# Formation of Specific Hydrophobic Sites for Incorporation of Methylene Blue by Laterally Arranged L-Glutamate Residues in Anionic, Crystalline Bilayer Aggregates

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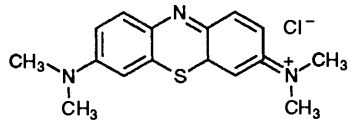
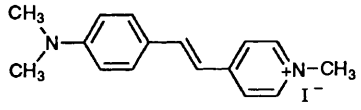
Bilayer membranes, formed from dioctadecyl L-glutamate-derived anionic amphiphiles, incorporated the cationic dye, methylene blue (MB), into their crystalline, hydrophobic region. The incorporated, monomeric MB was extraordinarily converted into a dimeric species during the gel-to-liquid crystalline phase transition by binding to carboxylates. A further increase in temperature upon the phase transition temperature led to the ordinary dimer-to-monomer transition of the bound, aggregated MB as well as the conventional various dye–polyion systems including micellar systems. However, such extraordinary behaviour strongly depends on the chemical structure of the bilayer-forming component amphiphiles. The molecular structure requirements of anionic amphiphiles for such incorporation has been investigated by varying the alkyl chain length, the average degree of polymerization ($x$), and the kind of amino acid residue in the connector moiety. Specific incorporation of MB into the crystalline, hydrophobic region is peculiar to molecular assemblies formed from L-glutamate amphiphiles with long alkyl chains and low $x$ values. Corresponding DL-glutamate derivatives showed ambiguous transitions and L-aspartate derivatives did not show such specific incorporation, indicating that the specific behaviour is peculiar to the L-glutamate derivatives. The same conclusion that the monomeric dye species in the crystalline bilayer state is incorporated in the hydrophobic region of the bilayer aggregates could be confirmed by using the solvatochromic dye, 4-[4-(dimethylamino)styryl]-*N*-methylpyridinium iodide (St-4C$_1$), as a micro-environmental probe instead of MB.

It is well-known that many kinds of ionic dye bind to oppositely charged natural and synthetic polyelectrolytes and, in general, aggregations are induced[1-4] or promoted.[4-39] For example, Hatano *et al.*[1] and Yamamoto *et al.*[2,3] have studied the chiral dimer formation of methyl orange (MO) bound to poly(L-lysin)[1-3] and poly(L-ornithine).[3] Acridine orange (AO) binds



Methylene Blue (MB)

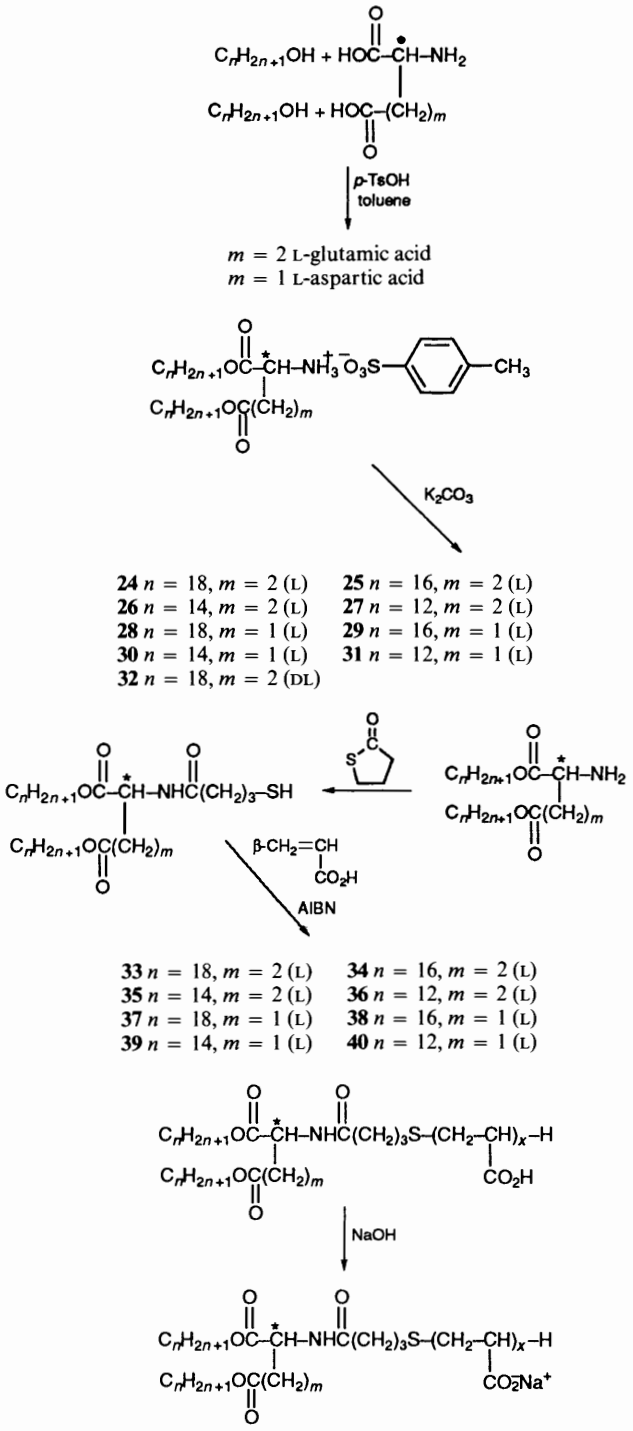


4-(4-Dimethylaminostyryl)-*N*-methylpyridinium iodide (St-4C$_1$)

to poly(L-glutamic acid) to form chiral dimers.[4-9] There have also been many other examples concerning dye aggregation, *e.g.*, poly(L-lysine)–azo dyes,[40] nucleic acid–AO,[18,26] nucleic acid–toluidine blue O (TB),[12] poly(acrylic acid)–MB,[20] poly(acrylic acid)–crystal violet (CV),[23] poly(methacrylic acid)–AO,[17] synthetic bilayer membranes–methyl orange (MO)[33-37,39] and synthetic bilayer membranes–cyanine dyes[35,37] systems. Nakashima *et al.*[33 37] investigated the binding behaviour of various dyes to chiral synthetic bilayer membranes. In all these aqueous systems including polymers and molecular assemblies,[1-40] the aggregated dyes (mainly dimers) are converted into monomeric species (namely, an aggregate-to-monomer transition) by heating the solutions,[16,41,42] dilution of their solutions,[20,42-46] addition of salts (KCl,[22] NaCl)[23] or a large excess of polyanion,[10,11,13,14,22,23] or by the addition of sufficient urea[20] or ethanol which has a lower relative permittivity[20,41] than water.

In our previous communication,[47] we briefly reported that the dimerization of MB and acridine orange (AO) was inhibited when the monomer was incorporated into the hydrophobic region of crystalline bilayer aggregates formed from dioctadecyl L-glutamate-derived amphiphilic telomers with oligo(sodium acrylate) head groups (**1**) and its simplified model amphiphile with succinate head group (**12**) corresponding to $x = 1$. The monomeric MB species were converted into dimers by gel-to-liquid crystalline phase transition of the bilayer aggregates of **1** and **12**. In the conventional dye solution systems, there has been no report about such an extraordinary monomer-to-aggregate transition upon heating in any dye–polyelectrolyte system[1-40] and pure aqueous dye solution system.[10,41-46,48-51]

Our current target is to construct a specific host by assembling component amphiphiles of relatively simple chemical structure, because the inclusion ability of conventional inclusion compounds such as cyclodextrins and calixarenes are restricted mostly by their primary structure. In this paper, we report the formation of specific hydrophobic sites for incorporation of MB in assemblies of L-glutamate-derived amphiphiles together with the detailed conditions concerning the previously reported[47] monomer-to-dimer transition of MB. Emphasis is placed on the specificity of the molecular




**Scheme 1**

assemblies formed from L-glutamate-based amphiphiles with longer alkyl chains and lower $x$ for incorporation of MB, using the corresponding DL-glutamate and L-aspartate derivatives as comparisons. The specific behaviour of MB[47] and AO[47] was also confirmed by using the solvatochromic dye, St-4$C_1$. The dye also showed incorporation and release behaviour of MB and AO, in crystalline and liquid-crystalline bilayer states, respectively.

## Experimental

*Materials.*—MB was a commercially available, guaranteed reagent and was used after being recrystallized twice from methanol. St-4$C_1$ was purchased from Aldrich and used after being recrystallized twice from methanol. Chloroform, tetrahydrofuran (THF) and benzene were distilled prior to use. Poly(acrylic acid) (PAA) (average molecular weight 2000; average degree of polymerization *ca.* 28) was from Aldrich and used without further purification. Azoisobutyronitrile (AIBN) was commercially available and used after recrystallization from methanol.

