CHEMISTRY LETTERS, pp. 959-962, 1989.

Stabilization of a Phosphate Molecular Bilayer in Organic Media by Complexation with Ca^{2+} Ion[1)]

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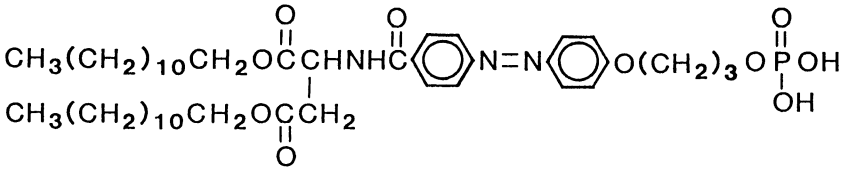
An aqueous phosphate bilayer membrane was transformed by addition of $CaCl_2$ to a stoichiometric, water-insoluble complex, of which dispersions in $CHCl_3$ and toluene retained the bilayer characteristics such as huge molecular weight, fiber-like morphology and regular chromophore orientation.

It has been shown that a large number of synthetic amphiphiles form stable bilayer membranes in aqueous media.[2)] The component molecules are highly oriented in these bilayers and, therefore, the bilayer aggregate is particularly suitable for preparing organized molecular systems. The bilayer organization is also maintained in cast films which are obtained by evaporation of water from aqueous bilayer dispersions.[3-5)]

Exciting possibilities would arise if these regular organizations could be maintained in organic media. The hydrophobic interaction which is a major driving force in producing stable aqueous aggregates, micelles and bilayer membranes, is not available in organic media. Polymerization was shown by Regen et al.[6)] and Roks et al.[7)] to render aqueous bilayer vesicles stable in ethanol-water mixtures.

In the present paper, we describe the complexation of the phosphate head group and Ca^{2+} ion as a means of stabilizing synthetic bilayer membranes in organic media.



1 $2C_{12}AspAzoC_3PO_4H_2$

Double-chain phosphate amphiphile 1 was synthesized by condensation of didodecyl L-aspartate and p-hydroxypropyl-p'-carboxyazobenzene in the presence of diethyl cyanophosphonate and the subsequent reaction with $POCl_3$.[8)] This is a typical bilayer-forming amphiphile. The aspartate connector and the azobenzene unit in the spacer portion act as chiral and absorption spectral reporter groups, respectively. Figure 1a is an electron micrograph of the aqueous (sonicated) dispersion (5 mM, pH adjusted to 7.5 by NaOH)(1 M = 1 mol dm^{-3}). Both of rods and single-walled vesicles are recognized. The fine rods have diameters of 100-200 Å

and the wall thickness of the vesicles is not greater than 100 Å. Differential scanning calorimetry (DSC) shows the gel-to-liquid crystal phase transition at 90°C (ΔH = 22 kJ/mol). These data are consistent with the bilayer formation in water.

Subsequently, the aqueous dispersion was added with stirring to two equivalents of 5 mM aqueous $CaCl_2$ solution. The precipitates were recovered, washed with water and methanol, and dried. The colorless powder dissolved slowly (ca. 1 day) in $CHCl_3$ or in benzene. An electron micrograph of the $CHCl_3$ solution (Fig. 1b) indicates the presence of flexible fibers of diameter of ca. 100 Å. A gel permeation chromatogram of this aggregate (Fig. 2a) shows the presence of a high molecular-weight fraction (peak top, 5.1×10^6; 86%) and a low molecular-weight fraction (peak top, 2.2×10^3; 14%). The atomic ratio of Ca and P is determined as 0.97 by the inductively coupled plasma-atomic emission spectrometry.[9] It is obvious from these data that the 1:1 Ca^{2+} complex remains in $CHCl_3$ as huge aggregates with a well-defined structure.

This is further supported by the spectral data. As shown in Fig. 3, the absorption peak of the amphiphile by itself is located at 355 nm in $CHCl_3$. This absorption is typical of that of the molecularly-dispersed species. The Ca^{2+} complex prepared at pH 7.5 shows an absorption peak at 340 nm in $CHCl_3$. This blue shift is not so large as that observed for the uncomplexed phosphate bilayer between water (λ_{max} 322 nm) and $CHCl_3$.

The uncomplexed phosphate bilayer has been found to show different molecular orientations at pH 7 and pH 12.[10] When $CaCl_2$ is added at pH 12, precipitation occurred faster and the resulting Ca^{2+} complex showed the Ca/P ratio of 0.75. In $CHCl_3$, its aggregate weight corresponds aggroximately to trimer (Fig. 2b), and λ_{max} is located at 350 nm. Therefore, highly-developed Ca^{2+} complexes are difficult to form at pH 12.

