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Temperature-induced Chirality Reversal of Induced Circular Dichroism of Premicellar Aggregates of Acridine Orange Derivatives and Dodecanoyl-L-threonine in Aqueous Solution

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Abstract Circular dichroism (CD) and absorption spectra were measured for acridine orange derivatives: 3,6-bis(dimethylamino) acridine (AO), 3,6-bis(dimethylamino)-10-methylacridinium bromide (C_1AO), and 3,6-bis(dimethylamino)-10-dodecylacridinium bromide (C_{12}AO) in aqueous dodecanoyl-L-threonine (C_{12}Thr) solutions at 30, 40, 50 and 60°C and pH 8-9. CD spectra were not induced for AO and C_1AO in C_{12}Thr aqueous solutions. Induced circular dichroism (ICD) based on the exciton interaction was found for premicellar aggregates of C_{12}AO with C_{12}Thr, but the micellar aggregates failed to induce circular dichroism for solubilized C_{12}AO at a higher concentration than C_{12}Thr critical micelle concentration. The maximum ICD intensity was observed for 1:1.2 aggregates of C_{12}AO and C_{12}Thr. The ICD spectrum indicated negative chirality at 30°C, but positive chirality at 50°C. The chirality transition occurred at 40~45°C. The slow change in both the absorption and ICD spectra is ascribed partly to the rearrangement of dye alignment and partly to the growth of the aggregate; the system reaches final phase separation after a few days.

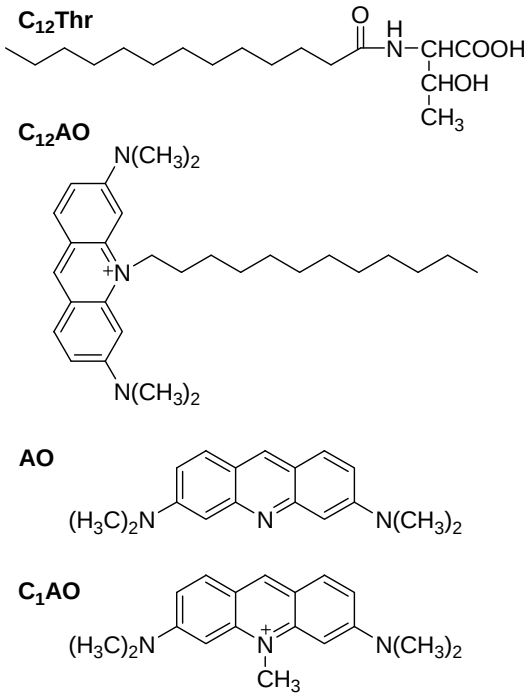
Keywords Induced Circular Dichroism, Acylamino acid, Alkyacridine orange, Aggregation of ionic complex, Absorption spectra

Introduction

Acylamino acids have special potential as amphiphiles because of the chirality of their hydrophilic head and the possibility of hydrogen bond bridges forming in the aggregate state. Circular dichroism (CD) has been observed for the liquid crystal state and lamellar phase in organic solvents.[1,2] The chirality of the aggregates reflects the molecular chirality. The chirality of the (–) isomer aggregate is the reverse of that of the

(+) isomer aggregate. In aqueous solutions, however, only a weak CD spectrum is observed for the micelles of acyl-L-glutamic acids. It is thought that strong hydrogen bonding of water molecules disturbs the formation of chiral aggregates of the acylamino acid.

