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DRASTIC CHANGES OF CIRCULAR DICHROISM OF SYNTHETIC PHOSPHATE BILAYERS DUE TO INTERACTION WITH METAL IONS[1)]

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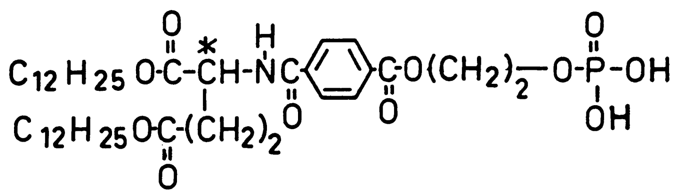
A chiral bilayer membrane containing the phosphate head group shows, in the crystalline phase, remarkably enhanced circular dichroism, which is suppressed specifically and sensitively by the interaction of the phosphate group with added ions.

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.



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Amphiphile 1 was prepared by the successive condensation of didodecyl L-glutamate with terephthaloyl chloride and ethylene glycol, accompanied by reaction with $POCl_3$ 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 $(CH_3)_4N^+Cl^-$, μ = 0.01) by warming and diluted to 1.0×10^{-4} M. This aqueous dispersion has an absorption maximum at 240 nm (ε 16 000). Addition of 1.0×10^{-4} M $CaCl_2$ does not cause any spectral change. Precipitates are formed when 5×10^{-4} M $CaCl_2$ 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°C) of the bilayer(Fig. 1): $[\theta]_{max}$(260 nm) = -350 000 deg·cm^2·dmol^{-1} at 15°C and $[\theta]_{max}$(240 nm) = +4 000 at 43°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 $CaCl_2$(5×10^{-6} M) can lower the CD intensity and the suppression is complete at 1×10^{-4} 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 ($\underline{P}$ value)[2] of diphenylhexatriene(1×10^{-6} M) embedded in the chiral bilayer (1×10^{-4} M) are 0.39 and 0.41, respectively, in the absence and the presence (1×10^{-4} 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 Mg^{2+} and Ba^{2+} ions are effective. Sr^{2+} ion is as effective as Ca^{2+}, and La^{3+} ion produces a noticeable decrease even at 10^{-6} M. The spectral changes are produced instantaneously in the case of Ca^{2+}, Sr^{2+}, and Ba^{2+}, but the change upon Mg^{2+} addition requires several minutes. It is reported on the basis of ^{31}P NMR spectroscopy[8] that the interaction of acidic phospholipids with Ca^{2+} is detectable at concentrations lower than that of Mg^{2+}. This is in agreement with the results of Fig. 3. Though not shown in the figure, Cu^{2+} is not effective, and Co^{2+} and Mn^{2+} display influences close to that of Ba^{2+}.

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 Mg^{2+} is removed completely in ca. 3 h and that of La^{3+} is not at all removed by excess EDTA.