*Lipid Membrane Preparation.*—The lipids were hydrated with 30 $cm^3$ of deionized water at 70 °C by ultrasound (Ultrasonic generator with a 4280S type vibrator produced by Kaijyo Denki Co. Ltd.), and then the suspension was kept at 20 °C for 1 h prior to use.

*Preparation of Lipid Membrane–Dye Solution.*—All the solutions of the mixture of bilayer and dye were prepared by addition of stock solution of dyes to aqueous dispersions of amphiphiles and sonicated. After adjustment of the pH to 9.0 with sodium hydroxide, the solutions were used for visible absorption spectra measurements.

*Visible Absorption Spectra Measurements.*—The samples in a 1 mm quartz cell were incubated for 15 min at selected temperatures. The visible absorption spectra were measured with a JASCO Ubest 35 spectrophotometer.

*Characterization of Amphiphiles.*—The chemical structures of all the compounds synthesized were confirmed by Fourier transform infrared spectroscopy (FTIR) measurement with a JASCO FT/IR-7000 and by $^1H$ NMR measurement with a JEOL JNM-EX-90. The average degree of polymerization ($x$) was estimated by $^1H$ NMR measurement ($D_2O$) and confirmed by elemental analysis with a Yanaco CHN Corder MT-3 from the ratio of %C to %N which is sensitive to the measure of $x$. The purity was estimated by observing the aqueous solutions visually to see whether or not they were homogeneous, birefringent and transparent.

*Characterization of Bilayer Aggregates.*—Aggregate morphologies of amphiphiles were observed by using a JEOL 2000FX transmission electron microscope. The aqueous samples ($6 \times 10^{-4}$ mol $dm^{-3}$) were spotted onto carbon-coated copper grids. The samples were air-dried at room temperature, after which they were stained with 2 wt% aqueous ammonium molybdate. The gel-to-liquid crystalline phase transition temperature was measured by differential scanning calorimetry (DSC) with a SEIKO I&E DSC 120. The sample solution (20 mmol $dm^{-3}$) was sealed in an Ag capsule and scanned using a heating rate of 2 °C $min^{-1}$. This technique is also applicable to the estimation of purity by noting the absence of an endothermic peak due to the melting point of starting materials from the syntheses.

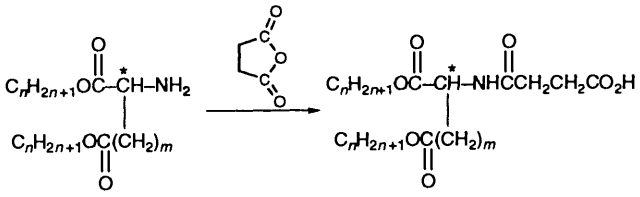
*Preparation of Amphiphiles (Scheme 1).—Dioctadecyl* L-*glutamate hydrotoluene*-p-*sulfonate* (**24**). L-Glutamic acid (5 g, $3.4 \times 10^{-2}$ mol), toluene-*p*-sulfonic acid monohydrate (7.8 g, $4.1 \times 10^{-2}$ mol) and *n*-octadecanol (20.3 g, $7.5 \times 10^{-2}$ mol) were suspended in 400 $cm^3$ of toluene and refluxed for 5 h, removing water azeotropically.[53] The reaction mixture was concentrated and diethyl ether was added. The mixture was cooled at 0 °C to obtain a white solid (**24**): yield 21.8 g, (78%); m.p. 64–67 °C; $\nu_{max}$(KBr)/$cm^{-1}$ 2920, 2854, 1736, 1470 and 1218; $\delta$($CDCl_3$) 0.81–0.93 (m, 6 H, $CH_3$), 1.07–1.43 (m, 64 H, $CH_2$), 2.08–2.65 (s, 3 H, $CH_3$), 3.82–4.30 (m, 4 H, $CH_2OC{=}O$),

7.10–7.85 (d, 4 H, $C_6H_4$). The related compounds were synthesized as described above: **25**, yield 80%, m.p. 65–67 °C; **26**, yield 80%, m.p. 58–62 °C; **27**, yield 70%, m.p. 58–61 °C; **32**, yield 90%, m.p. 58–61 °C. The corresponding L-aspartate derivatives were prepared as described above: **28**, yield 95%, m.p. 90–95 °C; **29**, yield 86%, m.p. 87–90 °C; **30**, yield 80%, m.p. 75–78 °C; **31**, yield 82%, m.p. 73–77 °C.

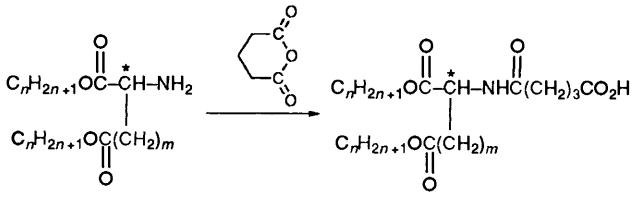
*Dioctadecyl* N-(*4-mercaptobutanoyl*)-L-*glutamate* (**33**). Dioctadecyl L-glutamate hydrotoluene-*p*-sulfonate **24** (5.0 g, $6.1 \times 10^{-3}$ mol) was dissolved in 100 $cm^3$ of chloroform. The solution was washed twice with 50 $cm^3$ of 5% aqueous $K_2CO_3$, and then dried over anhydrous $Na_2SO_4$. The solution was concentrated *in vacuo*, after which the residue was dissolved in 10 $cm^3$ of chloroform. γ-Thiobutyrolactone (0.75 g, $7.3 \times 10^{-3}$ mol) and triethylamine (0.74 g, $7.3 \times 10^{-3}$ mol) were added to the chloroform solution. The mixture was allowed to react at ambient temperature for 1 month to obtain white solid. The emergence of amide bond was confirmed by IR spectroscopy, after which the solid was recrystallized from methanol to give white malodorous solid (**33**): yield 4.0 g, (86%); m.p. 58–65 °C; $\nu_{max}$(KBr)/$cm^{-1}$ 2920, 2854, 1742, 1644, 1468 and 719; $\delta$($CDCl_3$) 0.81–0.93 (m, 6 H, $CH_3$), 1.07–1.52 (m, 64 H, $CH_2$), 2.25–2.50 (m, 4 H, $CH_2C{=}O$) and 3.92–4.30 (m, 4 H, $CH_2OC{=}O$) (Found: C, 71.75; H, 11.55; N, 1.85. Calc. for $C_{45}H_{87}NO_5S$: C, 71.71; H, 11.55; N, 1.87%).

Related compounds were synthesized as described above: **34**, yield 70%, m.p. 45–47 °C (Found: C, 70.5; H, 11.5; N, 2.05. Calc. for $C_{41}H_{79}NO_5S$: C, 70.54; H, 11.41; N, 2.01%); **35**, yield 72%, m.p. 44–47 °C (Found: C, 69.2; H, 11.2; N, 2.15. Calc. for $C_{37}H_{71}NO_5S$: C, 69.22; H, 11.15; N, 2.18%); **36**, yield 60%, m.p. 39–40 °C (Found: C, 67.35; H, 10.7; N, 2.4. Calc. for $C_{33}H_{63}NO_5S$: C, 67.36; H, 10.77; N, 2.39%). The corresponding aspartate derivatives were prepared as described above: **37**, yield 90%, m.p. 64–68 °C (Found: C, 71.45; H, 11.6; N, 2.05. Calc. for $C_{44}H_{85}NO_5S$: C, 71.45; H, 11.50; N, 1.89%); **38**, yield 86%, m.p. 64–66 °C (Found: C, 70.25; H, 11.3; N, 2.05. Calc. for $C_{40}H_{77}NO_5S$: C, 70.22: H, 11.35; N, 2.05%); **39**, yield 80%, m.p. 53–55 °C (Found: C, 68.8; H, 11.2; N, 2.2. Calc. for $C_{36}H_{69}NO_5S$: C, 68.85: H, 11.08; N, 2.23%); **40**, yield 75%, m.p. 50–51 °C (Found: C, 67.2; H, 10.8; N, 2.45. Calc. for $C_{32}H_{61}NO_5S$: C, 67.20; H, 10.75; N, 2.45%).