Circular dichroism can be induced in achiral molecules that are located in a chiral environment and aggregate with a chiral alignment.[3,4] This induced circular dichroism (ICD) has been observed for many dye aggregates with polymers that have a chiral conformation, such as DNA,[5-11] polypeptides,[12-16] and oligo- and polysaccharides.[17-25] However, there are few reports of ICD in aggregates of chiral amphiphiles.[1,2,26-28] ICD has been seen with anthracene and azurene in chiral lyotropic liquid crystals of benzene/acylglutamic acid.[1,2] In aqueous solutions, however, no ICD of achiral dyes solubilized in chiral aggregates of acylamino acid has been observed, presumably owing to the mobility of the solubilized dye and the strong hydrogen bonding of the acylamino acid with water, which weakens the formation of chiral aggregates of the acylamino acids via hydrogen bond bridges. Dyes form premicellar aggregates with a surfactant of opposite charge. Sodium dodecyl sulfate forms a complex with cationic dyes, such as methyl orange and acridine orange.[29-34] Spectroscopic studies have demonstrated dye aggregation in premicellar aggregates with surfactants.[33-36] Sato et al.[35] called premicellar aggregations dye-rich induced micelles, and found enhanced fluorescence energy transfer between donor and acceptor dyes. We investigated ICD in dyes solubilized in chiral micelles of acylamino acid, and found no ICD for the solubilized state of the dye in acylamino acid micelles. However, ICD resulting from exciton interaction of 3,6-bis(dimethylamino)-10-dodecylacridinium bromide (C_{12}AO) was found in premicellar aggregates with dodecanoyl-L-threonine (C_{12}Thr) in aqueous solutions, and temperature-induced chirality reversal was observed. This paper reports the dependence of the ICD of C_{12}AO on the temperature and concentration of C_{12}Thr over time.



Experimental section

Materials

N-dodecanoyl-L-threonine (C_{12}Thr) was synthesized using the (–) isomer of the corresponding amino acid. There was a single spot on HPLC. The purity of the chirality was not determined. C_{12}AO (Dojin), and 3,6-bis(dimethylamino)acridine (AO, Aldrich) were used without further

purification. The 3,6-bis(dimethylamino)-10-methylacridinium bromide (C_1AO) was a gift from Professor Shosenji, Kumamoto University.

Solution preparation and measurements

C_{12}Thr was dissolved in solutions adjusted to pH 9 using NaOHAt this pH, C_{12}Thr is anionic. The dye solutions were also adjusted at pH 9. No pH buffer was used to avoid any interference of the interaction of acylamino acid with acridine orange through the changing ionic strength. The dye and C_{12}Thr solutions were kept at a given temperature before mixing: one was kept in a spectroscopic cell thermo-regulated by circulating water and the other was in a block thermo-regulator. The dye concentration was kept at 0.05 mM. To obtain reproducible results, solution preparation was found to be very important. The absorption and CD spectra were measured using a JASCO V-560 spectrophotometer and a JASCO J-70 spectropolarimeter, respectively. Both had thermo-regulated cell holders. Electrophoretic Light Scattering Spectrophotometer ELS-800 (Otsuka Electronics) was used for the measurements of aggregation process.

Results and Discussion

Absorption and CD spectra of C_{12}AO/C_{12}Thr hetero-aggregates

The absorption spectra of 1.0 mM AO and C_{12}AO have bands near 500 and 280 nm (Fig. 1a and b). At this concentration a clear aggregation band was distinguished. The band near 280 nm shows the vibronic structure. The band near 500 nm shows a maximum at 495 nm, and was assigned to the monomeric forms of the dyes. The shoulder for AO and the maximum for C_{12}AO at 470 nm increased in intensity at higher concentrations. They were assigned to the dimeric or aggregate form of the dye and indicate the coexistence of aggregated forms of both dyes. Comparison of these two spectra indicates that C_{12}AO produces more aggregated forms than AO owing to the more hydrophobic nature of C_{12}AO. Figure 2 compares the absorption spectra of 0.1 mM C_{12}AO in the presence of ethyl alcohol (b-d) and excess C_{12}Thr (e) at 50°C. In ethanol, C_{12}AO showed an intense band at 495 nm and a shoulder at 470 nm, while in the aqueous solution, the dimer band at 470 nm was stronger than the monomer band at 495 nm. Increasing ethanol content induced a clear monomer band peak at 495 nm. The absorbance of the monomer band at 495 nm increased at higher temperatures and was maximal at 60°C in the presence of 3% EtOH, indicating dissociation of the dimer into the monomer. In the presence of 50 mM C_{12}Thr, the spectrum had an absorption maximum at 495 nm and a shoulder at 470 nm, suggesting that C_{12}AO dissociates into the monomeric form in a solution containing excess C_{12}Thr.

