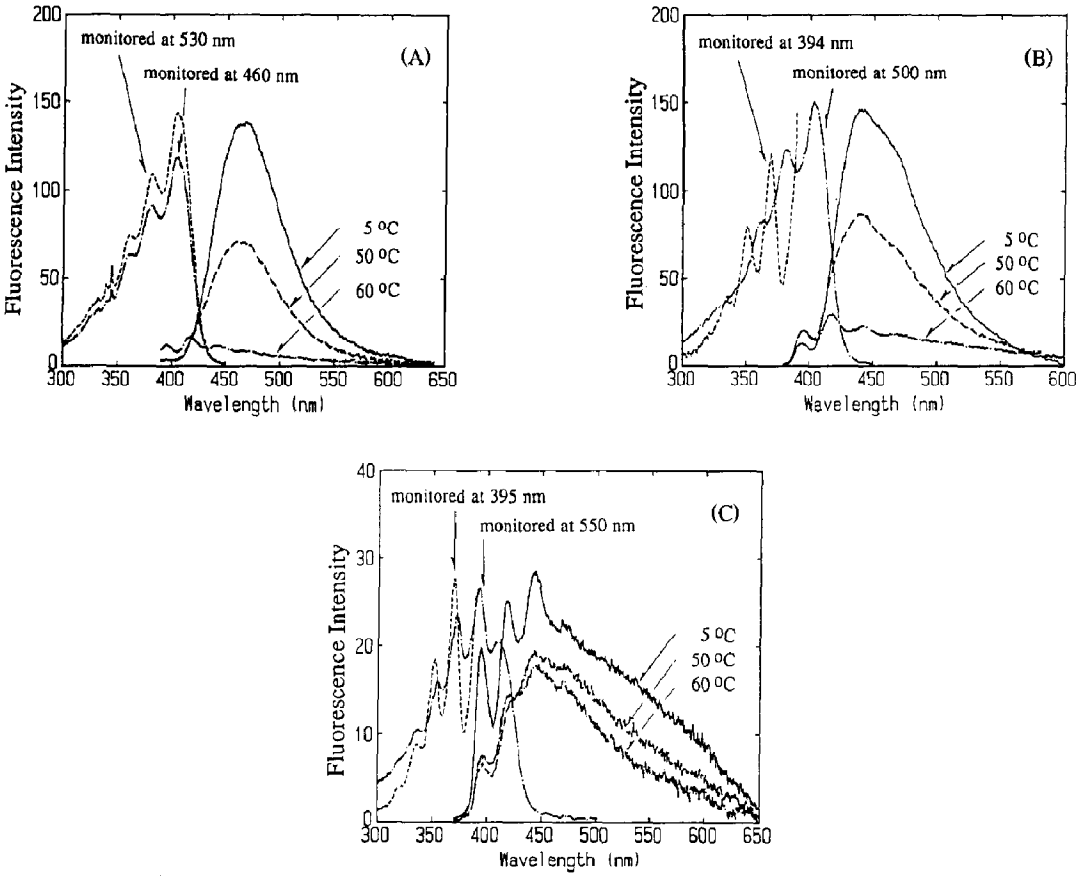


Fig. 9. Fluorescence spectra of (A) 5AG12(D/D), (B) 5AG12(D/L), and (C) 10AG12(D/D) in aqueous dispersion at three different temperatures. The excitation wavelength was 337 nm for 5AG12(D/D) and 10AG12(D/D) and 350 nm for 5AG12(D/L). The excitation spectra of each amphiphile at 5°C are also shown, which were monitored at the wavelength indicated in the figure.

reported that the fluorescence spectra of 5A18 in the assembly consist of monomer and excimer emissions [12], while those of 5Cz18 are nearly free from excimer emission [8]. Figure 8 shows fluorescence spectra of *m*CzG12 amphiphiles. In all spectra, a broad emission is observed at longer wavelength in addition to monomer emission. With 5CzG12 and 10CzG12, fluorescence excitation spectra monitored at the monomer emission and at the other emission did not completely coincide with each other, indicating that the broad emission is composed of excimer and excited dimer or higher aggregates. In the latter, the emission originates from the excitation of ground-state dimer or higher aggregates in the assembly. The same interpretation of the excitation spectra can be applied to 5AG12(D/D), 5AG12(D/L) and 10AG12(D/D) (Fig. 9). Especially in the case of 5AG12(D/L), the excitation spectrum monitored at 394 nm is markedly different from that monitored at 500 nm, and all peaks in the excitation spectrum monitored at 500 nm are present in the UV spectrum of 5AG12(D/L) assembly (Fig. 6B), which were assigned to dimer or higher aggregates. Therefore, the broad emission around 450 nm of 5AG12(D/L) assembly is due mainly to excited dimer or higher aggregate, and the contribution from excimer is low. The arrangement of anthryl groups in the dimer or higher aggregates is different among these lipids, which is shown in differences in UV absorption spectra and excitation spectra. The molecular packing of the lipids depends strong on the molecular structure that results the different photophysical properties.