*Telomerization.*[54,55]—Dioctadecyl *N*-(4-mercaptobutanoyl)-L-glutamate **33** (1.5 g, $2.0 \times 10^{-3}$ mol) was dissolved in 10 $cm^3$ of benzene. Acrylic acid (0.72 g, $1.0 \times 10^{-2}$ mol) and AIBN (10 mg) were added to the solution. The solution was purged with nitrogen for 40 min and then stirred with heating. After reflux for 1.5 h, the slightly turbid solution was cooled in an ice bath and diethyl ether (20 $cm^3$) was added. Methanolic NaOH (0.40 g, $1.0 \times 10^{-2}$ mol per 20 $cm^3$ MeOH) equivalent to the initial amount of acrylic acid was added dropwise to the solution with cooling. The white precipitate produced was collected by filtration and washed with methanol and diethyl ether to give a white waxy solid (**1**): yield 1.5 g (49%); $\nu_{max}$(KBr)/$cm^{-1}$ 3438, 2922, 2854, 1738, 1650, 1572, 1468 and 1412; $\delta$($D_2O$; internal sodium (2,2,3,3-$^2H_4$)-3-(trimethylsilyl)propionate; 70 °C) 0.70–1.00 (m, 6 H, $CH_3$), 1.00–1.44 (m, 64 H, $CH_2$), 1.46–1.83 (m, 14.4 H, $CH_2$ in PAA), 1.97–2.28 (m, 1 H, $CHCOO^-Na^+$), 4.6 (s, $H_2O$ in amphiphile and solvent). The signals derived from alkyl chains were somewhat diminished due to the formation of bilayer aggregates. The purity was confirmed by solubilization in water to give a slightly turbid solution and by DSC measurement.* The average degree of polymerization ($x$) was calculated from the elemental analyses from the ratio %C : %N and the values are summarized in Table 1.1. The related L-glutamate derivatives (**2**–**7**) and the corresponding L-aspartate derivatives (**8**–**11**) were synthesized similarly and their properties are summarized in Tables 1.1 and 1.2.



**12** $n$ = 18, $m$ = 2 (L) **13** $n$ = 12, $m$ = 2 (L) **14** $n$ = 18, $m$ = 1 (L)



**15** $n$ = 18, $m$ = 2 (L) **16** $n$ = 16, $m$ = 2 (L)
**17** $n$ = 14, $m$ = 2 (L) **18** $n$ = 12, $m$ = 2 (L)
**19** $n$ = 18, $m$ = 2 (DL) **20** $n$ = 18, $m$ = 1 (L)
**21** $n$ = 16, $m$ = 1 (L) **22** $n$ = 14, $m$ = 1 (L)
**23** $n$ = 12, $m$ = 1 (L)

**Scheme 2**

*Dioctadecyl*-N-(*3-carboxypropanoyl*)-L-*glutamate* (*Scheme 2*) (**12**). Dioctadecyl L-glutamate hydrotoluene-*p*-sulfonate **24** (5.0 g, $6.1 \times 10^{-3}$ mol) was dissolved in 100 $cm^3$ of chloroform. The solution was washed twice with 50 $cm^3$ of 5% $K_2CO_3$, and then dried over anhydrous $Na_2SO_4$. The solution was concentrated *in vacuo*, after which the residue was dissolved in 10 $cm^3$ of chloroform. Succinic anhydride (0.62 g, $6.2 \times 10^{-3}$ mol) and triethylamine (0.63 g, $6.2 \times 10^{-3}$ mol) were added to the solution. After being stirred at ambient temperature, the solution was washed with 1 mol $dm^{-3}$ HCl and dried over anhydrous $Na_2SO_4$. The solution was concentrated *in vacuo* and the residue was recrystallized from methanol to give white powders (**12**): yield 3.6 g (80%); m.p. 74–77 °C; $\nu_{max}$(KBr)/$cm^{-1}$ 2920, 2854, 1738, 1660, 1562, 1468 and 1207; $\delta$($CDCl_3$) 0.81–0.94 (m, 6 H, $CH_3$), 1.08–1.43 (m, 64 H, $CH_2$), 2.25–2.85 (m, 6 H, $CH_2C{=}O$), 3.93–4.30 (m, 4 H, $CH_2OC{=}O$) (Found: C, 70.45; H, 11.25; N, 1.75. Calc. for $C_{45}H_{85}NO_7{\cdot}H_2O$: C, 70.22; H, 11.31; N, 1.82%. The related compounds were synthesized as described above: **13**, yield 85%, m.p. 52–55 °C (Found: C, 67.9; H, 10.65; N, 2.45. Calc. for $C_{33}H_{61}NO_7$: C, 67.92; H, 10.46; N, 2.40%). The corresponding L-aspartate derivatives were prepared as described above: **14**, yield 87%, m.p. 60–64 °C (Found: C, 71.3; H, 11.45; N, 1.95. Calc. for $C_{44}H_{83}NO_7$: C, 71.64; H, 11.26; N, 1.90%).

*Dioctadecyl* N-(*4-carboxybutanoyl*)-L-*glutamate* (**15**). Dioctadecyl L-glutamate hydrotoluene-*p*-sulfonate **24** (5.0 g, $6.1 \times 10^{-3}$ mol) was dissolved in 100 $cm^3$ of chloroform. The solution was washed twice with 50 $cm^3$ of 5% aqueous $K_2CO_3$, and then dried over anhydrous $Na_2SO_4$. The solution was concentrated *in vacuo*, after which the residue was dissolved in 10 $cm^3$ of chloroform. Glutaric anhydride (0.83 g, $7.3 \times 10^{-3}$ mol) and triethylamine (0.74 g, $7.3 \times 10^{-3}$ mol) were added to the solution. After being stirred at ambient temperature, the solution was washed with 1 mol $dm^{-3}$ HCl and dried over anhydrous $Na_2SO_4$. The solution was concentrated *in vacuo*, and the residue was recrystallized from methanol to give a white powder (**15**): yield 3.7 g (81%); m.p. 73–76 °C; $\nu_{max}$(KBr)/$cm^{-1}$ 3320, 2920, 1736, 1651, 1473 and 1199; $\delta$($CDCl_3$) 0.81–0.93 (t, 6 H, $CH_3$), 1.08–1.44 (m, 64 H, $CH_2$), 2.20–2.56 (m, 6 H,

* Telomerization procedures for various vinyl monomers using single-chain thiols, alcohols and amines have been reported by Yamada *et al.*[54] Telomerization procedures for various vinyl monomers using various freshly synthesized dialkyl thiols have been established recently by us. Details including characterizations will be published elsewhere.

**Table 1.1** Elemental analyses of various telomers and their average degrees of polymerization ($x$). $\beta$ in parentheses denotes the initial molar ratio of acrylic acid to thiols

| Amphiphiles | Yield (%)[a] | | C(%) | H(%) | N(%) | C/N | $x$ |
|---|---|---|---|---|---|---|---|
| **1** ($n$ = 18, $m$ = 2) ($\beta$ = 5.0) | 49 | Found | 51.91 | 8.16 | 0.905 | 57.4 | 7.2 |
| | | Calc. | 51.91 | 7.85 | 0.909 | 57.1 | |
| | | Calc. for $C_{66.6}H_{108.6}NO_{19.4}SNa_{7.2}\cdot 6.1H_2O$ | | | | | |
| **2** ($n$ = 18, $m$ = 2) ($\beta$ = 12) | 60 | Found | 44.00 | 6.63 | 0.57 | 77.2 | 15.0 |
| | | Calc. | 44.00 | 6.74 | 0.57 | 77.2 | |
| | | Cal. for $C_{90}H_{132}NO_{35}SNa_{15}\cdot 16.2H_2O$ | | | | | |
| **3** ($n$ = 18, $m$ = 2) ($\beta$ = 12) | 72 | Found | 40.74 | 6.21 | 0.425 | 95.9 | 22.3 |
| | | Calc. | 40.70 | 6.18 | 0.424 | 95.9 | |
| | | Calc. for $C_{111.9}H_{153.9}NO_{49.6}SNa_{22.3}\cdot 25H_2O$ | | | | | |
| **4** ($n$ = 18, $m$ = 2) ($\beta$ = 15) | 75 | Found | 39.05 | 5.59 | 0.37 | 105.5 | 26.0 |
| | | Calc. | 39.05 | 6.12 | 0.37 | 105.5 | |
| | | Calc. for $C_{123}H_{165}NO_{57}SNa_{26}\cdot 32.4H_2O$ | | | | | |
| **5** ($n$ = 16, $m$ = 2) ($\beta$ = 5.0) | 45 | Found | 52.22 | 7.81 | 1.00 | 52.2 | 6.6 |
| | | Calc. | 52.22 | 7.89 | 1.00 | 52.2 | |
| | | Calc. for $C_{60.8}H_{98.8}NO_{17.2}SNa_{6.6}\cdot 5.3H_2O$ | | | | | |
| **6** ($n$ = 14, $m$ = 2) ($\beta$ = 5.0) | 40 | Found | 48.58 | 7.33 | 0.94 | 51.7 | 7.7 |
| | | Calc. | 48.60 | 7.28 | 0.94 | 51.5 | |
| | | Calc. for $C_{60.1}H_{94.1}NO_{20.4}SNa_{7.7}\cdot 6.6H_2O$ | | | | | |
| **7** ($n$ = 12, $m$ = 2) ($\beta$ = 5.0) | 42 | Found | 49.82 | 7.14 | 1.04 | 47.9 | 7.6 |
| | | Calc. | 49.80 | 6.80 | 1.04 | 47.9 | |
| | | Calc. for $C_{55.8}H_{85.8}NO_{20.2}SNa_{7.6}\cdot 2.5H_2O$ | | | | | |

[a] Yields were calculated based on the molar ratio of telomer to thiol used.

