The interaction of ubiquinone-3 with phospholipid membranes

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Abstract The effects of ubiquinone-3 (UQ) on dipalmitoylphosphatidylcholine (DPPC) membrane were studied by surface monolayer, differential scanning calorimetry (DSC) and fluorescence techniques. DPPC and UQ are proved to be freely miscible in the mixed monolayer at an air/water interface, and to be partially miscible in bulk phase, i.e. bilayer and solid phase. There is a condensing interaction between UQ and DPPC in the UQ/DPPC mixed monolayers. The solubility of UQ in the DPPC is about 20 mole% and the solubility of DPPC in UQ is about 10 mole%. The membrane fluidity of DPPC was increased by the addition of UQ and the phase transition temperature was decreased.

Key words: Ubiquinone-3; Dipalmitoylphosphatidylcholine; Monolayer-bilayer equilibrium; Solubility; Membrane fluidity

1. Introduction

Ubiquinone is a well known constituent of the mitochondrial inner membrane [1,2] where it is believed to function as a mobile carrier of electrons and protons in the mitochondrial electron transfer chain. The mobility, location and association of ubiquinone in phospholipid membrane have been investigated on the basis of surface monolayer [3], differential scanning calorimetry (DSC) [4], and fluorescence techniques [5]. There seems to be general agreement that a limited amount of ubiquinone-10 is incorporated into phospholipid bilayer membranes (less than 10 mole%), and that an appreciable fraction forms a separate phase located outside bilayers [3–5]. On the other hand, ubiquinone-3 (UQ) has appreciable solubility in phospholipid bilayers and the addition of some other neutral lipids, such as diglyceride [6,7], monoglyceride [8], and menaquione-4 [9], which have appreciable solubility in phospholipid bilayers to the bilayers, changes the hydrophilic-lipophilic balance and induces a phase transition from the bilayer to a hexagonal H_{II} or reversed cubic phase.

In this study, in order to clarify the interaction between UQ and phospholipid, we prepared mixed monolayer films from UQ and L-α-dipalmitoylphosphatidylcholine (DPPC) and determined their behavior. The miscibility and solubility of UQ and DPPC were evaluated by DSC. In addition, the effect of UQ on the fluidity and diffusion of fluorescence probe of pyrene in DPPC membrane was examined.

2. Materials and methods

2.1. Materials

UQ and DPPC were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Wako Pure Industrial Ltd. (Osaka, Japan).

2.2. Determination of the behavior of UQ in mixed monolayers with DPPC and measurements of collapse and spreading pressures

UQ, DPPC and UQ/DPPC mixtures were dissolved in benzene as the spreading solvent. The solution was added with an Agla micrometer syringe onto double-distilled water. After complete evaporation of the solvent, the surface pressures of the monolayers were measured by Wilhemy's method using a surface tensiometer (Model CBVP-A3, Kyowa Kaimenkagaku Co., Ltd., Tokyo, Japan), and a surface pressure-area per lipid molecule curve was obtained. The collapse pressures of the monolayer (surface pressures at the transition point from monolayer to bilayer or solid states) were determined from the inflection points on the curves. The spreading pressures of UQ/DPPC mixtures at an air/water interface (surface pressures at the transition point from bilayer or solid states to monolayer) were obtained from the steady value of the surface pressure at 12–24 h after the addition of the lipid or lipid mixture on water. Both the collapse and spreading pressures were determined at 25°C. Details of the monolayer techniques have been described elsewhere [10,11].

2.3. Thermotropic behavior of UQ/DPPC mixtures and solubility of UQ in DPPC membranes and of DPPC in UQ

In order to determine the solubility of UQ in DPPC membranes and of DPPC in UQ, DSC was performed using a Model DSC-100 (Seiko-Denshi Co., Ltd., Tokyo, Japan). UQ/DPPC mixtures (total 1.5×10^{-6} mole) in 40 μl of water were placed in a DSC pan and sealed. An equal volume of water was placed in the reference pan. Temperature scans were made from 10°C to 70°C with constant heating rates of 2°C/min. All calorimetric data were obtained from samples during the heating phase. The onset and completion temperatures for the phase transition, i.e. gel to liquid crystalline transition for hydrated lipid bilayers and melt-crystallization for non-hydrated lipid systems, were determined and the temperatures were used to define phase boundaries of the binary phase diagram, i.e. solidus and liquidus lines [12].

