Difference in Chiral Recognition of Gel and Liquid-Crystalline Phases of Phosphatidylcholine Vesicles as Determined by Circular Dichroism Spectroscopy

Hiroko NAKAGAWA* and Koh-ichi YAMADA

Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan.
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The circular dichroism (CD) spectra of three types of (L)phosphatidylcholine (PC) vesicles in aqueous solution showed differences in the sign and intensity of the Cotton effect compared with those of monomers in ethanol, indicating the existence of chiral environments in these vesicles. From the temperature dependence of CD intensities, the main phase transition temperatures between gel (Gel) and liquid-crystalline (LC) phases of the vesicles were estimated to be 40, 23, and 55 °C for dipalmitoyl PC, dimyristoyl PC and distearoyl PC, respectively. Furthermore, both low-fluidity Gel and high-fluidity LC phases recognized the chirality of incorporated 2-hydroxymethyl[5]thiaheterohelicene (5HM) with a helical structure, which undergoes a rapid racemization owing to a weak repulsion between the terminal hydrogen atoms. The ability for chiral recognition was evaluated using thermodynamic parameters for the equilibrium between P and M enantiomers of 5HM in the vesicles; the Gel phase manifested a higher recognition ability than the LC phase.

Key words chiral recognition; helicene; circular dichroism; phosphatidylcholine; phase transition temperature; vesicle

Circular dichroism (CD) spectroscopy is an effective tool for studying the stereochemical structures of macromolecules such as helixes and random coils of peptides and proteins.[1,2] The CD spectroscopy also demonstrates usefulness for estimating the chiroptical properties of aggregated systems of chiral molecules.[3—5] Artificial membranes and vesicles composed of chiral surfactants, such as phosphatidylcholine (PC) derivatives, afford chiral environments that induce CD absorptions different from those of monomers,[6—9] presumably because of their intermolecular interactions with one-sided twisting. The vesicles in aqueous solution are fluid at room temperature, but sufficiently tight to maintain guest molecules facing inward. However, the fluidity is easily affected by temperature variations, altering the aggregated state. The intensity of the induced CD depends on the fluidity of the vesicles; it will decrease with an increase in temperature. Therefore, the main phase transition temperature (Tc) of vesicles between the gel (Gel) and liquid-crystalline (LC) phases may play a decisive role in the induction of CD, because of the low-fluidity Gel and high-fluidity LC phases. This suggests that the measurement of the temperature dependence of the intensity of induced CD would facilitate the estimation of the Tc's of vesicles, in addition to the utilization of differential scanning calorimetry (DSC)[10] and ^{1}H-NMR spectroscopy.[11]

Furthermore, it is interesting to determine whether the vesicles can recognize the chirality of incorporated guest molecules. We have previously reported that aggregated systems, such as micelles and membranes formed with chiral surfactants,[12,13] discriminate between right-handed (P) and left-handed (M) helixes of 2-hydroxymethyl[5]thiaheterohelicene (5HM).[14] That is, this suggests that 5HM acts as a probe for determining whether the chiral environments can recognize the chirality of guest molecules. Although the 5HM molecule possesses a helical structure, the helix is sufficiently labile to be readily racemizable in solution, owing to a weak repulsion between the hydrogen atoms attached to both terminal rings. However, once 5HM is incorporated into a chiral environment, the equilibrium shifts to either the P or M side, inducing CD absorptions. The resulting bias in the population of P and M enantiomers can be estimated on the basis of the sign and intensity of the Cotton effect originating from 5HM at approximately 350 nm, where the absorptions seldom overlap those of many host molecules. These characteristics of 5HM may also enable the evaluation of difference in the chiral recognition ability of both the Gel and LC phases of the vesicles.

The PCs studied here are (L)dimyristoyl PC (DMPC), (L)dipalmitoyl PC (DPPC) and (L)distearoyl PC (DSPC), for which the main phase transition temperatures (Tc's) of the vesicles formed from individual PCs have been reported in the literature to be 24.0, 41.5 and 54.3 °C, respectively.[10,15]

Experimental

(L)DMPC, (L)DPPC and (L)DSPC were purchased from Sigma Chemical Co. and used without further purification. 5HM was prepared according to the procedures described in our previous article.[16] Water used was distilled and then passed through a Millipore Milli-Q Jr. purification system. The PC vesicle solutions were prepared by the injection method.[17—19] An aliquot of 200 μl of PC ethanol solution was added with vigorous stirring (700 rad/min) to 5 ml of HCl–Tris buffer (pH=7.0) using a Mini Chemi Pump (Nihon Seimitu Kagaku) at a rate of 30 μl/min. This procedure was carried out at 35 °C, 45 °C and 60 °C for (L)DMPC, (L)DPPC and (L)DSPC, re-

