Gel to Liquid Crystal Phase Transition of Black Lipid Films in Air as Studied by FT-IR Spectroscopy

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FT-IR spectra of black lipid films formed by dimyristoylphosphatidylcholine (DMPC) in air were recorded at various temperatures for the first time. Balck lipid films drawn from a DMPC aqueous dispersion and a DMPC+0.1 $MCaCl_2$ aqueous dispersion showed the gel to liquid crystal phase transition at about 24 and 27 °C, respectively.

Only a limited number of papers have dealt with the formation of black lipid films in air with insoluble surface-active agents such as fatty acids,[1] alkanols[2] or phospholipids.[3-7] In addition, no spectroscopic investigation has been made of phase transitions only induced by temperature (without electrolyte) in black lipid films formed with such insoluble monolayers, because it is very difficult to obtain stable black lipid films in the gel state. Therefore, we devised a new temperature-controlled cell and a method to obtain stable black lipid films in the gel state and first succeeded to record FT-IR spectra of the film at various temperatures. To prepare the aqueous dispersions, 50 mg of DMPC was sonicated (A) in 100 ml pure water and (B) in the presence of 0.1 M $CaCl_2$, respectively, after Vortex mixing at 40 °C above the gel to liquid crystal phase transition temperature (T_m=24 °C).[8]

The sample cell adopted in this experiment is illustrated in Figure 1. The main cell body (75 mm internal diameter and 93 mm high) is made of glass. The measuring cell has two CaF_2 windows (17 mm diameter) and a film-supporting rectangular frame of 33 mm wide and 14 mm high, which is made of a 1 mm ϕ platinum wire. The infrared beam normally passed through

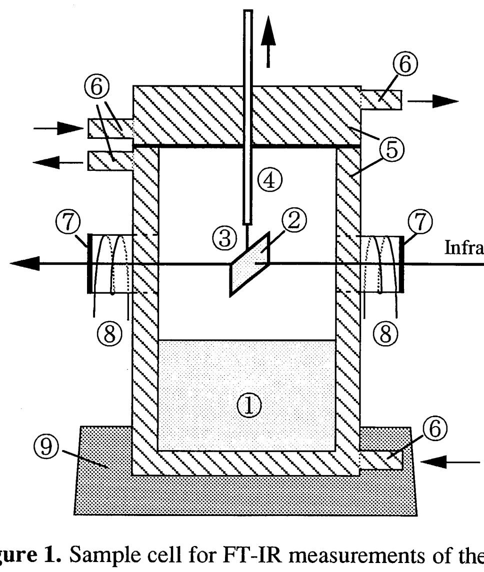

Figure 1. Sample cell for FT-IR measurements of the black lipid films: (1) sample solution containing lipid dispersions, (2) film, (3) platinum frame, (4) stainles steel rod, (5) water-thermostated glass cell consisting of a cover and a body to change the solutions, (6) inlet and outlet for thermostating water, (7) CaF_2 windows, (8) flexible ribbon-heater, (9) vibration dampers.

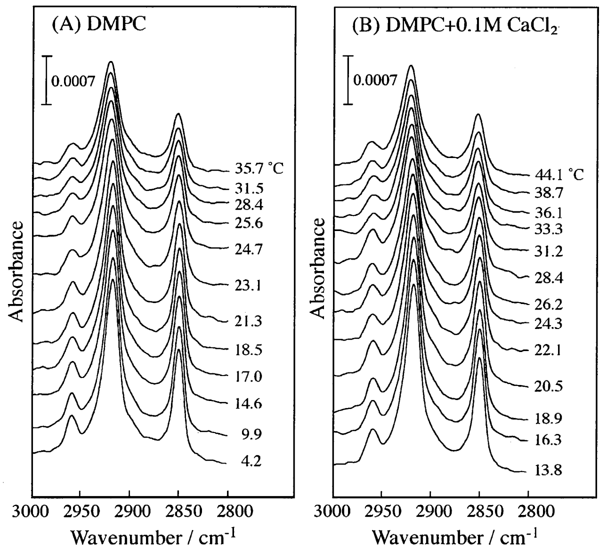

Figure 2. Temperature-induced changes of the infrared spectra in the region of the C-H streching vibrations of the black films drawn from (A) DMPC and (B) DMPC+0.1$MCaCl_2$ aqueous solutions.

the lipid film and two CaF_2 windows on the cell wall. This cell consists of a double wall except for CaF_2 windows and thermostated water was circulated to achieve the equilibrium of temperature and vapor pressure inside the cell. Temperature was monitored by a copper-constantan thermocouple inserted inside the cell. The overall accuracy of temperature controll and reading was within 0.1 °C. At high tempertures, CaF_2 windows were slightly heated by flexible ribbon-heaters coiled up on the cell, to avoid the condensation of water vapor on CaF_2 windows. The platinum frame was attached to a device which could be moved up at a controllable speed.