**Table 1.2** Elemental analyses of various telomers and their average degree of polymerization ($x$). $\beta$ in parentheses denotes the initial molar ratio of acrylic acid to thiols

| Amphiphiles | Yield (%)[a] | | C(%) | H(%) | N(%) | C/N | $x$ |
|---|---|---|---|---|---|---|---|
| **8** ($n$ = 18, $m$ = 1) ($\beta$ = 5.0) | 60 | Found | 45.48 | 9.10 | 0.89 | 51.1 | 5.0 |
| | | Calc. | 45.12 | 8.92 | 0.89 | 50.6 | |
| | | Calc. for $C_{59}H_{100}NO_{15}SNa_5\cdot 20H_2O$ | | | | | |
| **9** ($n$ = 16, $m$ = 1) ($\beta$ = 5.0) | 58 | Found | 47.92 | 8.82 | 1.01 | 47.4 | 5.1 |
| | | Calc. | 47.94 | 8.51 | 1.01 | 47.4 | |
| | | Calc. for $C_{55.3}H_{92.3}NO_{15.2}SNa_{5.1}\cdot 12.3H_2O$ | | | | | |
| **10** ($n$ = 14, $m$ = 1) ($\beta$ = 5.0) | 53 | Found | 51.42 | 7.59 | 1.10 | 46.8 | 6.2 |
| | | Calc. | 51.39 | 7.49 | 1.10 | 46.8 | |
| | | Calc. for $C_{54.6}H_{87.6}NO_{17.4}SNa_{6.2}\cdot 3.6H_2O$ | | | | | |
| **11** ($n$ = 12, $m$ = 1) ($\beta$ = 5.0) | 55 | Found | 52.33 | 7.52 | 1.25 | 41.9 | 5.6 |
| | | Calc. | 52.32 | 7.22 | 1.25 | 41.9 | |
| | | Calc. for $C_{48.8}H_{80.2}NO_{17.4}SNa_{5.6}\cdot 1.2H_2O$ | | | | | |

[a] Yields were calculated based on the molar ratio of telomer to thiol used.

$CH_2C{=}O$), 3.92–4.30 (m, 4 H, $CH_2OC{=}O$) (Found: C, 70.8; H, 11.4; N, 1.8. Calc. for $C_{46}H_{87}NO_7\cdot 0.8H_2O$: C, 70.79; H, 1.44; N, 1.80%). The related compounds were prepared similarly: **16**, yield 3.0 g (78%), m.p. 51–54 °C (Found: C, 71.0; H, 11.3; N, 2.0. Calc. for $C_{42}H_{79}NO_7$: C, 71.04; H, 11.21; N, 1.97%); **17**, yield 2.4 g (62%), m.p. 43–50 °C (Found: C, 68.1; H, 10.55; N, 2.15. Calc. for $C_{38}H_{71}NO_7\cdot 0.9H_2O$: C, 68.10; H, 10.95; N, 2.09%); **18**, yield 0.63 g (15%), m.p. 40–47 °C (Found: C, 66.8; H, 10.55; N, 2.35. Calc. for $C_{34}H_{63}NO_7\cdot 0.8H_2O$: C, 66.77; H, 10.63; N, 2.29%); **19**, yield 1.5 g (46%), m.p. 45–50 °C (Found: C, 70.0; H, 11.6; N, 1.8. Calc. for $C_{46}H_{87}NO_7\cdot 1.3H_2O$: C, 69.99; H, 11.44; N, 1.77%). The corresponding L-aspartate derivatives were prepared as described above: **20**, yield 89%, m.p. 59–62 °C (Found: C, 71.6; H, 11.5; N, 1.9. Calc. for $C_{45}H_{85}NO_7$: C, 71.85; H, 11.39; N, 1.86%); **21**, yield 78%, m.p. 58–61 °C (Found: C, 70.65; H, 11.3; N, 2.1. Calc. for $C_{41}H_{77}NO_7$: C, 70.75; H, 11.15; N, 2.01%); **22**, yield 74%, m.p. 41–46 °C (Found: C, 69.4; H, 10.9; N, 2.25. Calc. for $C_{37}H_{69}NO_7$: C, 69.44; H, 10.87; N, 2.19%); **23**, yield 74%, m.p. 62–66 °C (Found: C, 68.0; H, 10.8; N, 2.4. Calc. for $C_{33}H_{61}NO_7$: C, 67.89; H, 10.53; N, 2.40%).

## Results and Discussion

*Interaction of MB with Anionic Bilayer Aggregates.*—Bilayer formation of all the amphiphiles used were investigated by DSC, which nowadays is well known as a technique for confirming the formation of bilayer aggregates[33–38,56,57] by their phase transitions, although not all the kinds of bilayer aggregate show the endothermic peak and the technique is not a direct observation. The DSC data are summarized in Table 2. The representative aggregation morphologies of **1**, **3**, **8**, **12**, **15**

**Table 2** Phase transition parameters of aqueous dispersions of various amphiphiles and the direction of transition of MB in the presence of the amphiphiles

| Amphiphiles | $n$ | $m$ | Optical isomer | $x$ | Peak-top temperature[a] $T_c$/°C | Transition enthalpy $\Delta H$/kcal mol$^{-1}$ | Direction of transition of MB species in the presence of various amphiphiles $T(<T_c) \longrightarrow (T_c<)T$ [amphiphile]/[MB] = 4[b] | [amphiphile]/[MB] = 20[b] |
|---|---|---|---|---|---|---|---|---|
| **1** | 18 | 2 | L | 7.2 | 58.9, 51.8 | 8.0 | Monomer ⟶ Dimer | Monomer ⟶ Dimer |
| **2** | 18 | 2 | L | 15 | 57.6, 53.0 | 9.0 | Monomer ⟶ Dimer | Monomer ⟶ Dimer |
| **3** | 18 | 2 | L | 22 | 65.8, 55.9 | 7.1 | Dimer ⟶ Monomer | —[h] |
| **4** | 18 | 2 | L | 26 | 64.7, 56.1 | 3.0 | Dimer ⟶ Monomer | —[h] |
| **5** | 16 | 2 | L | 6.6 | 59.4 | 4.3 | Dimer (Trimer) ⟶ Monomer | —[h] |
| **6** | 14 | 2 | L | 7.7 | Not detected | Not detected | Dimer ⟶ Monomer | —[h] |
| **7** | 12 | 2 | L | 7.6 | Not detected | Not detected | Dimer ⟶ Monomer | —[h] |
| **8** | 18 | 1 | L | 5.0 | 72.4 | 5.2 | Dimer ⟶ Monomer | —[h] |
| **9** | 16 | 1 | L | 5.1 | 61.3 | 4.1 | Dimer ⟶ Monomer | —[h] |
| **10** | 14 | 1 | L | 6.2 | Not detected | Not detected | Dimer ⟶ Monomer | —[h] |
| **11** | 12 | 1 | L | 5.6 | Not detected | Not detected | Dimer ⟶ Monomer | —[h] |
| **12** | 18 | 2 | L | (1)[c] | 63.8, 51.3 | 5.6 | Trimer[e] ⟶ Dimer | Dimer ⟶ Monomer |
| **13** | 12 | 2 | L | (1)[c] | 14.5 | 0.3 | Dimer ⟶ Monomer[f] | Dimer ⟶ Monomer |
| **14** | 18 | 1 | L | (1)[c] | 68.6 | 4.9 | Polymer ($n$ > 3)[e] ⟶ Dimer | Dimer ⟶ Monomer |
| **15** | 18 | 2 | L | (1)[d] | 55.1, 62.0 | 8.7 | Monomer ⟶ Dimer | Monomer ⟶ Dimer |
| **16** | 16 | 2 | L | (1)[d] | 44.4, 37.3 | 7.9 | Monomer ⟶ Trimer[e] | Monomer ⟶ Dimer |
| **17** | 14 | 2 | L | (1)[d] | 32.1, 28.1 | 4.5 | Trimer[e,g] ⟶ Dimer | Monomer ⟶ Dimer |
| **18** | 12 | 2 | L | (1)[d] | 23.8 | 1.3 | Dimer ⟶ Monomer[f] | Dimer ⟶ Monomer |
| **19** | 18 | 2 | DL | (1)[d] | 60.0, 49.4 | 8.0 | Trimer[e,g] ⟶ Dimer | Monomer ⟶ Dimer ⟶ Monomer |
| **20** | 18 | 1 | L | (1)[d] | 63.9, 60.6, 50.4 | 5.3 | Dimer ⟶ Monomer | Dimer ⟶ Monomer |
| **21** | 16 | 1 | L | (1)[d] | 45.4, 38.5 | 6.0 | Polymer ($n$ > 3)[g] ⟶ Dimer | Dimer ⟶ Monomer |
| **22** | 14 | 1 | L | (1)[d] | 27.4, 30.1 | 1.7 | Dimer ⟶ Monomer | Dimer ⟶ Monomer |
| **23** | 12 | 1 | L | (1)[d] | Not detected | Not detected | Dimer ⟶ Monomer | Dimer ⟶ Monomer |