The intensity ratio of monomer emission against other emission is larger in the 10CzG12 assembly than in the 5CzG12 assembly and in the 10AG12 assembly than in the 5AG12 (D/D) and the 5AG12(D/L) assemblies. Therefore, the formation of excimer, excited dimer or higher aggregates in the assembly is enhanced in the amphiphiles with shorter spacer chain between the ammonium group and the amino acid residue in the molecular structure. The intensity of monomer emission increases with higher temperature, which can be explained in terms of increasing structural disorder in the assembly. This explanation is consistent with the temperature-dependent changes in UV and CD spectra.

In summary, 5*X*G12 amphiphiles have a molecular organization suitable for strong interactions between chromophores in the assembly. The factors for promoting a tight packing of chromophores in the assembly are as follows: (i) intermolecular hydrogen bondings, (ii) the presence of dual chiral centers resulting in reduction of molecular freedom, and (iii) close proximity of chromophores to polar groups to immobilize chromophores.

3.5 Excited energy transfer from carbazolyl to anthryl group in the bilayer assembly

The binary assembly of 5A18/5Cz18 was prepared with variable contents of 5A18. The fluorescence spectra were measured with an excitation wavelength of 298 nm, because most of the photoenergy is absorbed by the carbazolyl groups when the molar percentage of anthryl groups is less than 1%. The addition of 1 mol% of 5A18 significantly reduced the intensity of monomer emission and induced a strong emission from the anthryl group (Fig. 10). The energy-transfer efficiency was evaluated from the fluorescence spec-

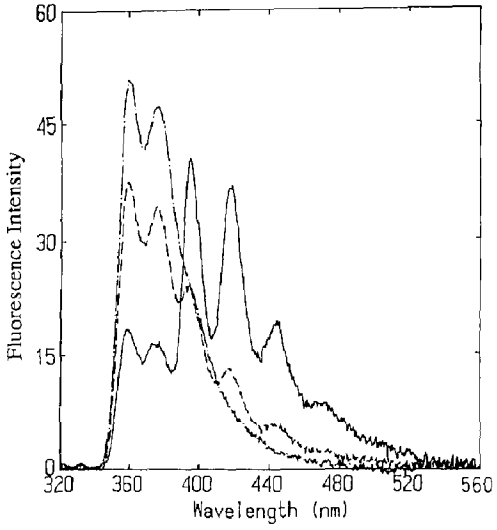


Fig. 10. Fluorescence spectra of 5A18/5Cz18 binary assembly. The excitation wavelength was 298 nm. Molar percentage of 5A18 = 0 (– · –), 0.1 (— — —), and 1.0 (—).

tra, and is shown in Fig. 11 against the surface density of 5A18. The solid line in the figure is the efficiency calculated for direct energy transfer without energy migration [13] taking 28.7 Å as the critical distance for energy transfer [14]. Obviously, the observed values are higher than the calculated values, suggesting the energy migration among carbazolyl groups in the assembly.

The occurrence of the energy migration in the 5A18/5Cz18 assembly is supported by quenching experiment using acrylamide. Since these lipids form a vesicular assembly, the chromophores only in the outer leaflet of the bilayer membrane are exposed to water-soluble quencher added to the dispersion. It has been reported that the relative amount of lipids in the outer leaflet of a vesicular bilayer membrane (α) is between 0.5 and 0.7 [15]. Figure 12 shows the Stern–Volmer plot of fluorescence quenching of 5Cz18 assembly and 5Cz18/5P18 (1/99 mol/mol) assembly (5P18 represents an amphiphile in which L-3-(5-*N*-ethylcarbazolyl)alanine of 5Cz18 is replaced with L-phenylalanine). The straight lines were obtained when α is taken to be 0.5. The emission from the 5Cz18 assembly was quenched more intensively than the mixed lipid assembly. This difference can be explained in terms of a facile energy migration in the 5Cz18 assembly.