The monomer band at 495 nm degenerated in the presence of C_{12}Thr at concentrations much below the critical micelle concentration (cmc) and the maximum was observed at 473 nm (Fig. 1c), corresponding the dimer band maximum. However, absorbance increases were observed at 400-450 nm and above 520 nm, suggesting dye aggregation in the presence of C_{12}Thr

(discussed later). CD spectrum was not observed in the absence of C_{12}Thr, but it was observed in the presence of C_{12}Thr, as shown in Figure 3. This induced CD spectrum was recorded 24 h after solution preparation at r = 1.0, where r is the mole ratio of C_{12}Thr/C_{12}AO.

AO and C_1AO showed similar absorption bands in the absorption spectrum in the presence of C_{12}Thr, but no ICD spectrum was observed. This fact suggests that the hydrophobic interaction between the long alkyl chains induced a constrained interaction of chromophore with amino acid. ICD bands of C_{12}AO appeared near 500 and 250 nm. These corresponded to the respective absorption bands of C_{12}AO (Fig. 1). Each ICD band consisted of a positive band at the longer wavelength and a negative band at the shorter wavelength, indicating that the ICD is based principally on exciton interaction among dye molecules in C_{12}AO/C_{12}Thr complexes. Light scattering measurements actually found particles of 440 nm size at 10 min after solution mixing.

The dependence of the ICD spectra near 500 nm on both time and concentration was investigated at various C_{12}Thr concentrations. Figure 4 shows the ICD spectra of the C_{12}AO hetero-aggregate with C_{12}Thr, recorded 6 h after solution preparation. The maximum ICD was observed at a C_{12}Thr/C_{12}AO mole ratio r = 1.2. The ICD pairing of a positive band at the longer wavelength and a negative band at the shorter wavelength indicates positive chirality of the exciton interaction among dye molecules.[37] The corresponding absorption spectra of these solutions indicated that precipitation occurs at a high r ratio and the weak ICD intensities at r = 1.6 and 2.0 are ascribed to partial phase separation. There were no significant differences in the absorption spectra, but there was a big difference in ICD intensity at r = 0.8, 1.0, and 1.2. This is ascribed to the ordering of the C_{12}AO aggregate sensitive to the C_{12}Thr/C_{12}AO ratio.

Goswami and Pal observed the biphasic negative ICD of a cationic cyanine dye, ethyl-2-[3-(1-ethylnaphtho[1,2-d]-thiazoline-2-ylidene)-2-methylpropenyl]naphtho[1,2-d]thiazolium bromide in the presence of sodium cholate at concentrations (0.05-1.0 mM) much below the cmc, but above r = 1.0, and at concentrations (12-16 mM) just below and above the cmc, and in the presence of sodium deoxycholate at concentrations (0.021-0.70 mM) much below the cmc, but above r = 1.0, and at concentrations (4.0-8.0 mM) just below and above the cmc.[27] The ICD intensity of both positive peaks was larger at concentrations just below the cmc than at the much lower concentrations of sodium cholate and sodium deoxycholate. They ascribed this r-dependence of the ICD intensity to aggregation of a dye-surfactant complex with regular interspersing, as the sharp metachromatic absorption peak was insensitive to the ratio r. We observed the ICD maximum at r = 1.2 for C_{12}Thr, but no ICD at r > 2.0, suggesting that aggregation of 1:1 complexes of C_{12}AO with C_{12}Thr is suitable for chiral arrangement of the dye. Bile salts form micelles with a very different structure from that of the micelles of common surfactants with a long hydrophobic tail,[38,39] and have a small

solubilization capacity (e.g., 0.046 to 0.023 for cholesterol, which has a structure very similar to cholate).[40] The solubilization capacities of the bile salts correspond to 22-43 bile molecules for each solubilizate, suggesting that a 1:1 complex of the bile salt and the cyanine dye is difficult to form. In our system, both the anionic acylamino acid and the cationic $C_{12}AO$ had a long hydrophobic tail and the 1:1 complex may have promoted self-aggregation, which contributed to inducing ICD. At a higher r ratio, the separation between chomophors interrupted the ordering of $C_{12}AO$ ions and microphase separation may decrease the ICD intensity.

