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DRASTIC CHANGES OF CIRCULAR DICHROISM OF SYNTHETIC PHOSPHATE BILAYERS DUE TO INTERACTION WITH METAL IONS¹⁾

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A chiral bilayer membrane containing the phosphate head group shows, in the crystalline phase, remarkably enhanced circular dichroism, which is suppressed specifically and sensitively by the interaction of the phosphate group with added ions.

The bilayer formation has been observed for a number of synthetic amphiphiles.²⁾ These bilayers show unique physicochemical properties that are typical of their aggregation structures. We already reported remarkable enhancements of circular dichroism(CD) in chiral bilayers, 3,4 and this observation was subsequently used for examination of the phase separation behavior of mixed bilayers.⁵⁾ In the present paper, we discuss the use of the CD technique for studying the interaction with metal ions of bilayers of a chiral double-chain amphiphile containing the phosphate head group. The interaction of Ca^{2+} and other metal ions with liposomes of acidic phospholipids has been investigated extensively in relation to membrane functions such as cell fusion, phase separation and ion transport. These investigations were conducted by using a large variety of the physical methods: electron microscopy, $^{6)}$ differential scanning calorimetry, $^{7)}$ X-ray diffraction, $^{7)}$ NMR spectroscopy, $^{8)}$ ESR spectroscopy, $^{9)}$ the fluorescence probe method, $^{10)}$ the surface tension measurement, $^{11)}$ turbidimetry, $^{12)}$ and the surface monolayer technique.¹³⁾ The CD technique described in this paper is convenient compared with these techniques, because it is applicable to very dilute solutions $(10^{-4} - 10^{-5}M)$ and the experimental procedure is straightforward.



Amphiphile 1 was prepared by the successive condensation of didodecyl Lglutamate with terephthaloyl chloride and ethylene glycol, accompanied by reaction with POCl₃ and by the treatment with water. The final product was identified by IR and NMR spectroscopies, thin layer chromatography, and elemental analysis.¹⁴⁾ Amphiphile 1 can be dispersed in aqueous buffers(pH 9) by warming or by sonication to give transparent solutions. Electron microscopy(instrument, Hitachi H-600, phosphotungstate staining) indicated the formation of bilayer lamellae, and differential scanning calorimetry(instrument, Seiko Electronics, model SSC/560) showed the presence of the crystal-to-liquid crystal phase transition at 43°C.

In the case of the spectroscopic measurements, amphiphile 1 was dispersed in Tris buffer(pH 9.0, 0.01 M (CH₃)₄N⁺Cl⁻, μ = 0.01) by warming and diluted to 1.0 x 10⁻⁴ M. This aqueous dispersion has an absorption maximum at 240 nm (ϵ 16 000). Addition of 1.0 x 10⁻⁴ M CaCl₂ does not cause any spectral change. Precipitates are formed when 5 x 10⁻⁴ M CaCl₂ is added.

In contrast, the CD spectrum is very sensitive to ion addition. In the absence of added metal ions, large CD enhancements are observed at temperatures below $T_c(43^{\circ}C)$ of the bilayer(Fig. 1): $[\theta]_{max}(260 \text{ nm}) = -350\ 000\ \text{deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$ at 15°C and $[\theta]_{max}(240 \text{ nm}) = +4\ 000\ \text{at}\ 43^{\circ}C$. This large temperature dependence is similar in magnitude to those observed for chiral bilayers of double-chain ammonium amphiphiles.^{3,4}

Figure 2 demonstrates the suppression of the enhanced CD by Ca^{2+} addition at $T < T_c$. A very small concentration of $CaCl_2(5 \times 10^{-6} \text{ M})$ can lower the CD intensity and the suppression is complete at $1 \times 10^{-4} \text{ M} CaCl_2$: $[\theta]_{260} = -4 000$. The Ca^{2+} effect resembles very closely the influence of the phase transition shown in Fig. 1. However, this is not attributable to the change in the membrane fluidity which may be caused by the interaction of Ca^{2+} with the phosphate head group. The extents of fluorescence depolarization (P value)²) of diphenylhexatriene($1 \times 10^{-6} \text{ M}$) embedded in the chiral bilayer ($1 \times 10^{-4} \text{ M}$) are 0.39 and 0.41, respectively, in the absence and the presence ($1 \times 10^{-4} \text{ M}$) of $CaCl_2$. These results clearly indicate that complexation of Ca^{2+} with the phosphate head group altered, without lowering the bilayer fluidity, the chromophore orientation in the bilayer in such a way as to lose the coupling of the electrical transition moment. This situation is schematically illustrated in Fig. 2. The effect of Ca^{2+} is not recognizable at 50 °C(above T_c) where no CD enhancement is present.

Suppression of the CD enhancement is produced by addition of other metal chlorides. Figure 3 describes the decrease in $[\theta]_{250}$ with metal ion concentrations. Na⁺ and K⁺ ions do not affect the CD spectrum even at high concentrations up to 0.1 M, while Mg²⁺ and Ba²⁺ ions are effective. Sr²⁺ ion is as effective as Ca²⁺, and La³⁺ ion produces a noticeable decrease even at 10⁻⁶ M. The spectral changes are produced instantaneously in the case of Ca²⁺, Sr²⁺, and Ba²⁺, but the change upon Mg²⁺ addition requires several minutes. It is reported on the basis of ³¹P NMR spectroscopy⁸ that the interaction of acidic phospholipids with Ca²⁺ is detectable at concentrations lower than that of Mg²⁺. This is in agreement with the results of Fig. 3. Though not shown in the figure, Cu²⁺ is not effective, and Co²⁺ and Mn²⁺ display influences close to that of Ba²⁺.

The enhanced CD spectrum can be regenerated by masking these metal ions by ethylenediaminetetraacetic acid(EDTA). As shown in Fig. 4, the CD recovery is composed of fast and slow processes. The fast processes occur in ca. 1 min, but the slow processes are not over even after 7 h for Ba^{2+} , Ca^{2+} , and Sr^{2+} . It appears that the two processes are related to metal ions bound at outer and inner layers of the bilayer aggregate. The influence of Mg²⁺ is removed completely in ca. 3 h and that of La³⁺ is not at all removed by excess EDTA.



Fig. 3. $[\theta]_{250}$ value of CD spectra vs. metal ion concentration. $[\underline{1}] = 1.0 \times 10^{-4} \text{ M}, 30^{\circ}\text{C}$





Fig. 4. Recovery of enhanced CD by addition of EDTA. $[1] = 1.0 \times 10^{-4} \text{ M},$ $[EDTA] = 1.0 \times 10^{-3} \text{ M}, 30^{\circ}\text{C}$

In conclusion, circular dichroism is used to detect the interaction of chiral bilayer membranes with metal ions. The CD change is recognizable at ion concentrations as low as $10^{-5}-10^{-6}$ M. The minor structural change at the membrane surface becomes detectable due to perturbation of the strong dipolar coupling of the chiral chromophore. On the basis of the present finding, we can develop new bilayer systems which are responsive to ions and molecules. Delicate modulation of the bilayer assembly by added ions would also be an interesting aspect of application.

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- 14) Mp rt \longrightarrow 120 °C (the liquid crystalline behavior was observed in this temperature range); Found: C, 61.21; H, 8.87; N, 1.94%. Calcd for $C_{39}H_{66}NO_{11}P$ 0.5H₂O: C, 61.24; H, 8.83; N, 1.83%.

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