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Crystallization of Two-Dimentional Chromophore Arrays in Aqueous Chiral Bilayer Membranes

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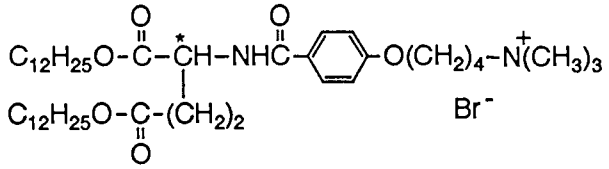
Crystallization behavior for the alignment of an aromatic chromophore in the aqueous chiral bilayer was examined based on its enhanced circular dichroism (CD). It was found that the existence of crystalline phase in the fluid bilayer promotes rapid crystallization of chromophere arrays in the bilayer. We also found that CD spectra oscillate at phase transition region of the bilayer.

Studies on the regulation of two-dimentional (2-D) alignments of chromophores in organized bilayer membranes have been the subject of considerable attention.[1-6] As we reported before,[1] L-glutamate-based chiral bilayer of 1 which contains phenyl moiety as a chromophore exhibits marked enhancement of CD at temperatures below phase transition and the enhancement disappears according to the phase transition of the bilayer. In this communication we report crystallization (transition from liquid crystalline- to crystalline phase) of 2-D chromophore arrays in the aqueous chiral bilayer of 1 based on its enhanced CD. During the study we have found that the CD spectra of the bilayer oscillate at phase transition region. A mechanism for this behavior is also described.



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Chiral amphiphile 1 was synthesized by the same method described elsewhere[1] and was identified by ^{1}H-NMR, IR and elemental analyses. Aqueous bilayer of 1 was prepared by gentle warming with hot water.

Figure 1 shows differential scanning calorimetry (DSC) thermograms

(instrument: Shimazu DSC-50 equipped with LTC-50 as a cooling unit) of bilayer 1 (40 mM) at heating and cooling cycles. Phase transition temperature (Tc) at heating cycle is observed at 30.7 °C which is identical with the value we reported before.[1] Cooling cycle measurement gave exothermic peak at 7.6 °C. Twenty-three degree's temperature difference between endo- and exothermic peaks is not unusual both for synthetic[7] and liposomal[8] bilayer membranes.

Figure 2 shows the suppression and the recovery of CD intensity of aqueous bilayer of 1 (1.0×10^{-4} M). CD behavior for the heating cycle was the same as in the previous paper[1]; drastic enhancement observed at temperatures below Tc ($[\theta]$=-400000 deg·cm^2·decimol^{-1} at 260 nm) disappeared completely at 29 °C indicating that the enhancement is derived from strong chiral interaction between the chromophores of the bilayer.

Trace B-D show cooling cycles. Except trace E, the bilayers were kept at each start temperatures for 30 min. Trace B exhibits the cooling cycle from 35 °C which corresponds to terminal temperature of the phase transition. It is clear that CD intensity does not recover at all even at 8 °C and supercooling at 3 °C was required for 100%- recovery. This result is in good correspondence with that of DSC shown in Fig. 1. Both in heating and cooling cycles, the change in the chromophore orientation in the bilayer is associated with the conformational change of dialkyl chains. Measurements which start cooling at T > 35 °C gave similar results with trace B. Trace C shows the cooling cycle from 30 °C, almost peak maximum temperature of DSC peak of 1 bilayer; here almost no CD recovery was observed until 22 °C. The data of trace E and F represent that cooling start temperatures are very sensitive to the CD recovery.

The results mentioned above indicate that at once the bilayer was heated at temperatures above the terminal temperature of Tc, where the crystalline phase disappears completely, large supercooling is required for the crystallization of the aromatic moiety in the bilayer, while no such supercooling is needed when cooling is started from mixed phase of crystalline- and liquid crystalline phase. This suggests that the crystal phase in the mixed phase acts as "crystalline nucleus" for the crystallization of the chromophore arrays.

We have found that CD spectra of 1 bilayer exhibit oscillation behavior at phase transition region both in heating and cooling cycles. Typical data are shown in Fig. 3. Following reasons are considered for the explanation of the oscillation behavior: i) influence of CD with very small temperature change, ii) self-oscillation of the bilayer and iii) a mechanical noise. In this experiment it would be very difficult to propose the mechanism for the spectral self-oscillation. Mechanical noise

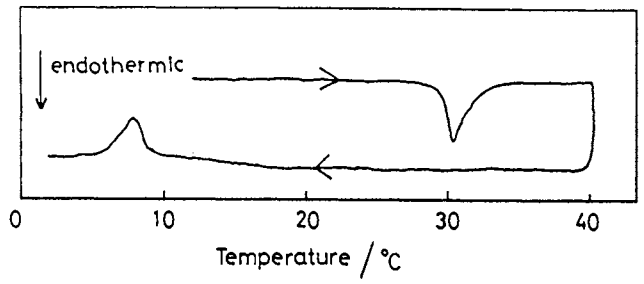

Fig. 1. DSC thermograms of an aqueous bilayer of 1 (40 mM) at heating and cooling cycles.