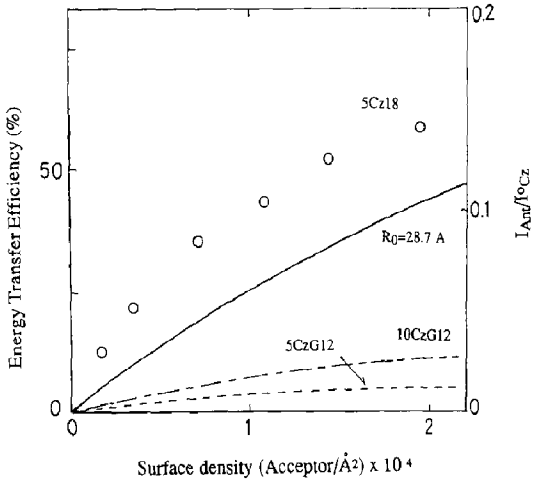


Fig. 11. Energy-transfer efficiencies in the binary assemblies of 9A18/5Cz18 (○), 5AG12(D/D)/5CzG12(D/D) (— — —), and 10AG12(D/D)/10CzG12(D/D) (–·–). In the latter two cases, the emission from excited dimer or higher aggregates of *m*CzG12 overlapped with the emission from anthryl group of *m*AG12 in the fluorescence spectra. Therefore, the excited energy-transfer efficiency was evaluated by the intensity ratio of anthryl emission against monomer emission of *m*CzG12 in the absence of acceptor in the assembly. Solid line indicates the energy-transfer efficiency calculated for direct energy transfer (without energy migration).

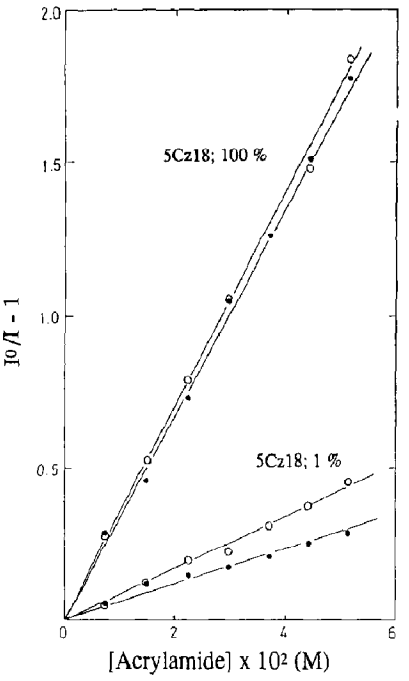


Fig. 12. Stern–Volmer plot of fluorescence quenching of 5Cz18 and 5Cz18/5P18 (1/99 mol/mol) assemblies with acrylamide in aqueous dispersion at (●) 15°C and (○) 45°C. Fluorescence intensities in the absence and the presence of acrylamide, I^0 and I, respectively, are corrected observed values under the assumption that acrylamide molecules are accessible to carbazolyl groups only at the outer leaflet of bilayer membranes.

On the other hand, the 5AG12/5CzG12 and 10AG12/10CzG12 assemblies showed much lower energy-transfer efficiencies than the 5A18/5Cz18 assembly (Fig. 11). Probably, the photoenergy absorbed by carbazolyl groups should be trapped by dimer or higher aggregates in the assembly. The ground-state interaction in the mXG12 assembly is so strong that the excited dimer or higher aggregates must be formed immediately after absorption of photoenergy. This type of energy trapping is considered a static process, and it works effectively as an energy-dissipating process. Taking these results into consid-

eration, an assembly with a high efficiency in photoenergy trapping should be free from ground-state interactions leading to dimer or higher aggregates. Thus, in order to inhibit the formation of dimer and higher aggregates, the chromophoric amphiphiles should be molecularly dispersed as far as possible in the assembly, however, within Forster's r_0 value for energy migrations among the chromophores. This situation also avoids self-quenching of chromophores caused by a high concentration of chromophores in the assembly [16]. The low probability of excimer formation is favorable, but not prerequisite for the efficient energy transfer in the assembly.

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