Circular dichroism (CD) is effective for probing the chromophore organization in chiral bilayers.[11-13] The phosphate bilayer in water shows a much enhanced CD spectrum compared with that in $CHCl_3$ where the uncomplexed bilayer is totally destroyed (Fig. 4). The Ca^{2+} complex gives enhanced CD spectra in $CHCl_3$ and toluene. The large $[\theta]_{max}$ values ($[\theta]_{345}$, -8×10^4 in $CHCl_3$ and -1.1×10^5 in toluene) indicate that there is strong exciton coupling among the well-aligned azobenzene units. The sign of CD spectra is reversed between the aqueous bilayer and the Ca^{2+} complex in organic solvents. Therefore, the spatial arrangement of the azobenzene unit relative to the chiral center may be reversed between the two systems. Again, the Ca^{2+} complex prepared at pH 12 showed a much reduced CD intensity ($[\theta]$, ca. 10^4) that is comparable to that of the monomeric dispersion.

In conclusion, we demonstrated that the Ca^{2+} complex of the phosphate bilayer retained the organized assemblage in $CHCl_3$ and toluene. Interaction of Ca^{2+} ion with phospholipid membranes has been extensively studied in relation to their physiological implications.[14] The property of dialkyl phosphate bilayers was shown to be affected by Ca^{2+} binding.[15] However, Ca^{2+} binding was never used for the immobilization purpose. The exact structure of the Ca^{2+} complex in organic solvents is not yet clear. The spectroscopic data suggest considerable side-chain alignment, and the electron microscopic observation (100 Å diameter) is consistent with 2-4 molecular layers, depending on the tilting of the molecular axis in the

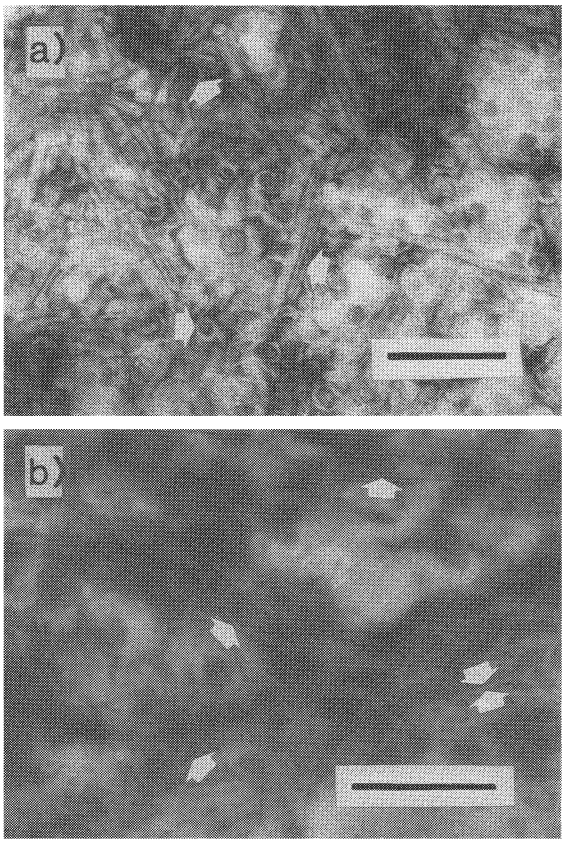


Fig. 1. Electron micrographs, post-stained with ammonium molybdate. Scale bar, 2000 Å.
a) aqueous dispersion of 1.
b) Ca^{2+}/1 complex in $CHCl_3$.

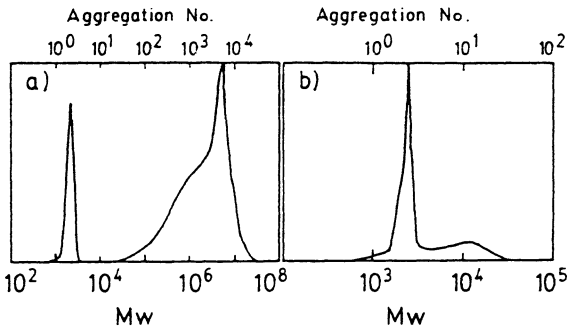


Fig. 2. Gel permeation chromatograms, of Ca^{2+}/1 complex. 30 °C.
Solvent, $CHCl_3$.
The complexes were prepared from aqueous dispersions of 1 at pH 7.5(a) and at pH 12(b).

