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Hirotaka Ihara[a]; Hiroshi Hachisako[a]; Chuichi Hirayama[a]; Kimiho Yamada[a]
[a] Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto, Japan

To cite this Article Ihara, Hirotaka , Hachisako, Hiroshi , Hirayama, Chuichi and Yamada, Kimiho(1987) 'Specific bindings of methyl orange to chiral bilayer membranes with β-alanyl-L-glutamoyl head groups [1]', Liquid Crystals, 2: 2, 215 — 221
To link to this Article: DOI: 10.1080/02678298708086292
URL: http://dx.doi.org/10.1080/02678298708086292

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LIQUID CRYSTALS, 1987, VOL. 2, NO. 2, 215–221

Specific bindings of methyl orange to chiral bilayer membranes with β-alanyl-L-glutamoyl head groups [1]

by HIROTAKA IHARA, HIROSHI HACHISAKO, CHUICHI HIRAYAMA and KIMIHO YAMADA

Department of Applied Chemistry, Faculty of Engineering, Kumamoto University Kumamoto 860, Japan

(*Received 4 July 1986; accepted 7 October 1986*)

Interactions between methyl orange and cationic bilayer membranes constructed from a dialkyl amphiphile with a β-alanyl-L-glutamoyl head group were investigated. The chiral bilayers undergo a gel to liquid crystal phase transition to give rise to the reversible chirality change from -8×10^4 to $+11 \times 10^4$ deg cm^2 dmol^{-1} near the phase transition temperature. Methyl orange combines with the cationic membranes to give three kinds of spectra, belonging to the surface-oriented chiral dimer and the monomer and incorporated achiral monomer, in response to the phase transition of the bilayer membrane.

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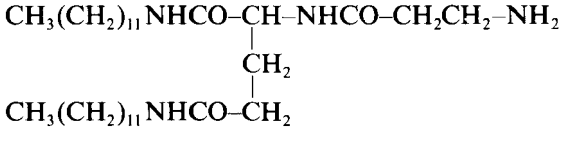
1. Introduction

It is known that a number of achiral dyes combine to polyions such as polyamino acids including proteins and chiral aggregates to provide optical activity [2–10]. The binding mode of the dyes depends on the chemical and structural asymmetries of the polyions. For example, poly(L-lysine) induces the asymmetric *H*-like stacking of methyl orange [5, 6]. On the other hand, cyanine dyes combine with trimethylammonium chiral bilayers to provide the *J*-like aggregate of dyes in a gel state [7–10].

In this article, we describe the specific dispersion behaviour of methyl orange in the presence of a new chiral amphiphile with a β-alanyl-L-glutamoyl head group, which produces unique micro-environments. The amphiphile is dispersed in water at pH 2–8 to form bilayer membranes in which primary ammonium groups are placed in order on the membrane-surface. Methyl orange combines with the membranes to give three kinds of spectra in response to the physical states of the membranes.



(G-1)

2. Experimental

2.1. *Materials*

Amphiphile (G-1), N-aminopropanoic-L-glutamic acid didodecyl amide, was synthesized as follows: L-glutamic acid didodecyl amide (G-2) was prepared by coupling of *N*-benzyloxycarbonyl-L-glutamic acid and dodecyl amine and by the debenzyloxycarbonylation and described briefly elsewhere [11]; m.p. 115–118°C. The dodecyl amide was coupled with *N*-benzyloxycarbonyl β-alanine by use of diethylphosphoro

cyanidate and recrystallized from methanol to give a waxy solid; yield 93 per cent. Analytically calculated for $C_{40}H_{70}N_4O_5$: C, 69·93 per cent; H, 10·27 per cent; N, 8·16 per cent. Found: C, 69·60 per cent; H, 10·25 per cent; N, 7·95 per cent. This compound was debenzyloxycarbonylated on Pd/H_2. The removal of the *N*-benzyloxycarbonyl group was followed by I.R. spectroscopy and recrystallized from methanol to give a colourless powder **(1)**; yield 90 per cent, m.p. 195–196°C. (G-1) was identified by N.M.R. and I.R. spectroscopy and elemental analysis. Calculated for $C_{32}H_{64}N_4O_3 3H_2O$: C, 63·30 per cent; H, 11·63 per cent; N, 9·23 per cent. Found: C, 63·37 per cent; H, 11·23 per cent; N, 9·24 per cent.

Methyl orange (MO) was obtained from Wako Pure Chemicals Ind. Co., Ltd and recrystallized from water.