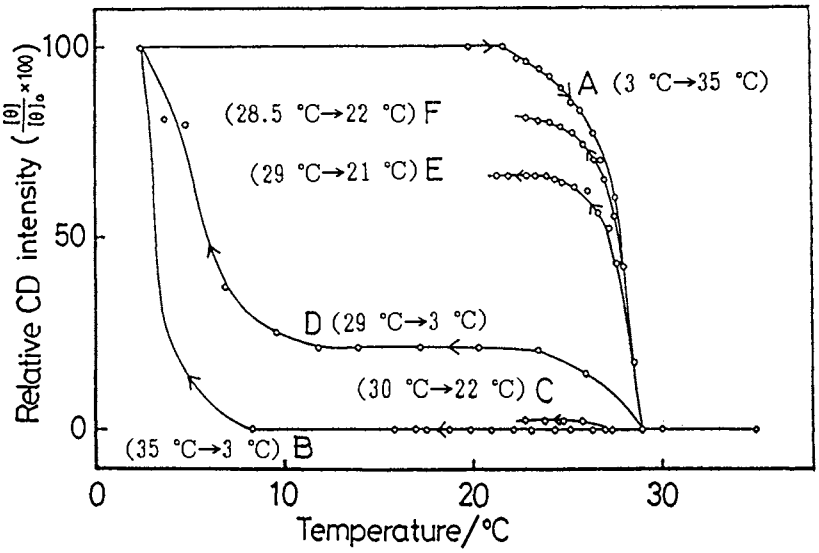

Fig. 2. Suppression and Recovery of CD spectra of an aqueous bilayer of 1 (1.0×10^{-4} M). $[\theta]_\circ$ corresponds 400000 deg·cm^2·decimol^{-1} which is CD intensity at the rigid crystalline phase bilayer and $[\theta]$ is the CD intensity at given temperatures.

should be excluded because the oscillation is not spike-like and it occurs only phase transition region. Reason i) is most plausible and thus in order to ascertain this, we measured the time courses of the minute temperature change in an UV quvette with a precise thermometer (Yokogawa Denki, Model IM2585-01 digital thermister thermometer). The sensor tip of the thermometer was immersed in the center or an inner wall of the quvette(path length, 10 mm) and measurements were conducted every 5 °C from 20 to 45 °C. An example for the time course of the temperature change was shown in Fig. 4, indicating that temperature changes with time at the center and at the inner wall were 0.02 °C and 0.08 °C, respectively. Temperature changes within the range of 20-45 °C are: (at the quvette center) 0.02-0.03 °C and (at an inner wall) 0.08-0.09 °C.

It is evident by comparison with the data of Figs. 3 and 4 that the CD spectral oscillation and the time course of the temperature of the solution is closely associated. As shown in Fig. 2, Tc dependence of the CD spectra is dramatic; heating from 27.9 to 28.5 °C causes 110000 deg·cm^2·

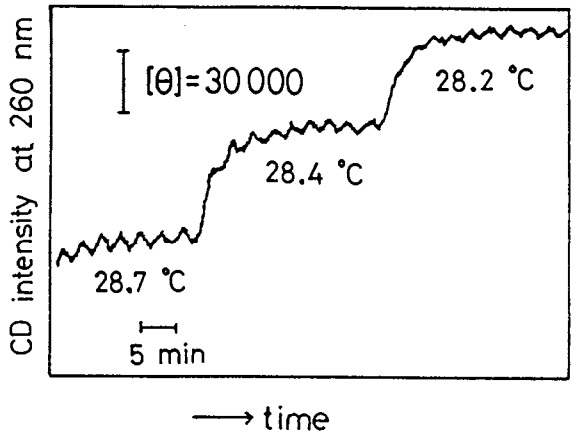

Fig. 3. A typical example of a CD spectral oscillation of an aqueous bilayer of 1 (1 × 10^{-4} M).

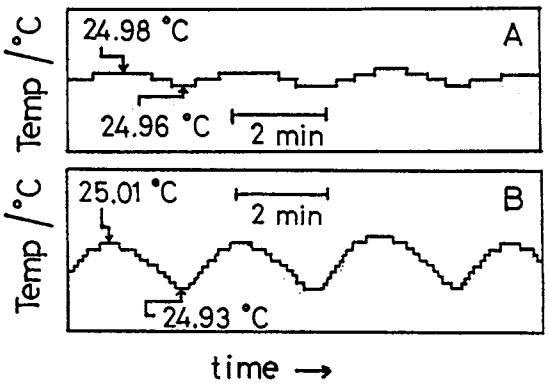

Fig. 4. Time course of temperatures at the center (A) and an inner wall (B) of a UV-quvette.

decimol^{-1}-change, or 9200 deg·cm^2·decimol^{-1}/0.05 °C. This change is not in conflict with the amplitude of the oscillation exhibited in Fig. 3.

In conclusion, we demonstrated that the existence of the crystalline phase in the fluid bilayer phase which would act as "crystalline nucleus" promotes rapid crystallization of the 2-D alignment of the chromophore in the aqueus chiral bilayer membrane. We also found that the CD spectra of the chiral chromophoric bilayer is responsive dynamically to the very small temperature change (≈0.05 °C) in the solution.

References

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