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Oriented Intercalation of Myoglobin into Multilayered Films of Synthetic Bilayer Membranes[1)]

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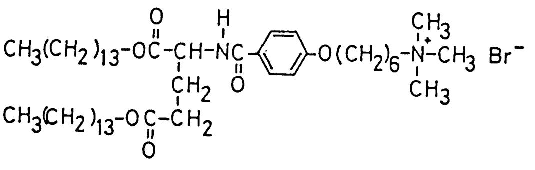
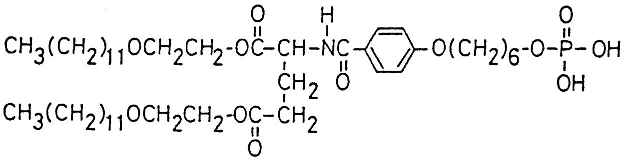
Myoglobin (Mb) was intercalated in a specific orientation into a multilayered cast film of a phosphate bilayer membrane, whereas Mb intercalated in an ammonium multibilayer film was denatured.

Layered compounds such as clay and graphite are prone to intercalate various molecules into their interlayer space to form novel composite materials.[2)] Guest molecules are widely varied from fluorine, amines, alcohols and organometals to polymers. We have shown that cast films of synthetic bilayer membranes constitute a novel class of the versatile layered templates.[3-6)] Compared with the conventional layered compounds, the cast film is characterized by ready modifications of the kind, density and distribution of interlayer charges, in addition to the flexibility of the layered structure. We describe herein oriented intercalation of myoglobin(Mb) molecules as a new example. This method complements our previous results that casting of aqueous mixtures of bilayer membrane and myoglobin produce regular multi-layered films in which myoglobin molecules are aligned in specific ways.[7)]



1 2C$_{14}$-L-GluPhC$_6$N$^+$



2 2(C$_{12}$OC$_2$)-L-GluPhC$_6$P

Ammonium amphiphile **1**, phosphate amphiphile **2** and distearoyl phosphatidylcholine (DSPC) were used as representative bilayer-forming amphiphiles. Cast films of these bilayer membranes were prepared as previously reported.[6)] Given amounts of amphiphiles were sonicated in 10 mM Tris-HCl buffer (pH 7.5) to give homogeneous 40 mM dispersions, which were cast onto glass plates or Fluoropore membranes (Sumitomo Electric, pore size 0.10 μm).

Drying at room temperature for 2-3 days gave self-supporting films.8) Intercalation of Mb (met-Mb from horse heart, Sigma) into these films was conducted by soaking the films in aqueous Mb (0.2 mM) for given periods of time. The cast films were then taken out, blotted by filter paper and dried at room temperature.

Intercalated Mb was detected by UV-visible spectroscopy of the cast film. Figure 1 shows absorption spectra of cast films which are soaked in aqueous Mb. In the case of ammonium (**1**) and phosphate (**2**) films, there exist absorption maxima at 409 nm that are ascribed to the Soret band of met-Mb.9) In contrast, this peak is not observed in the film of zwitterionic DSPC. Since the isoelectric point (pI) of horse Mb is reported to be 6.99,10) the net charge of Mb is approximately neutral under the present experimental conditions. It is clear that the overall charge of the Mb molecule is not important in the intercalation process. They are apparently intercalated into the polar interbilayer space by the Coulombic interaction between local surface charges of Mb and the oppositely-charged bilayer surface. Intercalation of Mb proceeds readily during the initial 10 min, passes a maximum and is lessened again, as shown in Fig. 2. Swelling of the cast film occurred upon prolonged soaking and the film was partially peeled off. This explains the decrease in Mb at the later stage.