ICD spectra of $C_{12}AO/C_{12}Thr$ at r = 1.2

The ICD spectrum of $C_{12}AO/C_{12}Thr$ hetero-aggregates was investigated at r = 1.2 in detail at different temperatures and in solutions containing a small amount of ethanol. Figure 5 shows the time dependence of the ICD spectrum at 30°C. The ICD spectrum results from an exciton interaction with positive chirality and showed strong time dependence. The ICD spectrum changed in both intensity and shape. After mixing $C_{12}AO$ and $C_{12}Thr$, the intense positive CD band near 475 nm increased slightly initially and reached a maximum at 10 min. Then, it decreased with time and deformed in shape, showing a CD maximum near 510 nm. The corresponding absorption spectra are given in Fig. 6. The absorption maximum at 466 nm decreased and shifted to a longer wavelength. Interestingly, an isosbestic point was observed at 405 nm, but no isosbestic point appeared at a longer wavelength, owing to the absorbance increase above 550 nm. This suggests that the absorbance increase above 550 nm arose from the growth of fine particles. The aggregation process was followed with light scattering measurements for three hours at 30°C. The average size increases with time: 443 nm at 10 min, 504 nm at 25 min, 582 nm at 40 min, 663 nm at 60 min, and 757 nm at 180 min. The quick growth of aggregates and turbidity was found at 60°C at 60 min. High temperature induced the dissociation of $C_{12}AO$ dimer in the stable monomer-dimer equilibrium, but accelerates the growth of hydrophobic $C_{12}AO/C_{12}Thr$ complexes. We infer that the absorbance increase above 500 nm corresponds to this size growth of the $C_{12}AO/C_{12}Thr$ hetero-aggregates.

The ICD spectra of $C_{12}AO/C_{12}Thr$ hetero-aggregates at 50°C are shown in Figure 7. The spectra had a negative CD band at a longer wavelength and a positive CD band at a shorter wavelength, which indicates an exciton interaction with negative chirality. The time dependence of the ICD spectrum was very similar to that at 30°C, but with the reverse sign. The chirality was generally determined by the molecular chirality such as right-handed helix by L-amino acid and left-handed helix by D-amino acid. In this case, C_{12}-L-Thr induced both chiral aggregates of $C_{12}AO$ depending on the solution temperature. The change in the absorption spectra (Fig. 8) was very similar to the one observed at 30°C (Fig. 6). The double minimum in spectrum f was similar to the one observed for a helical polypeptide and suggests helical rotation of the transition moment of the dye aggregates.

The drug dicumarol shows ICD reversal in the α1-acid glycoprotein depending on the presence of imipramine.[41] The dicumarol molecule has two chromophores connected by a methylene group. This can rotate, changing the relative configuration of the two chromophores. The high affinity of imipramine induces a configuration change of the two chromophores in the dicumarol-protein interaction, leading to ICD reversal. They also measured the ICD spectra of AO and C_{12}AO in protein solutions and found no chirality reversal. The temperature-dependent chirality of the C_{12}AO/C_{12}Thr system may have been induced via a weak interaction of C_{12}AO with C_{12}Thr that is perturbed at room temperature or 40°C. Figure 9 compares the ICD spectra at the maximum intensity. ICD reversal occurred between 40 and 50°C. At 60°C, phase separation appeared quickly and a weak ICD spectrum with negative chirality was observed. The conformational ordering of C_{12}AO/C_{12}Thr aggregates may change around 45°C. The Gibbs energy of a transient state of aggregates with positive and negative chirality may be inferred, as shown in Figure 10. The transient Gibbs energy of C_{12}AO/C_{12}Thr aggregates is smaller for the ordering with positive chirality at lower temperatures and larger at higher temperatures. The crossover point is around 45°C. This chirality reversal may be based on the small energy difference between the two ordered conformations of the dye in the aggregates. This small energy difference may arise from the aggregate grouwth and hydrogen bond bridging among the heads of acyl amino acid.

Conclusion

Chiral aggregates were found only for C_{12}AO with C_{12}Thr and the ICD spectra with a pair of positive and negative bands resulted from the exciton interaction of dye molecules. A slow change was observed in both the absorption and ICD spectra, presumably owing to the aggregation growth. The ICD spectrum of the aggregates had positive chirality at 30 and 40°C and negative chirality at 50°C.