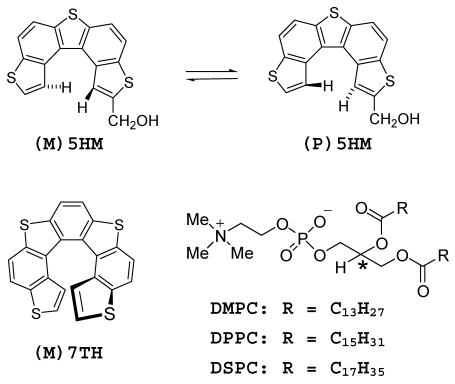

Chart 1

* To whom correspondence should be addressed. e-mail: hnaka@josai.ac.jp

spectively. The vesicles containing 5HM were prepared by diluting 20 μl of the 5HM ethanol solution with the vesicle solution and sonicating for 5 s. Then, the CD spectra of the solution thus obtained were measured using a JASCO J720W spectropolarimeter equipped with a PTC-348WI temperature controller.

Results and Discussion

CD Spectra of PC Monomers and Vesicles Phosphatidylcholine having an asymmetric carbon atom, exists in a monomeric form in an organic solution and in an aggregated form in an aqueous solution.[20] Thus, the differences in CD absorption of both forms were examined. In ethanol, (L)DMPC and (L)DPPC gave a weak and broad absorption band at approximately 200 to 220 nm with a negative Cotton effect (Figs. 1a, b). In the case of (L)DSPC, however, the band was too faint to be observed. On the other hand, the vesicles of (L)PCs in an aqueous solution demonstrated positive Cotton effects with much greater intensities as shown in Figs. 1c ((L)DMPC), d ((L)DPPC) and e ((L)DSPC). In addition, the absorption maxima shifted to a long-wavelength region in comparison with those of monomers. These results suggest that the aggregate of (L)PC in vesicles takes a supramolecularly twisted form, yielding chiral environments. The molecular ellipticity ([θ]) value of $+950$ deg cm^2 dmol^{-1} of (L)DPPC vesicles at the peak of 220 nm was much larger than that of $+380$ deg cm^2 dmol^{-1} of the vesicles reported by Walde *et al.*[9] This difference may be ascribed to the bigger size of their vesicles, owing to the difference in the preparation method in which they used a higher concentration of PC. Walde *et al.* employed a large unilamellar vesicle (LUV) extrusion technique[21] with an (L)DPPC concentration of 39 mM, yielding large vesicles with diameters from 60 to 100 nm,[21] whereas we employed an injection method with an (L)DPPC concentration of 0.92 mM, forming relatively smaller vesicles with diameters less than 60 nm.[18] It is conceivable that the smaller vesicles may afford tighter and more twisted environments, and thus, induce stronger CD absorptions.

Temperature Dependence of CD Intensity of PC Vesicles The molecular alignment and fluidity in PC vesicles change in harmony with the critical main phase transition between the gel (Gel) and liquid crystal (LC) phases. These changes are considered to affect the CD absorptions of the molecular aggregate. Figure 2 demonstrates the temperature dependence of the CD spectra of (L)DPPC vesicles in the range of 5 to 50 °C. The absorption intensity of the vesicles decreased with increasing temperature, although the absorption shapes did not show any large alterations. In Fig. 3, the molecular ellipticities of three types of vesicle at their absorption maxima are depicted against temperature. The intensity changes became linear and manifested a discontinuous point for each of the vesicles. The temperatures at the discontinuities were 40, 23 and 55 °C for (L)DPPC (black circles in Fig. 3), (L)DMPC (open circles) and (L)DSPC (stars), respectively. These temperatures are in good agreement with the main phase transition temperatures (Tc's) of these vesicles as described in the literature. The fluidity of the vesicles increased with increasing temperature, which caused a decrease in CD intensity. Therefore, the variations of molecular ellipticities may be considered as a useful measure for the assessment of Tc's, because of the distinct difference between the low-fluidity Gel phase and the high-fluidity LC phase.