To ensure the supply of DMPC molecules from the bulk reservoir, we obeyed the new protocol as given below. After the frame was withdrawn by 1~2 mm from the solution within two minutes, we waited 15~20 min, and again withdrawn 1~2 mm from the solution within 2 min and so on. Withdrawing the frame from aqueous dispersions below T_m and the film being attached to the dispersed solution through meniscus below T_m caused the black lipid films to be broken. Thus, we made the film above T_m and perfectly withdrew the frame out of the solution and the black lipid films thus obtained were subjected to FT-IR measurements during the cooling process as they were. The rate of cooling of the bath circulator was ~1 °C/h. After the temperature of the bath circulator reached each set point, 30 min was wasted to assure the thermal equilibrium of the black film. FT-IR spectra were recorded on a Nicolet 710 FT-IR spectrophotometer equipped with an MCT detector with a resolution of 4 cm^{-1} and 1000 -

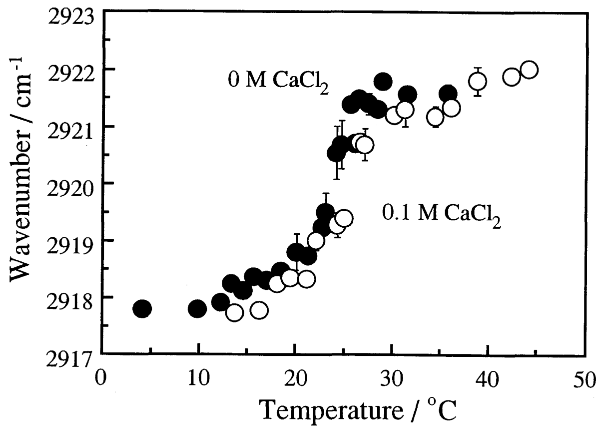

Figure 3. Temperature dependence of the infrared frequency of the CH_2 antisymmetric streching band in the spectra of the black films drawn from (●) DMPC and (○) DMPC+0.1MCaCl$_2$ aqueous solutions.

2000 scans.

Figures 2 (A) and (B) represent FT-IR spectra of the black lipid films which are withdrawn from the aqueous DMPC solution and in the presence of 0.1M $CaCl_2$, respectively. In this frequency region, we can observe the asymmtric CH_3 streching band (ν_aCH_3), antisymmetric and symmetric CH_2 streching bands (ν_aCH_2 and ν_sCH_2) at ca. 2955, 2920, and 2850 cm^{-1}, respectively, with an appreciably good S/N.[9] Remarkable changes in spectral feature with temperature are seen in this figure.

The temperature dependence of the wavenumber of ν_aCH_2 is given in Figure 3. It is found that there are drastic changes centered at about 24 and 27 °C for DMPC films drawn from aqueous dispersions without and with $CaCl_2$, respectively, in the plots. These changes correspond to the gel to liquid crystal transitions which are usually observed in the lipid dispersions in water,[9] the temperatures agreeing well with the values obtained from measurements of the lipid dispersions,[10-12] except for small vesicles.[8] These results suggest that the conformational change of the hydrocarbon chain predominantly contributes to T_m of both black lipid films in air and the large vesicles.[8] Since the electrostatic interaction of Ca^{2+} with the lipid polar group leads to a tighter packing and an increased order of the hydrocarbon chains, the increase in the phase transition temperature occurs.[13] The correspondence of the phase transition temperatures of the lipid films with T_m of the lipid bilayers are supported by the results obtained by the measurements of surface diffusion coefficient of phospholipids in black lipid films[6] or by the measurements of the film drainage transition temperatures of lipid films.[4] The decrease in wavenumber of the CH_2 stretching bands of the methylene chain upon transitions from a higher temperature phase to a lower temperature phase is a general trend of lipids.[9] In this case, area of the films is fixed by the frame. Therefore, we can conclude that the decrease in wavenumber is responsible for the decrease in *gauche* conformer. We are investigating molecular orientations in these lipid films by FT-IR polarization measurements and hope to present these results in the near future.

References and Notes

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