2.2. *Measurements*

The aqueous solutions for differential scanning calorimetry (D.S.C.), circular dichroism (C.D.) and absorption spectroscopy were prepared by sonicating (2 A, 1–2 min), using an ultrasonic generator with a 4280 S type vibrator (Kaijo Denki Co., Ltd). D.S.C. thermograms were obtained using a heating rate of 2°C/min with a Seiko I & E SSC–580/DSC–10 instrument according to Okahata *et al.* [12]. C.D. spectra were measured with a JASCO J–500C spectrophotometer. Absorption spectra were measured with a JASCO UVIDEC 660 spectrophotometer. The pH value of each solution was determined with a Toa digital pH meter (type HM–18 ET) with an accuracy of better than $\pm 0{\cdot}05$.

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3. Results and discussion

3.1. *Characterization of (G-1) aggregates*

(G-1) was dispersed in water at pH 2–8 by sonicating to give a slightly turbid solution. Generally it is known that amphiphiles with double-chain alkyl groups can form bilayer membranes which show a gel to liquid crystal phase transition. The D.S.C. of aqueous (G-1) aggregates at pH 7·2 gave two endothermic peaks (32°C and 41°C) as shown in figure 1. Some examples with multiple peaks are well known as pretransitions or similar phenomena [12, 13].

The formation of the bilayer structure is made clear by direct observation of the molecular layers by electron microscopy. In the case of cationic amphiphiles such as (G-1), uranyl acetate is used as a stain. However, the original morphology from (G-1) may be difficult to reproduce because of complex formation with metal ions and hydrophilic amino groups of (G-1) [14]. For reference it is noted that aqueous (G-1) at pH 2 and 7 shows a single-walled tubular morphology with a diameter 150–200 Å and a length 400–1000 Å in the presence of uranyl acetate. The layer thickness is 50 Å to 60 Å and nearly equal to the bimolecular length of (G-1). L-Glutamic acid didodecyl amide (G-2) without the β-alanyl-group shows typical multi-walled vesicles under the same conditions [15].

Chiral amphiphiles show enhanced optical activity by self-assembling to bilayer membranes in aqueous solutions [16, 17], therefore, C.D. spectra were examined. The (G-1) bilayers gave large C.D. bands as shown in figure 2. Since the C.D. decreased remarkably on addition of ethanol, the enlargement of the optical activity is attributed to molecular assembling of (G-1) in aqueous solution. The shape of the C.D. spectra is remarkably dependent on the temperature. According to Kunitake *et al.* [16, 17], a large C.D. is observed only in the gel state: in the liquid crystal state, chiral

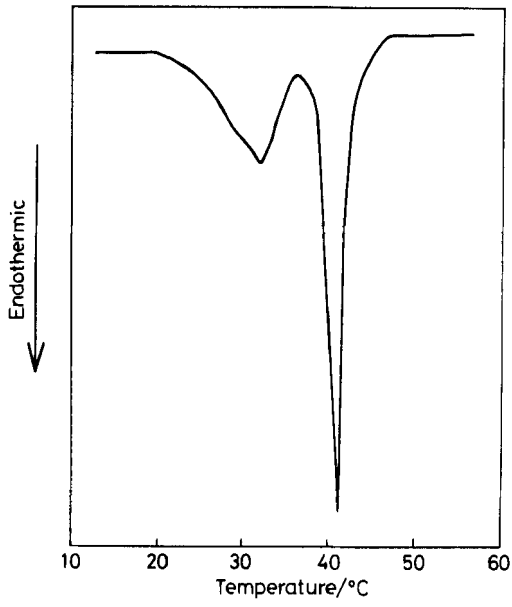

Figure 1. D.S.C. thermogram of aqueous (G-1) aggregates.