[a] Multiple peaks are listed in order of magnitude. [b] [MB] = 1.5 × 10$^{-4}$ mol dm$^{-3}$ = constant. [c] Model compound of $x$ = 1 with a succinate head group. [d] Model compound of $x$ = 1 with a glutarate head group. [e] Absorption maximum of trimeric MB located at 580 nm. See ref. 45. [f] Hardly changed. [g] With a slight shoulder due to polymeric species ($n$-mer, $n$ > 3) at *ca.* 550 nm. [h] — Indicates no measurement because of running out of the telomers.

and **19** in water were observed by transmission electron microscopy (TEM). Amphiphile **1** formed a mixture of short fibrillar aggregates and vesicular aggregates. Amphiphile **8** formed small particles. Amphiphile **7** showed no structure, in accordance with the absence of an endothermic peak due to the gel-to-liquid crystalline phase transition in DSC. Amphiphiles **12**, **15** and **19** formed vesicular aggregates. Amphiphile **1** (average $x$ = 7.2) showed the mixture of rod-like aggregates and vesicular aggregates, somewhat similar to those of **12**, **15** and **19** with a simple head group corresponding to $x$ = 1, whereas amphiphile **3** with $x$ = 22 formed large vesicles with an outer diameter of *ca.* 2000 Å. Other kinds of aggregate were not observed in this system. In the electron micrographs of **3**, the border between the hydrophilic black part and hydrophobic white part of the aggregates of **3** on negative staining is obscure, probably due to the higher degree of polymerization ($x$ = 22) than that of **1** ($x$ = 7.2). The thickness of the aggregates of **1** and **12** corresponds to the bimolecular length as well as to the conventional considerable number of synthetic bilayer membranes described by Kunitake *et al.*[33-38,56,57]

The absorption maxima ($\lambda_{max}$) of MB in water are located at 600 and 660 nm which are assigned to dimeric and monomeric species.[10,20,38,41,45] The dimer/monomer composition depends on the total MB concentration in water and the temperature. In general, when polyanions, *e.g.*, poly(acrylic acid) (PAA) were added to the aqueous MB solution, dimeric and trimeric[45] species became predominant with a slight shoulder due to monomeric species present in the visible absorption spectra. However, when anionic **1** was added to the aqueous MB solution at 20 °C, the dimer/monomer composition hardly changed as shown in Fig. 1, in spite of the general aggregation behaviour of MB in PAA system. However, upon raising the temperature to 60 °C, the dimeric species increased at the expense of the monomeric ones. Such a promotion of aggregation by increasing the temperature is unusual and has

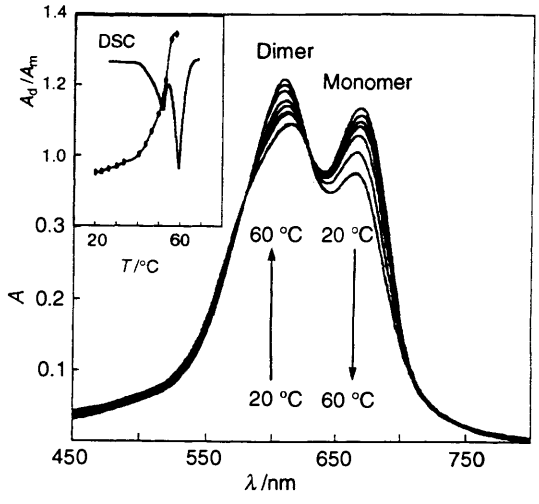



**Fig. 1** Temperature dependence of visible absorption spectra of MB in the presence of **1**: [**1**] = 6.0 × 10$^{-4}$ mol dm$^{-3}$, [MB] = 1.5 × 10$^{-4}$ mol dm$^{-3}$, pH 9.0, 20 °C, path length of quartz cell, 0.1 cm

never been seen in any conventional dye–polyelectrolyte system[1-40] or pure aqueous dye solution system.[10,41-46,48-51] The isosbestic point (625 nm) in Fig. 1 indicates that a simple equilibrium exists between the monomeric and dimeric MB species. Similar phenomena were observed when molar ratios of **1** to MB were varied at 20 °C. The temperature dependence of the spectral change was investigated in detail. As shown in the inset of Fig. 1, the DSC measurement revealed that aqueous **1** aggregates underwent the gel-to-liquid crystalline phase transition in the temperature range 40–65 °C [peak-top temperature ($T_c$); 51 °C and 60 °C]. The temperature dependence of absorption ratios of dimer to monomer is in good accord with the DSC thermogram, indicating that

dimerization of MB is promoted at the same time as the gel-to-liquid crystalline phase transition of aggregates of **1**. In general, however, aggregated dyes are converted into monomeric species upon heating.[16,33,35,36,41,42] According to Nakashima *et al.*, synthetic bilayer membranes induce aggregation in various dyes in the gel state, and these species are converted into monomeric species due to the gel-to-liquid crystalline phase transition of the bilayer host. These changes are reversible and have been observed in various conventional synthetic bilayer systems.[33,35,36] Therefore, such an increase in monomer composition of MB below $T_c$ of bilayer aggregates of **1** is quite extraordinary.

*Dispersion States of Monomeric and Dimeric MB.*—It is known that dyes are molecularly dispersed in media of low relative permittivity.[20,41] The hydrophobic region of molecular assemblies such as conventional micelles and synthetic bilayer membranes are regarded as a kind of hydrocarbon medium, and therefore, it can be assumed that these hydrocarbon assemblies provide a microenvironment of low relative permittivity. It is assumed that monomeric MB species at 20 °C are incorporated into the hydrophobic region of aggregates of **1** and molecularly dispersed. To verify this assumption, the molar ratios of various amphiphiles to MB were varied. As shown in Fig. 2, the composition of monomer increased when the concentration of amphiphile **1** was increased at constant dye concentration ([MB] = 1.5 × $10^{-4}$ mol $dm^{-3}$). When the temperature is increased, the dimer concentration is increased in the liquid crystalline bilayer state irrespective of the molar ratios (although the data are not shown in Fig. 2). Similarly in various amphiphile systems (**5–7** and **12–19**) the dimer-to-monomer transition occurred on increasing the amphiphile concentration at 20 °C. Similar tendencies were obtained in the case of L-aspartate derivatives **8–11**, **14** and **20–23**. It has been reported that metachromatic dye cations tend to cluster linearly along a single polyanion chain, resulting in polymerization as is seen in more concentrated aqueous dye solutions.[15,16] It has also been reported that the dye molecules are not randomly distributed among the available sites but prefer to occupy sites adjacent to each other.[21] Therefore, it seems that addition of an excess amount of polyanion is not very effective at dissociating aggregated dyes into monomers. In fact, in our experiments, when PAA, which cannot form a hydrophobic region in which to incorporate monomeric MB, was used instead of amphiphile **1**, a drastic increase in monomeric species was not observed in the [$COO^-$]/[MB] range 8–800, indicating that an increase in monomer concentration depends not on the [$COO^-$]/[MB] ratio but on the [**1**]/[MB] ratio. In the spectra of PAA–MB, absorption bands due to dimeric MB predominated over those due to monomeric MB (small peaks or shoulders), and their shapes were similar to the spectrum of the **1**–MB system measured at 60 °C in Fig. 1. Therefore, it was concluded that the unusual increase in the concentration of monomeric MB species at 20 °C is due to those monomers incorporated into hydrophobic region of aggregates of **1**, and by raising temperature, dimerization of MB is induced due to electrostatic binding to carboxylates, which is likely to predominate over hydrophobic interactions between MB and **1**. This conclusion is supported by the following, additional experimental data: (1) the molar extinction coefficient ($\varepsilon$) of the visible absorption band due to monomeric MB and the concentration of the monomeric MB are increased as the molar ratio of **1** to MB is increased, and (2) the fluorescence intensity of MB in the presence of **1** at 20 °C is slightly greater than that of MB alone in water.