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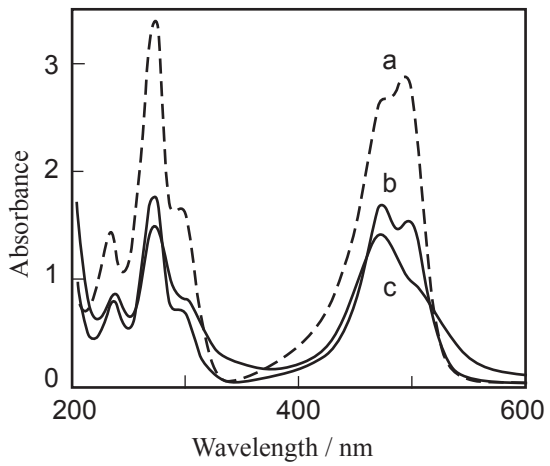

Fig. 1 Absorption spectra of AO (a) and $C_{12}AO$ (b, c). a and b: in water, c: in 1.0 mM $C_{12}Thr$ solution.

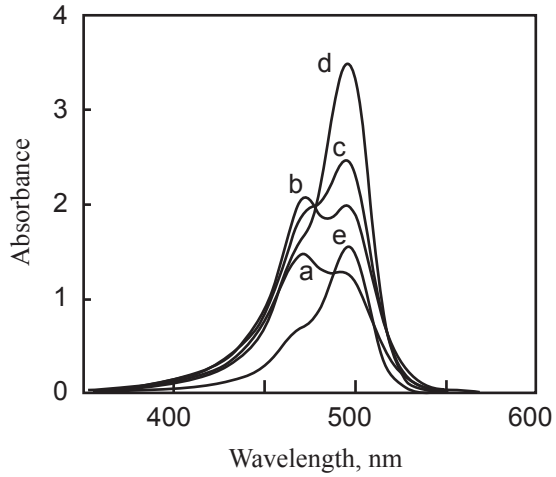

Fig. 2 Absorption spectra of $C_{12}AO$ in various solutions at 50°C. a: aqueous, b: 3% EtOH, c: 10% EtOH, d: EtOH, e: 50 mM $C_{12}Thr$

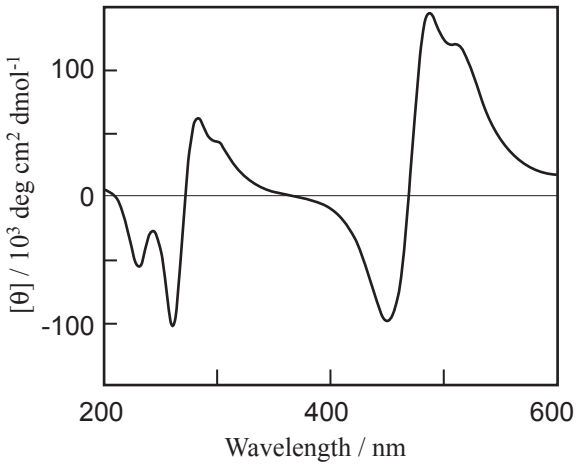

Fig. 3 Induced CD spectra of $C_{12}AO$ in the presence of $C_{12}Thr$ at $C_{12}Thr/C_{12}AO$ ratio (*r*) = 1.0.

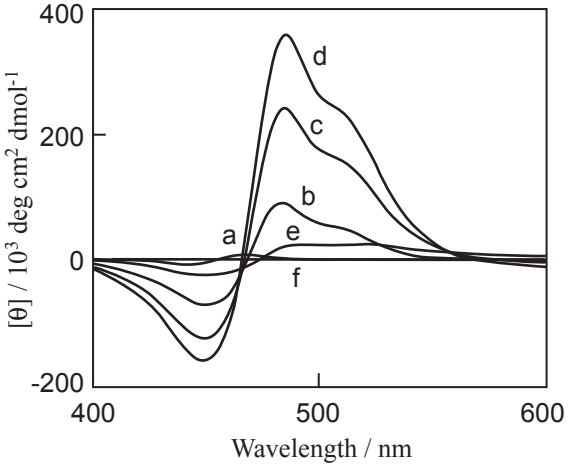

Fig. 4 The ICD spectra of C12AO/C12Thr hetero-aggregates in 1.5% EtOH solutions. The ratio *r*: a 0.4, b 0.8, c 1.0, d 1.2, e 1.6, f 2.0