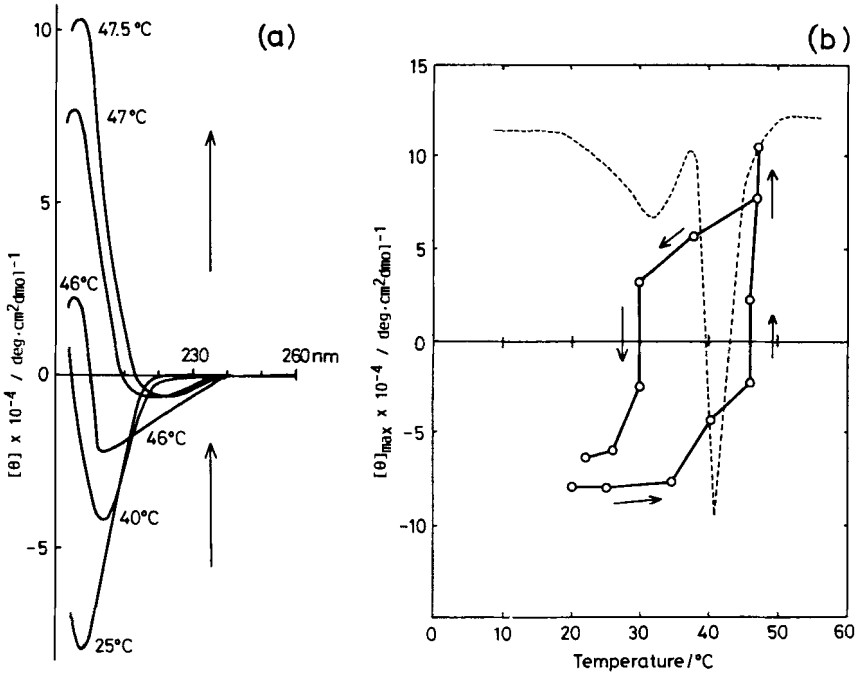

Figure 2. Temperature dependence of C.D. spectra (*a*) and $[\theta]_{max}$ values (*b*) of aqueous (G-1) bilayers. pH 7·2, [G-1] = $6{\cdot}2 \times 10^{-4}$ mol l^{-1}.

stacking among amphiphiles decreases with deterioration of molecular orientation to lower the optical activity to that of the homogeneous state. On the contrary, the (G-1) bilayers showed a drastic chirality change from -8×10^4 to $+11 \times 10^4$ deg cm^2 dmol^{-1} below and above the critical temperature. This change is

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reversible but with a thermal hysteresis (cf. figure 2). The appearance of a large C.D. in the liquid crystal state may be related to multiple amide bonding of the (G-1) molecule. The large C.D. belongs to the absorption band of the carboxamide groups and disappears in ethanol which destroys a highly ordered structure. Consequently, it indicates that chiral stackings among the (G-1) molecules are produced both in the gel and liquid crystal state, but the direction of the chiral interaction is different. Although the formation of a 'hydrogen belt' in bilayer membranes has been discussed by Murakami *et al.* [18] and Kunitake *et al.* [19], the reversible change of optical activity such as in the case of the (G-1) bilayers is unprecedented.

3.2. *Spectral behaviour of MO*

The absorption spectra of MO changed remakably in the presence of the (G-1) bilayers. As shown in figures 3 and 4, the change of λ_{max} is closely related to the phase transition of the (G-1) bilayers: in the gel state condition, a sharp peak (B) with a λ_{max} value of 357 nm was observed together with a broad peak (A) with a λ_{max} value of 455 nm, which was a normal species of MO; but in the liquid crystal state condition these peaks disappeared to shift to a new peak with a λ_{max} value of 400 nm. It is known that MO combines with polycations such as poly(L-lysine) [4, 6] and chiral ammonium bilayers [7] in the gel state to form chiral dimers. However, the two systems differ in their detailed behaviour and the matrix effect of the (G-1) bilayers in the gel state resembles that of poly(L-lysine) in the sharpness of absorption spectra and the shape of induced C.D. spectra (cf. figure 5). According to Hatano *et al.* the induced chirality in a poly(L-lysine)-MO system corresponds to the S-chirality resulting from coupling between the transition dipole moments of the MO molecules [4]. Therefore, it is considered that the (G-1) bilayer produces a micro-environment similar to that of poly(L-lysine) and species B combined with this is a S-chiral dimer.