To investigate the site to which MB binds, amphiphiles **12–23** with simplified head groups were prepared. In general, increasing the molar ratio of amphiphile to dye promotes the

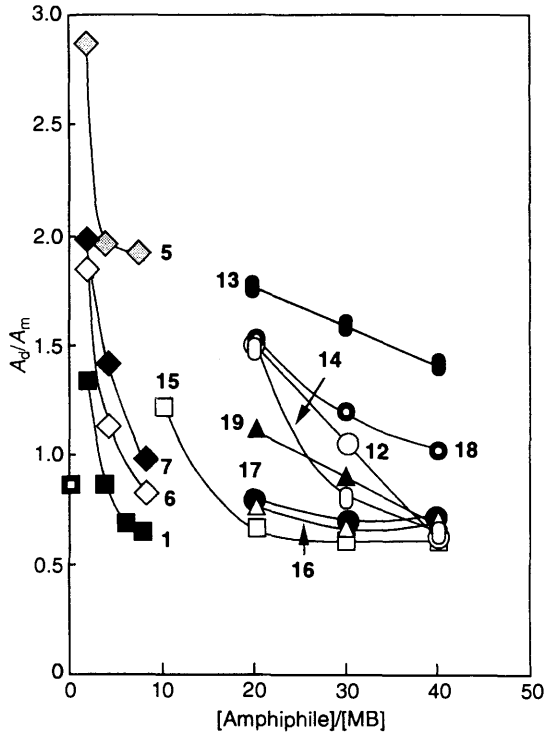



**Fig. 2** Relationship between the molar ratio of various amphiphiles to MB and absorption ratio ($A_{dimer}/A_{monomer}$): [MB] = 1.5 × $10^{-4}$ mol $dm^{-3}$, pH 9.0, 20 °C

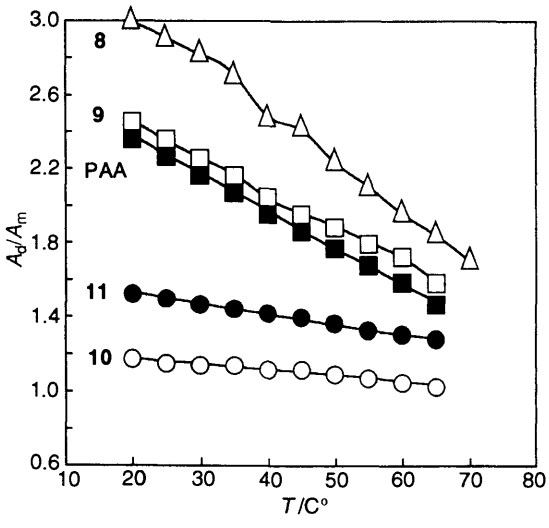



**Fig. 3** Effect of alkyl chain length and L-aspartate residue on absorption ratio ($A_{dimer}/A_{monomer}$) of MB: [amphiphile] = 6.0 × $10^{-4}$ mol $dm^{-3}$, [PAA-unit mol] = 4.3 × $10^{-3}$ mol $dm^{-3}$, [MB] = 1.5 × $10^{-4}$ mol $dm^{-3}$, pH 9.0

transformation of dye species from aggregates into monomers by increasing the number of amphiphiles capable of incorporating the monomers into the hydrophobic region, and the $\lambda_{max}$ value reaches a constant value close to those in organic solvents.[36] As shown in Fig. 2, amphiphile **15** with a glutarate head group converts aggregates MB into monomeric MB much more easily than the corresponding amphiphile **12** with a succinate head group, indicating that monomeric MB resides in the region between the polar carboxylate group and the L-glutamate residue. This is also supported by the following results. (1) When the L-aspartic acid derived telomer **8**, corresponding to **1**, was used, the monomer-to-dimer transition was not observed as shown in Fig. 3. As with the PAA–MB system raising the temperature did not lead to an

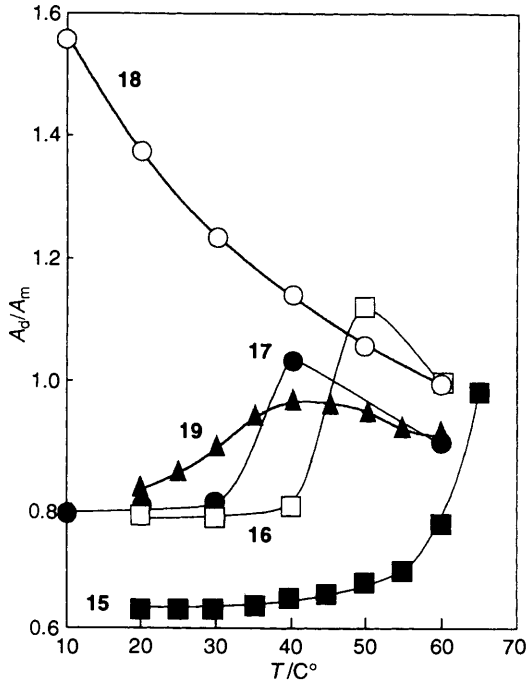



**Fig. 4** Temperature dependence of absorption ratio ($A_{dimer}/A_{monomer}$) of MB in the presence of **15**–**19** with different alkyl chain lengths: [amphiphile] = $3.0 \times 10^{-3}$ mol dm$^{-3}$, [MB] = $1.5 \times 10^{-4}$ mol dm$^{-3}$, pH 9.0

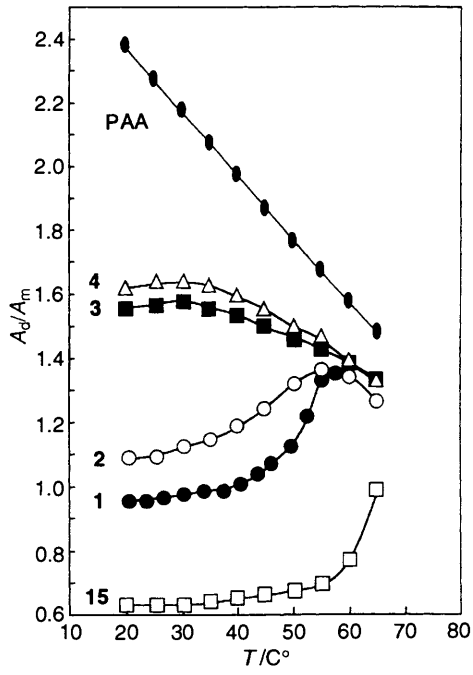
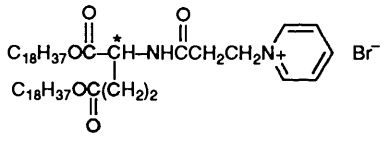



**Fig. 5** Effect of degree of polymerization of the oligo(acrylic acid) moiety on monomer-to-dimer transition of MB: [**1**] = [**2**] = [**3**] = [**4**] = $6.0 \times 10^{-4}$ mol dm$^{-3}$, [**15**] = $3.0 \times 10^{-3}$ mol dm$^{-3}$, [PAA-unit mol] = $4.3 \times 10^{-3}$ mol dm$^{-3}$, [MB] = $1.5 \times 10^{-4}$ mol dm$^{-3}$, pH 9.0

increased dimeric MB concentration, indicating that MB cannot be incorporated into the hydrophobic region of these aspartate amphiphiles, probably because of the tighter molecular packing among the L-aspartate residues,* unlike the corresponding glutamate amphiphile **1**. (2) When a DL-mixture of **19** was used instead of the L-isomer **15**, different temperature dependence as seen from the visible absorption spectra were obtained as shown in Fig. 4, in spite of the phase transition parameters being almost the same, as shown in Table 2. (3) Both the oligomer-type amphiphile **1** and the simplified amphiphile **15** show the same specific incorporation of MB as a monomer, suggesting that MB binds to the carboxylate group which is nearest to the glutamate residue of **1**. In addition, it is notable from the results in Fig. 2 that telomers with oligo-acrylate head groups are more advantageous than the model compounds with one carboxylate corresponding to $x = 1$ for the formation of monomeric species at lower molar ratios, probably because of the looser packing among the glutamate residues. (4) When poly(sodium acrylate) was added to the **1**–MB system, no significant spectral change was observed, indicating that hydrophobic interaction between MB and **1** is one of the necessary conditions. (5) When the cationic, bilayer-forming amphiphile **41** [58] was used instead of **1** or **15**, no interaction with MB was observed: MB showed the same behaviour as that of MB alone in water at the same concentration, indicating that the electrostatic interaction between **1** or **15** and MB is crucial. From these five findings, it is suggested that (1) monomeric MB exists in the hydrophobic region, between the polar head group and amino acid residue, of bilayer aggregates of **1** in the crystalline state, and (2) selection of appropriate spacers [57]




**41**

between the polar head group and the connector [57] moiety leads to facile incorporation of MB in the gel state, as suggested by the difference in behaviour between amphiphiles **12** (succinate type) and **15** (glutarate type) at [amphiphile]/[MB] = 20 (Fig. 2 and Table 2). On the basis of these results, it seems that assemblage of glutamate residues and spacer moieties in a specific manner form appropriate cavities for MB.