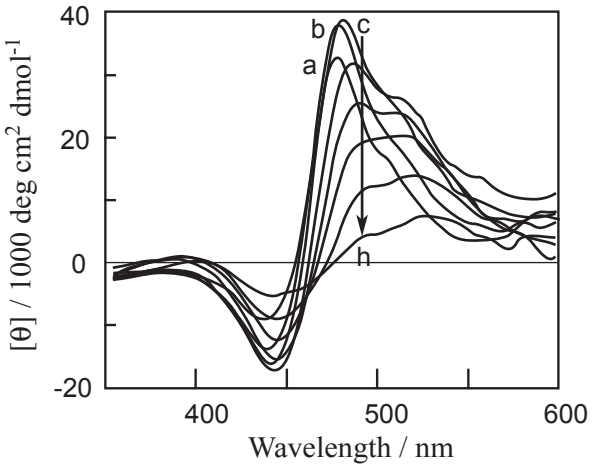

Fig. 5 ICD spectra of $C_{12}AO/C_{12}Thr$ hetero-aggregates at *r* = 1.2 at 30°C. Time: a 0, b 10, c 20, d 40, e 60, f 90, g 150, h 270 min

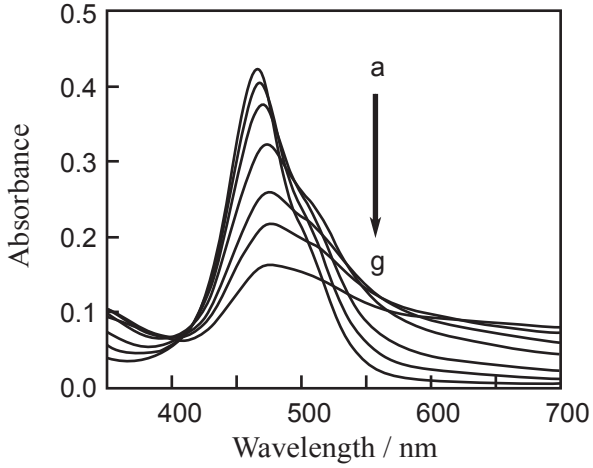

Fig. 6 Absorption spectra of C12AO/C12Thr mixture in aqueous solutions at 30°C. Time: a 0, b 5, c 15, d 48, e 96, f 150, g 270 min

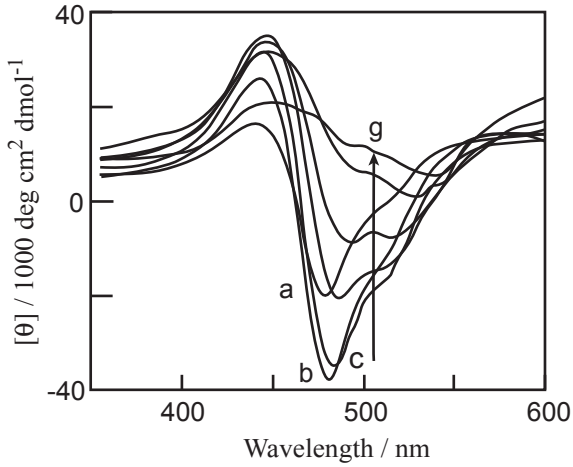

Fig. 7 ICD spectra of $C_{12}AO/C_{12}Thr$ hetero-aggregates at r = 1.2. 50°C; Time: a 0, b 5, c 10, d 20, e 35, f 70, g 130 min