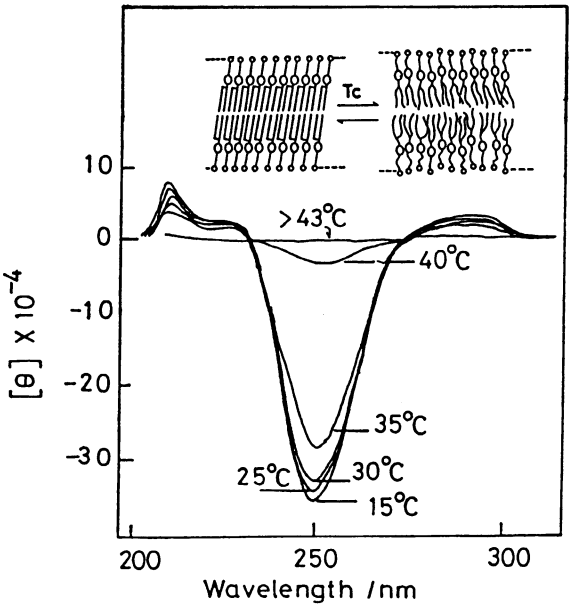

Fig. 1. CD spectra of chiral bilayers in aqueous buffer. [1] = 1.0 x 10^{-4} M

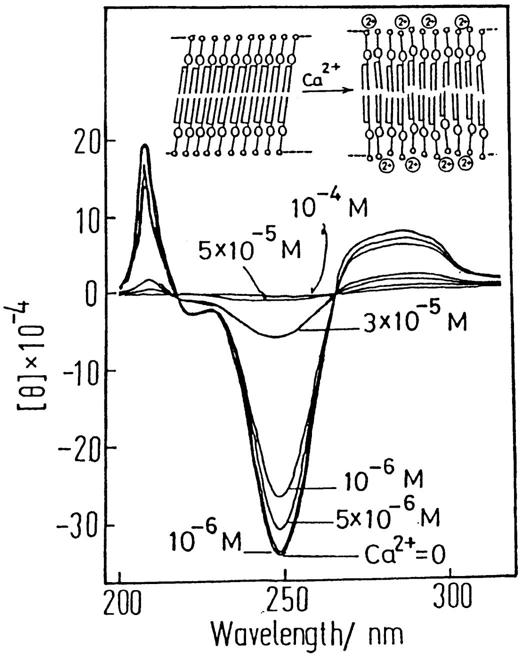

Fig. 2. CD spectral change induced by $CaCl_2$. [1] = 1.0 x 10^{-4} M, 30°C

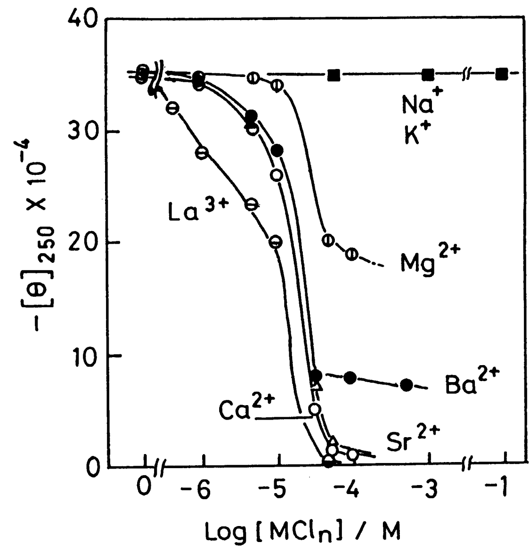

Fig. 3. $[\theta]_{250}$ value of CD spectra vs. metal ion concentration. [1] = 1.0 x 10^{-4} M, 30°C

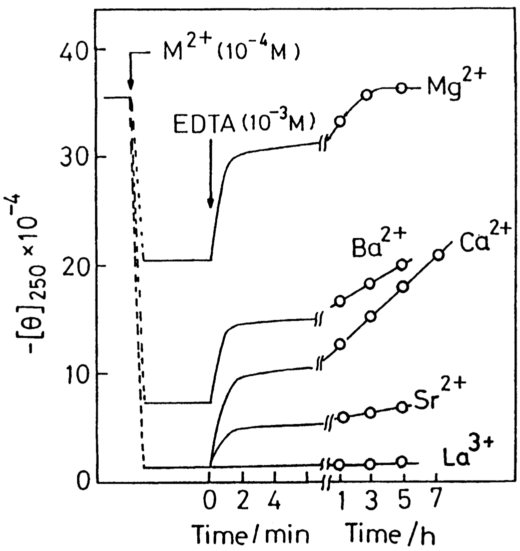

Fig. 4. Recovery of enhanced CD by addition of EDTA. [1] = 1.0 x 10^{-4} M, [EDTA] = 1.0 x 10^{-3} M, 30°C

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 $C_{39}H_{66}NO_{11}P\cdot 0.5H_2O$: C, 61.24; H, 8.83; N, 1.83%.

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