*Monomer-to-dimer Transition and Subsequent Dimer-to-monomer Transition during the Heating Process.*—The monomer-to-dimer transition occurred sharply at the critical temperatures. These temperatures correspond to the phase transition temperature ($T_c$) of the respective amphiphiles (Table 2). It is noteworthy that further increase in temperature above $T_c$ showed ordinary dimer-to-monomer transitions as shown typically in Figs. 4 and 5. This is consistent with a mechanism in which (1) the incorporated monomers are released from hydrophobic region of bilayer matrix during the phase transition, resulting in binding to carboxylates and aggregation in hydrophilic region, and (2) the carboxylate–MB complex thus formed in the hydrophilic region can be regarded as an ordinary poly(acrylate)–MB complex, therefore, further increase in temperature leads to the ordinary dimer-to-monomer transition in MB. These interactions are represented schematically in Fig. 6. It is noteworthy that the behaviour of the L-aspartate derivates **20**–**23** in Fig. 7 contrasts with that of the L-glutamate derivatives **15**–**19** in Fig. 4. This provides evidence for the specificity of molecular assemblies formed from L-glutamate amphiphiles.

*Molecular Structure Requirements of Amphiphiles for Monomer-to-dimer Transition.*—*Effect of degree of polymerization*

* This consideration was also supported by the different behaviour of **12** and **14** at a molar ratio of 4 in Table 2, namely, that the L-aspartate derivative **14** could induce MB polymerisation in the gel state although the L-glutamate derivative **12** gave rise only to dimeric MB at most. Presumably, this results from the difference in the distance between adjacent carboxylates on the highly ordered membrane surface of the bilayer aggregates.

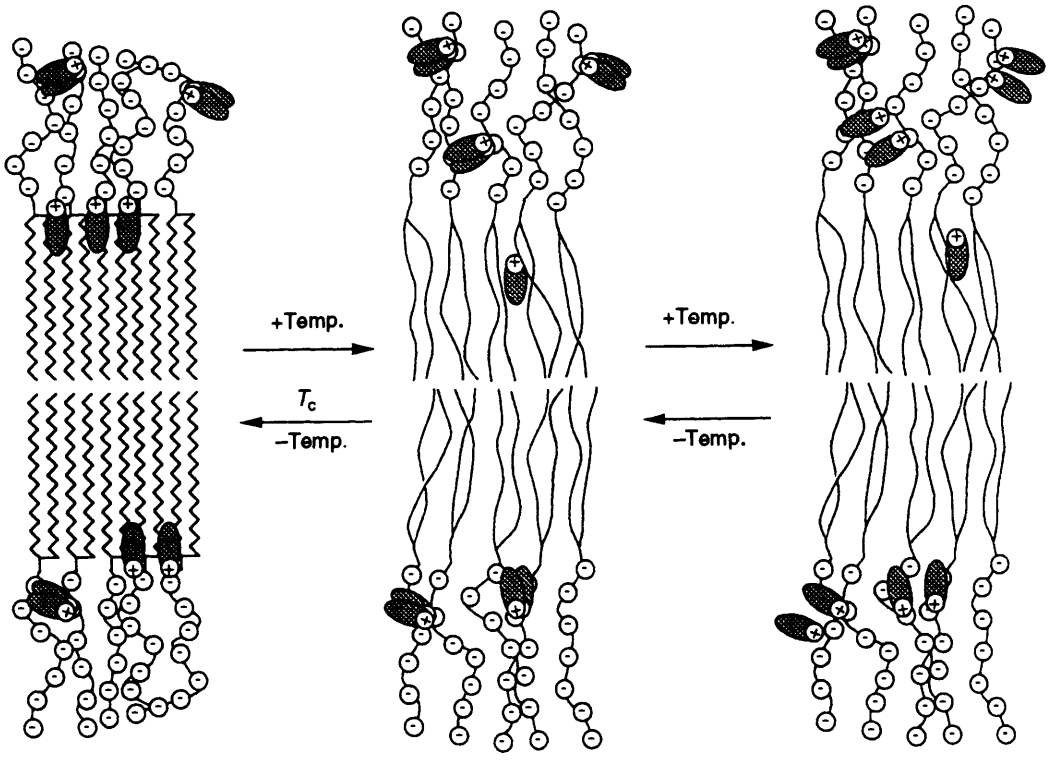



**Fig. 6** Schematic representation of dispersion states of MB in the presence of bilayer aggregates of **1**: ⊕ and ⊖ represent MB molecule and carboxylate, respectively

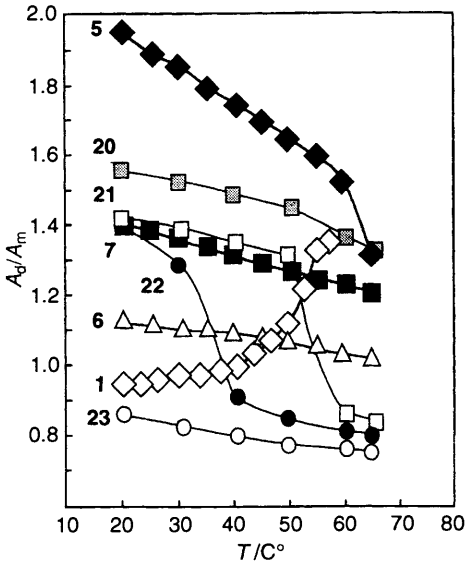



**Fig. 7** Temperature dependence of the absorption ratio ($A_{dimer}/A_{monomer}$) of MB in the presence of **1**, **5**–**7** and **20**–**23** with different alkyl chain lengths: [**1**] = [**5**] = [**6**] = [**7**] = $6.0 \times 10^{-4}$ mol dm$^{-3}$, [**20**] = [**21**] = [**22**] = [**23**] = $3.0 \times 10^{-3}$ mol dm$^{-3}$, [MB] = $1.5 \times 10^{-4}$ mol dm$^{-3}$, pH 9.0

($x$). To clarify the effect of the average degree of polymerization ($x$) of the hydrophilic head group on incorporation of MB, a series of anionic bilayer aggregates from telomers **1**–**3** of different degrees of polymerization and the simplified model amphiphile **15** corresponding to $x = 1$ were investigated. Fig. 5 shows the temperature dependence of absorption ratios of dimer to monomer ($A_{dimer}/A_{monomer}$). **1**–**3** showed monomer-to-dimer transitions. However, the individual behaviour was different. For example, the higher the $x$ value, the higher became the relative amount of dimer to monomer at 20 °C. It is also notable that the temperature dependence of absorption ratios of dimer to monomer in the presence of amphiphiles of higher $x$ values resembles that of PAA, indicating that promotion of monomerization in MB is closely related to the $x$ value in the case of fixed length hydrophobic alkyl chains.

*Effect of alkyl chain length.* The effect of alkyl chain length was also investigated. As shown in Fig. 7, among L-glutamate-derived telomers **1**, **5**–**7** which have almost the same $x$ values (7–8) and different alkyl chain lengths, telomer amphiphiles **5** ($n = 16$), **6** ($n = 14$), **7** ($n = 12$) did not induce the monomer-to-dimer transition of MB upon heating to liquid crystalline states. This indicates that monomer-to-dimer transition is peculiar to the amphiphile **1** among these oligomer-type amphiphiles. Furthermore, as already shown in Fig. 3, none of the L-aspartic acid derived telomers **8**–**11** induced the monomer-to-dimer transition of MB at all.