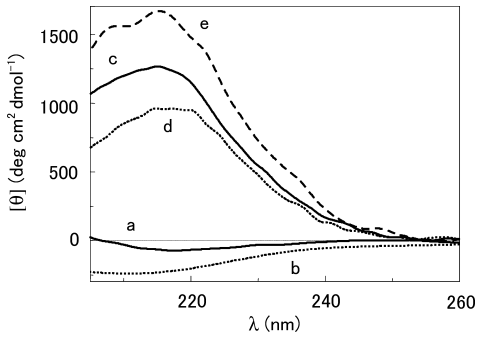

Fig. 1. CD Spectra of PC Monomers and Vesicles

5.62×10^{4} M of (L)DMPC (a) and 5.00×10^{4} M of (L)DPPC (b) in ethanol. 5.85×10^{-4} M of (L)DMPC (c), 9.24×10^{-4} M of (L)DPPC (d) and 1.20×10^{-4} M of (L)[DSPC] (e) in aqueous solution.

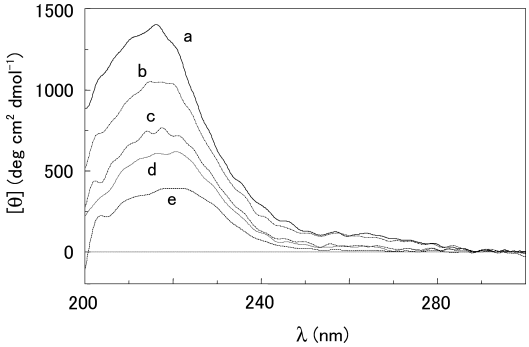

Fig. 2. CD Spectra of (L)DPPC in Vesicles at 5 (a), 25 (b), 35 (c), 40 (d) and 50 °C (e)

[(L)DPPC]$=9.24\times10^{-4}$ M.

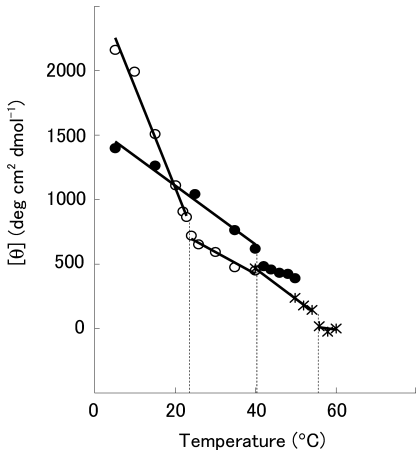

Fig. 3. Temperature Dependence of CD Intensities of (L)DMPC (○), (L)DPPC (●) and (L)DSPC (*) Vesicles Measured at the Absorption Maxima around 216 nm

[(L)DMPC]$=5.85\times10^{-4}$ M, [(L)DPPC]$=9.24\times10^{-4}$ M and [(L)DSPC]$=1.20\times10^{-4}$ M. Broken lines represent phase transition temperatures.

Induced CD of 5HM in (L)PC Vesicles Chiral environments formed by (L)PC vesicles may recognize the chirality of incorporated guest molecules. A 5HM molecule as a guest in the vesicles used here biases its helix moiety to either the P or M enantiomer side in chiral environments, inducing CD absorptions, although this molecule exists in a racemic form in organic solvents and in solid.

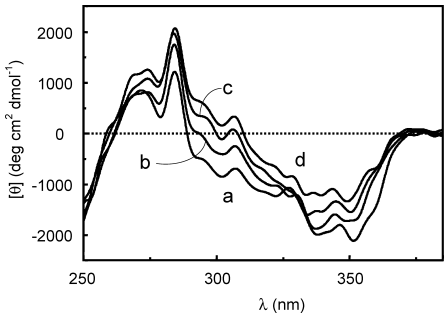

Fig. 4. CD Spectra of 5HM in (L)DMPC Vesicles at 0 (a), 10 (b), 20 (c) and 50 °C (d)
[(L)DMPC]=7.19×10^{-4} M. [5HM]=7.43×10^{-5} M.

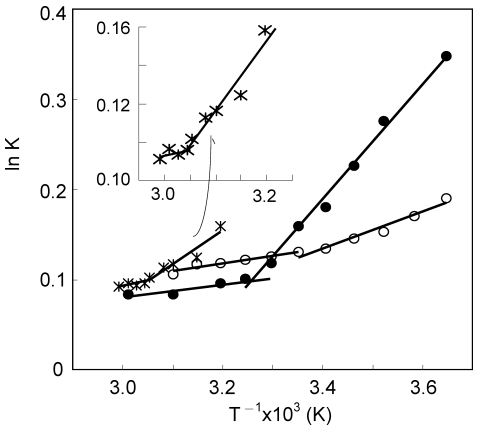

Fig. 5. van't Hoff Plots of the Equilibrium between (P)5HM and (M)5HM in (L)DMPC (○), (L)DPPC (●) and (L)DSPC (∗) Vesicles
[DMPC]=7.19×10^{-4} M, [DPPC]=9.24×10^{-4} M, [DSPC]=1.21×10^{-4} M and [5HM]=7.58×10^{-5} M.