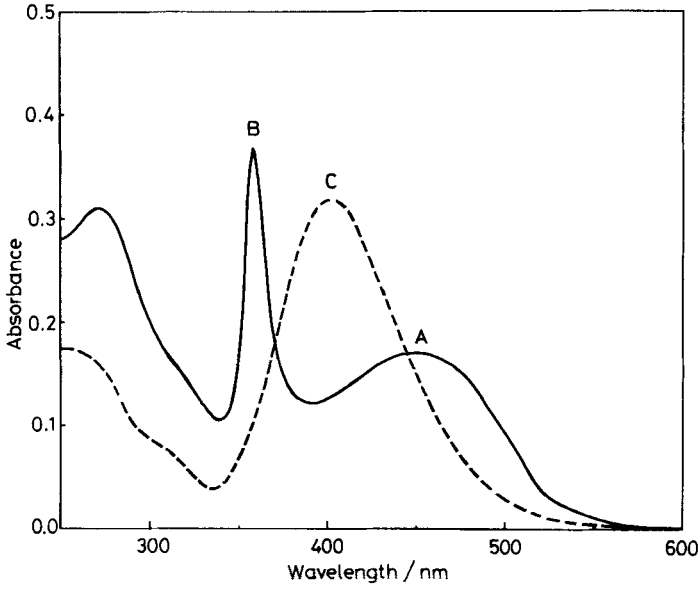

Figure 3. Typical absorption spectra of MO in the presence of (G-1) bilayers. pH 7·2, [G-1] = 6·0 × 10^{-4} mol l^{-1}, [MO] = 1·2 × 10^{-4} mol l^{-1} ——, 20°C, – – – –, 50°C.

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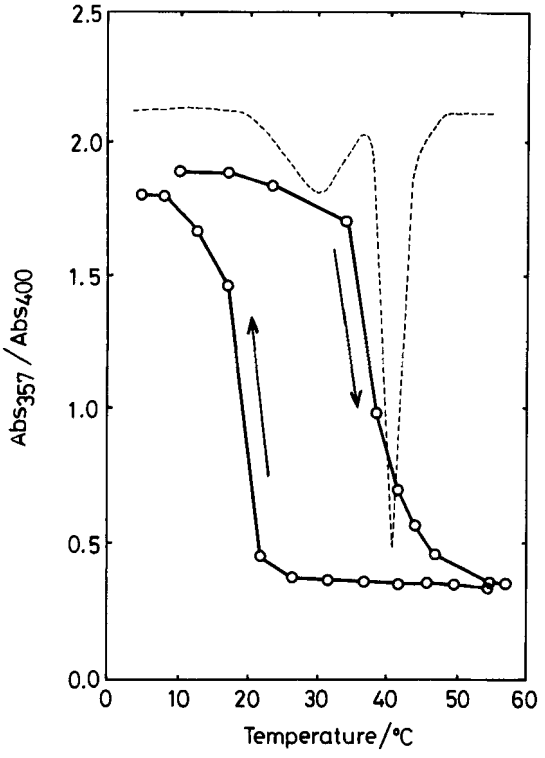

Figure 4. Relationship between the temperature and the absorption ratio (Abs_{357}/Abs_{400}). pH 7·2, [G-1] = 6·0 × 10^{-4} mol l^{-1}, [MO] = 1·2 × 10^{-4} mol l^{-1}.

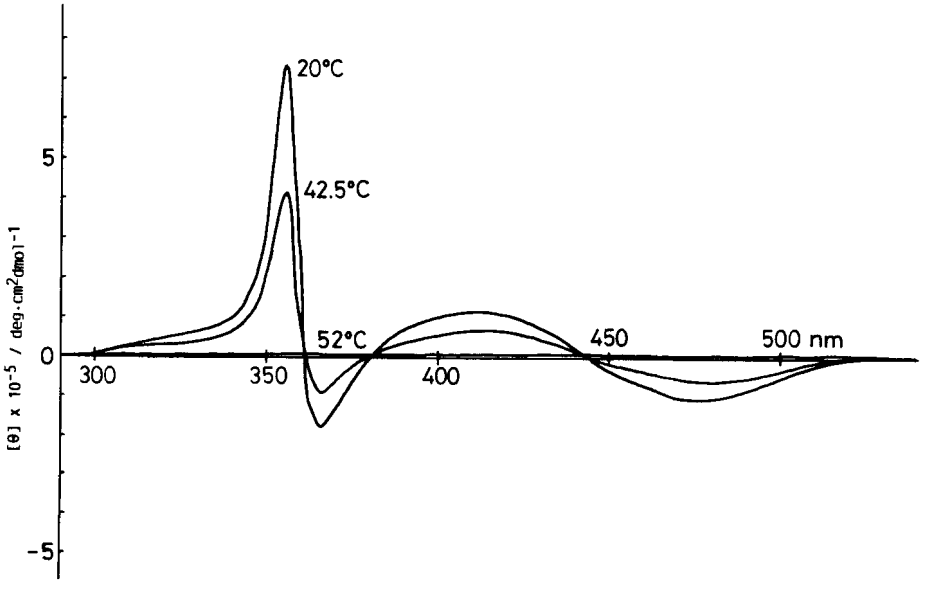

Figure 5. Typical induced C.D. spectra of MO in the presence of the (G-1) bilayers. pH 7·2, [G-1] = 6·0 × 10^{-4} mol l^{-1}, [MO] = 1·2 × 10^{-4} mol l^{-1}.