The molar ratio of intercalated Mb and an amphiphile component was determined by redispersion of the composite films in buffer solutions and measurement of the Mb concentration by UV-visible spectra. The ratio was 1/615, 1/250 and 1/10000 in the cationic (**1**), the anionic (**2**) and the zwitterionic (DSPC) films, respectively. The cast films can accommodate 1/40-1/20 (mol/mol) of Mb per amphiphilic component (the molecular cross section, 60-100 Å).10,11) However, Mb molecules are not incorporated in such large amounts in the present method. The ratio of 1/250 corresponds to about 10% occupation of the total bilayer surface. These molar ratios cannot be explained by simple adhesion of Mb molecules to the surface of cast films such as discussed by Pachence and others.13)

Electron spin resonance spectroscopy (ESR) of the composite film demonstrated a remarkable difference between phosphate bilayer **2** and ammonium bilayer **1**. The heme unit of native met-Mb is in the ferric high spin state, and is a very useful probe for monitoring the orientation as well as denaturation of Mb. ESR spectra of Mb in the anionic film gave g values that are in satisfactory agreement with those of native Mb.14) In addition, macroscopic anisotropy was noticed as shown in Fig. 3. The $g_{\parallel}$ component does not appear when the normal of the film plane is parallel to the applied magnetic field ($\phi = 90°$). It displays a maximum intensity at $\phi = 15°$. The intensity of the $g_{\perp}$ component gradually decreases from $\phi = 90°$ to 0°. This angular dependence undoubtedly indicates that the heme plane

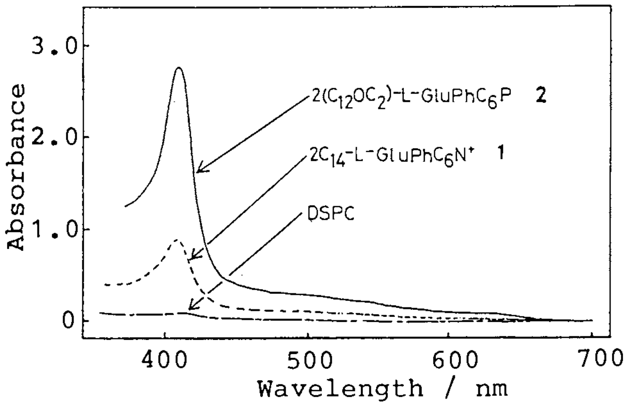


Fig. 1. UV-visible spectra of cast films which were soaked in 0.2 mM Mb in 10 mM Tris-HCl buffer, pH 7.5 for 3 h. Cast films were attached to glass plates for the spectral measurement.

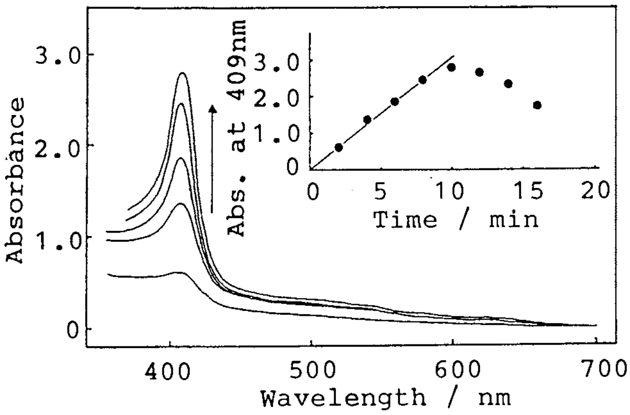


Fig. 2. Time course of intercalation of Mb into the phosphate cast film. [Mb]=0.8 mM. The other conditions are identical to those in Fig. 1.

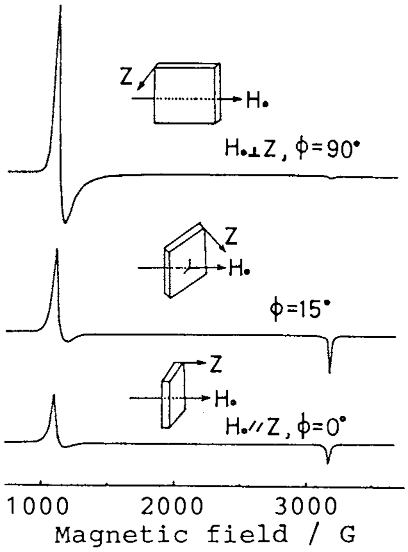


Fig. 3. Anisotropic ESR spectra of Mb (Mb/**2**=1/330) intercalated into the phosphate cast film (JEOL JES 2 X X-band spectrometer, at 4 K, microwave power 5 mW, microwave frequency 9.044 GHz, modulation amp. 7.9 G).