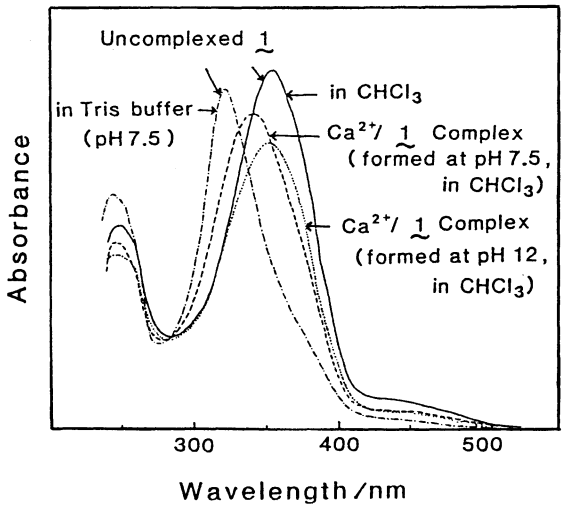


Fig. 3. Absorption spectra of 1 and its stoichiometric Ca^{2+} complex. 20 °C.

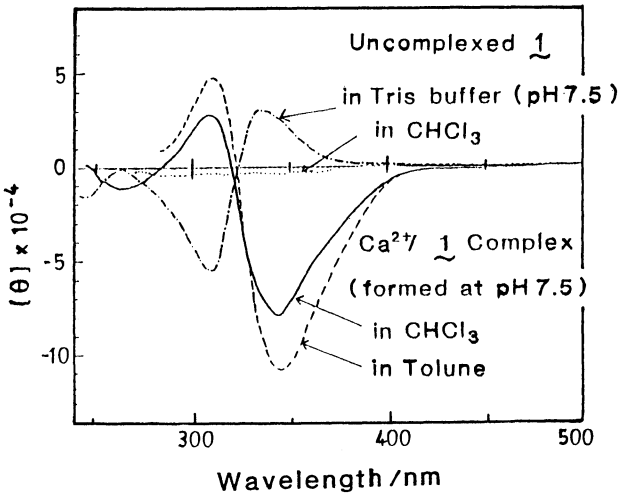


Fig. 4. Circular dichroism spectra of 1 and its stoichiometric Ca^{2+} complex.

aggregate. It is probable that the reversed bilayer assembly as illustrated in Fig. 5 or its multilayers are formed in organic solvents. Extention of the present finding to other systems should be interesting.

The authors thank Dr. Ishikawa for his stimulating discussion and suggestions.

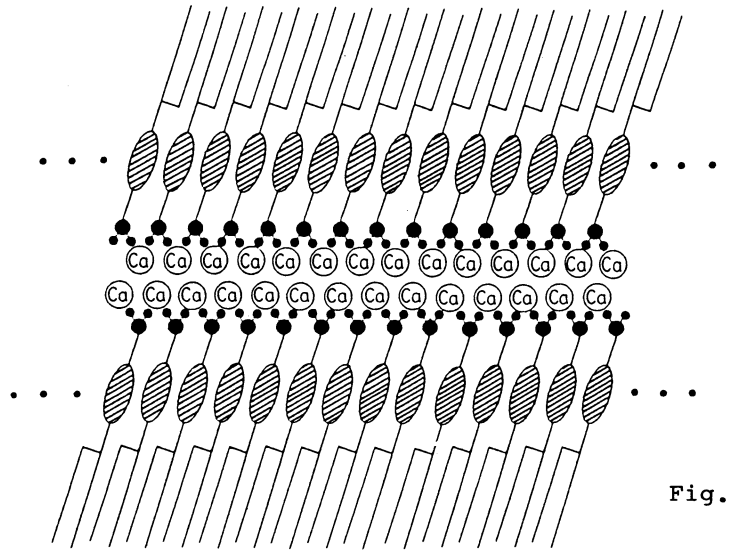


Fig. 5. A schematic illustration of the $Ca^{2+}/\underset{\sim}{1}$ complex.

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8) Mp 85-93 °C; Found: C, 62.13; H, 8.51; N, 4.91%. Calcd for $C_{44}H_{70}N_3O_{10}P\cdot 2H_2O$: C, 62.17; H, 8.54; N, 4.95%. IR, 3300 cm^{-1} (ν_{NH} amide), 1740 cm^{-1} ($\nu_{C=0}$, ester), 1640 cm^{-1} ($\nu_{C=0}$, amide), 1020 cm^{-1} (ν_{P-O-P}, phosphate ester).
9) Instrument, Seiko Electronics, SPS 1200 VR Plasma Spectrometer. We are grateful to Prof. S. Morooka of Kyushu University for the use of a plasma spectrometer and kind technical advice.
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(Received February 28, 1989)