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On the other hand, the (G-1) bilayers possess unique functions which cannot be found in poly(L-lysine). As shown in figure 5, monomeric MO (A) also gives C.D. spectra with θ_{max} values of 412 nm and 477 nm corresponding to the absorption band in the gel state temperature range. Consequently, it is estimated that species A is also electrostatically combined to the (G-1) bilayer. Poly(L-lysine) and conventional chiral bilayers do not induce chirality in the case of monomeric MO.

Absorption and induced C.D. spectra of MO are dependent on the molar ratio for the matrix (G-1) molecules. In the gel condition, both chiral monomer A and dimer B disappeared with the increase of the molar ratio of (G-1) molecules (for example, [MO]:[G-1] = 1:100) to change to species C with the λ_{max} value of 400 nm analogous to the case under liquid-crystalline conditions. Conversely, dimer B was induced even at the liquid crystal temperature when the molar ratio of MO increased ([MO]:[G-1] = 1:2). In addition, species C is achiral and the spectrum is similar to that of MO in organic solvents. Therefore, it is estimated that species C is monomeric MO incorporated into the (G-1) bilayer.

In conclusion, the new amphiphile (G-1) can form bilayer membranes in aqueous dilute solution which give rise to the reversible chirality change, which cannot be found in conventional systems, in response to the phase transition. The three kinds of MO (species A, B and C) produced by interacting with the bilayers are schematically illustrated in figure 6. When the (G-1) bilayer is in the rigid (gel) state, most MO are combined on the highly ordered membrane surface to induce the stacking of MO molecules. Optical activity is induced to both monomeric A and dimeric B by the chiral perturbation from the (G-1) bilayer. The low solubility of MO into the hydrophobic layer in the gel state may be related to the 'hydrogen belt' among amide bondings of the (G-1) bilayers. When the orientation of the (G-1) bilayers is

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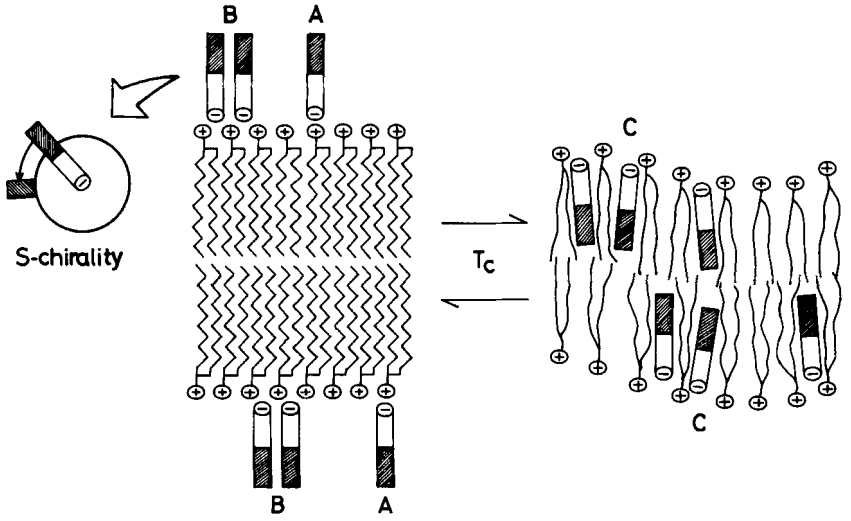
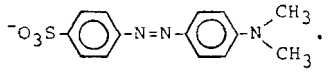


Figure 6. Schematic illustration of the dispersion state of MO in the presence of the (G-1) bilayers, where T_c is the phase transition temperature from gel to liquid crystal states and ⊖▭▨ stands for a MO molecules,



loosened above the critical temperature to a liquid crystal state, MO is incorporated into the comparatively hydrophobic site of bilayers to give the spectrum C. This species is monomeric and achiral. The drastic change from A and B to C is reversible depending on the temperature.

The previously mentioned unique behaviour of MO is attributable to the binding to specific assemblies in which primary ammonium groups are placed in order on the surface, and its physicochemical states are regulated by bilayer properties.

This work is partially supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of Japan.

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