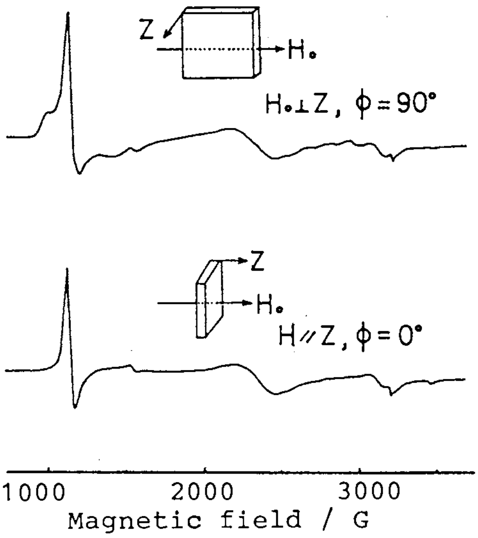


Fig. 4. ESR spectra of Mb intercalated into the ammonium cast film (Mb/**1**=1/615). The spectral conditions are identical to those in Fig. 3.

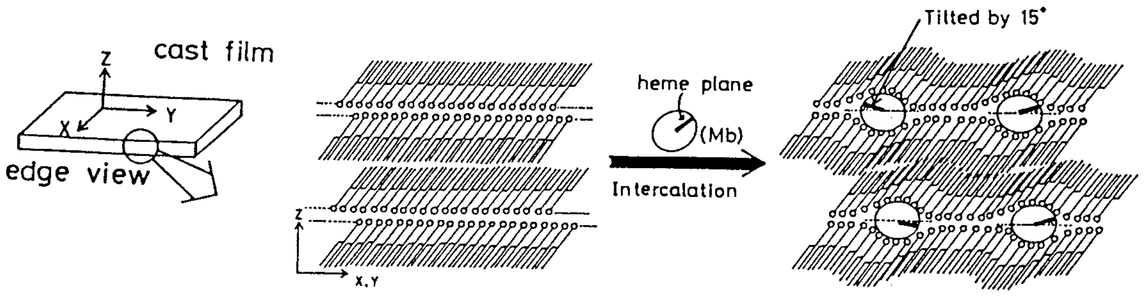


Fig. 5. Schematic illustration of the intercalation process.

of intercalated Mb molecules is tilted regularly against the film plane by 15°. This tilt angle coincides with that observed in the case of direct cocasting,7) and its standard deviation (20-25°) is slightly improved relative to that of the cocasting (30°). Interestingly, extra peaks are observed at g = 6.8, 4.3, and 2.5 in ESR spectra of Mb intercalated in the cationic film (Fig. 4). The spectral pattern is independent of angle ϕ. Therefore, we conclude that intercalation of Mb into the cationic film is nonspecific adsorption with concomitant denaturation.

A DSC thermogram (Seiko Instruments SSC 5200, scan speed = 1°C /min) of the cast film of **2** gave an endothermic peak at 59°C (ΔH = 52.2 kJ/mol) due to gel-to-liquid crystal phase transition. The phase transition temperature (peak top) after Mb intercalation was found at 57°C (ΔH = 90.5 kJ/mol). The side-chain alignment in the bilayer membrane is not essentially affected by Mb intercalation.

As for the selective intercalation into layered compounds, Yamagishi reported that optically active tris(bipyridyl) metal chelate ($M(bpy)_3$) can be selectively intercalated into smectite clays.15) Kijima and coworkers showed that a copper complex of β-cyclodextrin was oriented in montmorillonite clay.16) To the best of our knowledge, the present example is unprecedented in that biological macromolecules are incorporated into layered compounds in specific manners. The present methodology would be applied widely to artificial organization of biomolecules together with the casting method previously reported by us.

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