It is notable that monomeric species formed by increasing molar ratio of amphiphile to MB in the crystalline bilayer state (Fig. 2) are not necessarily converted into aggregated species: bilayer **1** showed the monomer-to-dimer transition under all the molar ratios shown in Fig. 2. On the other hand, amphiphiles with shorter alkyl chains did not induce the monomer-to-dimer transition in MB under any conditions in spite of the initial higher concentration of monomeric species as shown in Figs. 3 and 7.

The interactions with MB are summarized in Table 2. It is notable that, of the telomer amphiphiles, only dioctadecyl L-glutamate type amphiphiles **1** and **2** with lower $x$ values showed obvious monomoer-to-dimer transitions, while amphiphiles **16** ($n = 16$) and **17** ($n = 14$) with glutarate head group showed monomer-to-dimer transitions in contrast with the dimer-to-monomer transition exhibited by the corresponding telomers **5** ($n = 16$) and **6** ($n = 14$), indicating that control of either the average degree of polymerization ($x$) or alkyl chain length ($n$) of glutamate amphiphiles can form specific microenvironments for the monomer-to-dimer transition of MB. Incidentally, HLB [59]

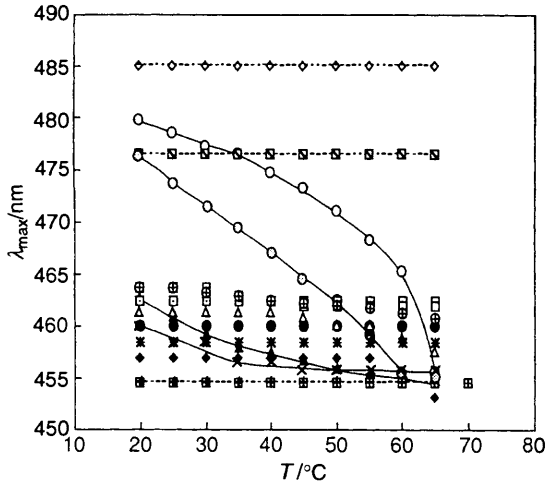



**Fig. 8** Temperature dependence of $\lambda_{max}$ of St-4C$_1$ in the presence of various media: [amphiphile] = 6.0 × 10$^{-4}$ mol dm$^{-3}$, [St-4C$_1$] = 1.5 × 10$^{-4}$ mol dm$^{-3}$, pH 9.0. St-4C$_1$ alone in water (+), methanol (▨), *n*-butanol (◇), in the presence of **1** (○), **2** (⊙), **3** (▲), **4** (×), **5** (△), **6** (□), **7** (⊡), **8** (⊞), **9** (◆), **10** (⊕), **11** (✳) and poly(acrylic acid) (PAA) (●)

(abbreviated form of hydrophile–lipophile balance) values calculated for the free acid form (COOH) could not be interpreted systematically, because amphiphiles derived from various glutamates and aspartates showed quite different behaviour in spite of having almost identical HLB values,* indicating that the packing mode of amphiphiles in molecular assemblies is much more important than the primary structure of the component amphiphiles.

*Other Conditions for Monomer-to-dimer Transitions.—Molar ratio of amphphile to MB.* It is also notable that dilution of MB also effects the monomer-to-dimer transition. For example, even amphiphiles **12** and **14** with succinate head groups, which showed dimer-to-monomer transitions under the same conditions as those in Table 2 could induce monomer-to-dimer transition by diluting MB and increasing amphiphile concentration ([MB] = 7.5 × 10$^{-5}$ mol dm$^{-3}$, [**12**] = [**14**] = 1.8 × 10$^{-3}$ mol dm$^{-3}$, [amphiphile]/[MB] = 24). This indicates that once MB can be incorporated into the crystalline state of bilayer aggregates of **12** and **14** with longer alkyl chains, it can aggregate according to the phase transition regardless of the kind of head group and amino acid residue. It was noted that longer alkyl chains are required because amphiphile **13** with shorter alkyl chains did not induce the monomer-to-dimer transition in MB under the same conditions. This indicates that tighter molecular packing by lengthening two alkyl chains is a necessary condition for incorporation of dyes into crystalline bilayer states.

*Evidence by using the solvatochromic dye* (St-4C$_1$). To confirm the above-mentioned incorporation mechanism independently, the solvatochromic dye (St-4C$_1$) was used instead of the metachromatic and non-solvatochromic MB described above. St-4C$_1$ shows the $\lambda_{max}$ change according to the solvent polarity. For example, $\lambda_{max}$ of St-4C$_1$ in water, methanol and *n*-butanol are at 455, 477 and 485 nm, respectively, regardless of the temperature. This is ascribed not to thermochromism but to solvatochromism. As shown in Fig. 8, the temperature dependence of the $\lambda_{max}$ changes, which reflect the change in the microenvironment in which the dye exists correlates well with the results in Figs. 3–5, and 7.

In our previous communication,[47] we reported that monomeric MB is incorporated into the crystalline hydrophobic region of bilayer aggregates as if there were no electrostatic interactions between MB cations and the carboxylate groups. According to our current findings, as shown in Fig. 6, it is correct to say that the monomeric species are bound to carboxylates from the inner site of the crystalline bilayer membranes. Our supposition that monomeric MB exists near the glutamate residue in the crystalline bilayer state with electrostatic interactions is also supported by the data in our previous communication (see Fig. 4).[52] Thus, the results of experiments using solvatochromic St-4C$_1$ together with those with metachromatic MB support the incorporation of cationic dyes as monomers in the crystalline hydrophobic region of bilayer membranes formed from the amphiphiles such as **1** and **15**.

## Conclusions

It may be concluded that the monomeric and dimeric MB species in crystalline, anionic bilayer aggregates of L-glutamate-derived **1** can be described as follows: the former is incorporated at the inner surface by binding to the carboxylate nearest to the L-glutamate residue in the bilayer aggregates, and the latter is bound electrostatically to carboxylates without hydrophobic interactions. According to the gel-to-liquid crystalline phase transition, the incorporated MB monomers are converted into dimeric species by binding to carboxylates electrostatically in the hydrophilic region. This results in further promotion of dimerization of MB. However, further increase in temperature leads to the ordinary dimer-to-monomer transition of bound MB as well as the various conventional polyanion–MB systems including PAA and synthetic bilayer membranes.

The molecular structure requirements of anionic, amphiphilic, double-chained telomers for the monomer-to-dimer transition can be summarized as follows. (1) When the structures of the head-groups are similar, a longer alkyl chain is preferred. (2) When the number of carbons (*n*) of hydrophobic alkyl chain is the same, a lower average degree of polymerization (*x*) is preferred in the case of the same kind of amino acid residue (connector). (3) The type of the amino acid residue in the connector moiety is crucial for the formation of specific hydrophobic sites for incorporation of MB, since it was found that L-aspartate derivatives were not able to incorporate MB under the same conditions. (4) Selecting appropriate molar ratios of amphiphile to MB produces monomeric species in the crystalline state and heating up to the liquid crystalline state leads to the monomer-to-dimer transition in the case of amphiphiles with longer alkyl chains regardless of the amino acid residues. (5) Amphiphiles with shorter alkyl chains (such as **7** and **13**) induced no monomer-to-dimer transition on heating regardless of the initial composition of monomeric species in the crystalline state, indicating that tight molecular packing is one of the necessary conditions for the incorporation of MB. (6) Bilayer aggregates formed from amphiphiles with glutarate head groups incorporate MB much more easily than do the corresponding bilayer aggregates of succinate amphiphiles into the hydrophobic region of the crystalline bilayer aggregates, which suggests that the distance between head group and L-glutamate residue is significant. (7) L and DL-glutamate derivatives give the different results for the transition behaviour of MB, suggesting that packing mode among laterally arranged glutamate residues is significant. It is also suggested that the incorporated MB monomers reside near the glutamate residue and are not deeply incorporated.

These results obtained using the metachromatic dye, MB,

* HLB (calculated as free acid-form): 10.9 for **1**, 14.5 for **2**, 16.5 for **3**, 17.3 for **4**, 11.2 for **5**, 12.6 for **6**, 13.5 for **7**, 9.5 for **8**, 10.3 for **9**, 11.9 for **10**, 12.3 for **11**, 5.8 for **12**, 7.9 for **13**, 5.9 for **14**, 5.7 for **15**, 6.2 for **16**, 6.8 for **17**, 7.7 for **18**, 5.7 for **19**, and 5.8 for **20**, 6.3 for **21**, 7.0 for **22**, 7.9 for **23**.

were confirmed independently by use of the solvatochromic dye, St-4$C_1$.

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