When water-insoluble 5HM was incorporated into (L)DMPC vesicles, the solution was transparent. In the CD spectra of the solution, new absorptions appeared in the long-wavelength region (Fig. 4). In particular, negative Cotton effects at approximately 350 nm were observed. An ethanol solution containing a mixture of (L)PC and racemic 5HM exhibited no CD absorption at approximately 350 nm, because of the equal occurrence of the P and M enantiomers. Thus, the newly appearing negative Cotton effects of 5HM in the vesicles were ascribed to the excess of the M enantiomer over the P enantiomer, in comparison with the Cotton effect of (M)[7]thiaheterohelicene (7TH),[14,22] a higher congener of 5HM. 7TH possesses molecular dissymmetry owing to a skeletal overlap. The intensity of induced CD of 5HM decreased with increasing temperature, because of the increasing fluidity of the vesicles and the weakening fixation of the helix of 5HM.

A similar tendency was observed in the experiments using 5HM in (L)DPPC and (L)DSPC vesicles. Furthermore, the combination of 5HM and (D)PC vesicles yielded the same results except for the opposite signs of the Cotton effects.

Thermodynamic Parameters of 5HM in (L)PC Vesicles Since chiral PC vesicles can recognize the chirality of guest molecules as mentioned above, the chiral discrimination ability for the Gel and LC phases was examined by obtaining the thermodynamic parameters for the equilibrium between P and M enantiomers of 5HM. The temperature dependence of the CD intensities shown in Fig. 4 yielded the M enantiomeric excess (e.e.), which was tentatively calculated by considering the value of $[\theta]=30300$ at 347 nm as a $[\theta]$ value of pure (P)5HM. This $[\theta]$ value has been obtained for (P)5HM in bovine serum albumin at 0 °C.[23] From these estimated e.e. values, the equilibrium ((P)5HM ⇆ (M)5HM) constants (K) were calculated for each of the temperatures. Figure 5 shows the van't Hoff plots of the K's of 5HM against 1/T for three types of (L)PC vesicle. In each case, two straight lines with different slopes were found above and below the Tc of the vesicles.

Therefore, two sets of thermodynamic parameters for each case can be calculated, that is, for the Gel and LC phases of the vesicles. Table 1 summarizes the $\Delta H°$ and $\Delta S°$ values thus obtained. Although the values of $\Delta H°$ and $\Delta S°$ are, on the whole, low, it may be predicted that the M enantiomer of 5HM is more stable in (L)PC vesicles than its antipode, because of the negative values of both $\Delta H°$ and $\Delta S°$. An examination of Table 1 indicates that for all vesicles, the low-fluidity Gel phase possesses smaller $\Delta H°$ and $\Delta S°$ values than the high-fluidity LC phases. In other words, the Gel phase manifests a higher ability for chiral recognition than the LC phase and this ability was of the order of DMPC<DSPC<DPPC. This order seems to exhibit no significant relationship with the order of alkyl chain lengths of the PC molecules. However, this suggests that there presumably exist appropriate sizes between PC molecules and 5HM molecules. The LC phase shows no significant differences in the values of $\Delta H°$ and $\Delta S°$, taking into account the relatively large standard deviations, among the three types of PC vesicles. However, it is surprising that LC phase formed in the region of higher temperatures still has the ability for chiral discrimination between enantiomers of 5HM.

Table 1. Thermodynamic Parameters of Conversion from (P)5HM to (M)5HM in PC Vesicles and Main Phase Transition Temperatures (Tc)

		$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	Tc (lit.[10])
DMPC	Gel	−1.92±0.16	−5.44±0.58	23 (24.0)
	LC	−0.73±0.09	−1.35±0.29	
DPPC	Gel	−4.93±0.31	−15.15±1.04	40 (41.5)
	LC	−0.57±0.30	−1.05±0.93	
DSPC	Gel	−3.06±0.52	−8.53±1.62	55 (54.3)
	LC	−0.99±0.46	−2.21±1.39	

Conclusion

It was proved that the Gel and LC phases of three types of PC vesicles formed distinct chiral environments, which was confirmed by measuring the CD spectra at various temperatures. Furthermore, both phases can recognize the chirality of incorporated guest molecules. This ability for chiral recognition was evaluated by determining the thermodynamic parameters; the Gel phase showed a higher recognition ability than the LC phase. However, it was surprising that the LC phase of PC vesicles possessing high fluidity still has the ability for chiral discrimination between the enantiomers of 5HM.

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