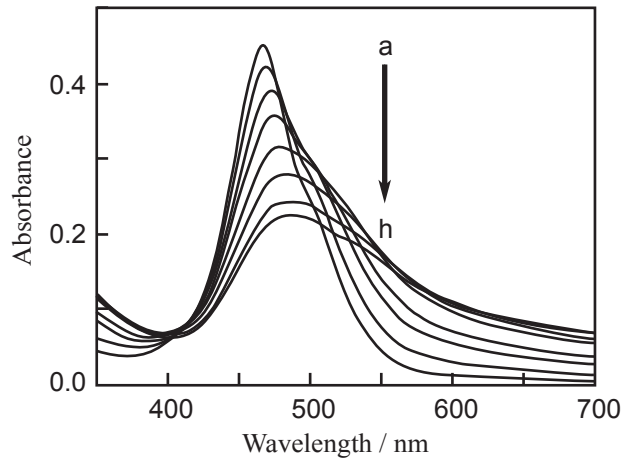

Fig. 8 Absorption spectra of $C_{12}AO/C_{12}Thr$ mixture in aqueous solutions at 50°C. Time: a 1, b 5, c 11, d 19, e 45, f 94, g 213, h 314 min

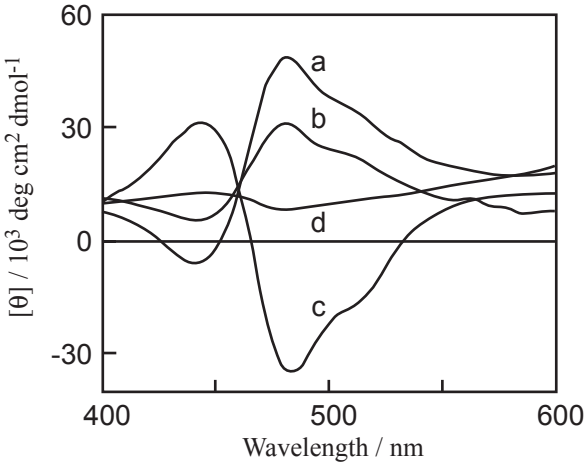

Fig. 9 Temperature dependence of ICD spectra of $C_{12}AO$ in $C_{12}Thr$ solutions at the maximum ICD intensity. Temparature (°C): a 30, b 40, c 50, d 60.

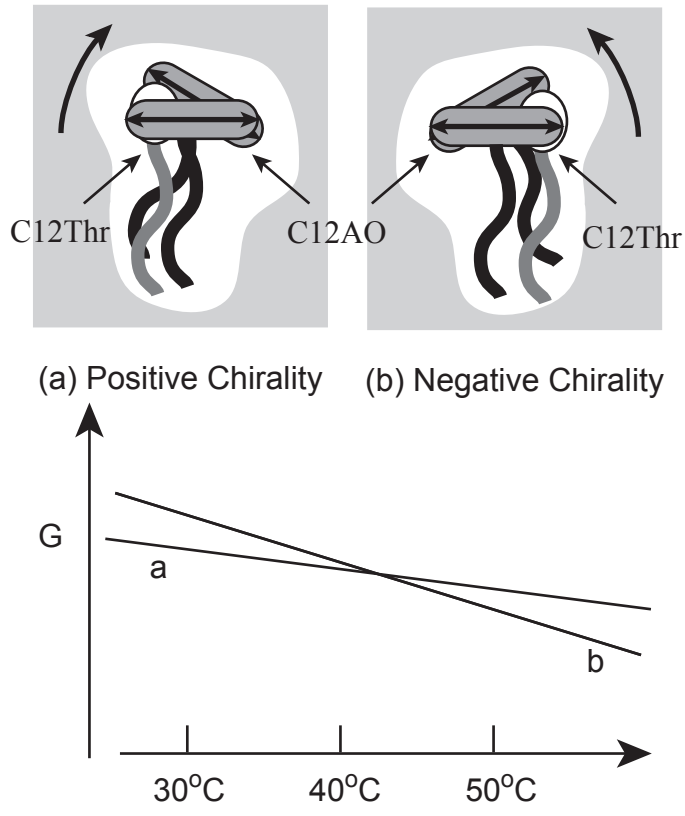

Fig. 10 Schematic diagram of Gibbs energy and a relative coordination of neighboring $C_{12}AO$ chromophores in the 1:1 $C_{12}AO/C_{12}Thr$ aggregates. a: the ordering of positive chirality and b: the ordering of negative chirality.