2.4. The effect of UQ on membrane fluidity

The effect of UQ on the membrane fluidity of DPPC was determined using a fluorescence polarization technique (probe: DPH). DPH was added at 1 mole% of total lipids. All fluorescence measurements were carried out using a Model F-4500 fluorescence spectrophotometer (Hitachi Co., Ltd., Tokyo, Japan) equipped with a thermoregulated cell compartment, Atago Coolnics Model REX-C10 (Atago Co., Ltd., Tokyo, Japan). The degree of polarization (P) was calculated using the following equation;

$$P = (I_{VV} - C_f \cdot I_{VH})/(I_{VV} + C_f \cdot I_{VH})$$

where I is the fluorescence intensity and subscripts V and H indicate the vertical and horizontal orientations of excitation (first) and analysis (second) polarizers, respectively. C_f ($= I_{HV}/I_{HH}$) is the grating correction factor.

2.5. Pyrene fluorescence spectra

In order to determine the effect of UQ on the phase transition temperature of DPPC membrane, pyrene fluorescence spectra were measured. DPPC and UQ were dissolved in chloroform and then mixed at a suitable ratio. Pyrene was dissolved in tetrahydrofuran and added to the lipid mixture to achieve 1 mole% of total lipids. The solvents were evaporated under a steam of nitrogen gas at 70°C. The lipid film was hydrated to give a total concentration of the total lipids of 1 mM with 4.25 mM phosphate-NaOH buffer (pH 7.3). The

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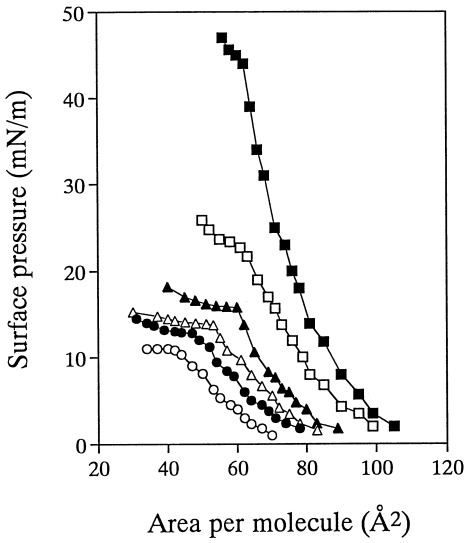

Fig. 1. Surface pressure-area curves for mixed monolayers of UQ and DPPC at different molar ratios. ○: $X_{UQ}=1.0$; ●: $X_{UQ}=0.67$; △: $X_{UQ}=0.5$; ▲: $X_{UQ}=0.33$; □: $X_{UQ}=0.2$; ■: $X_{UQ}=0$.

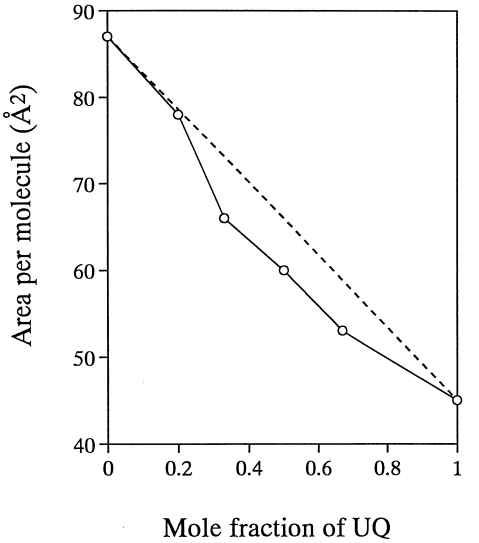

Fig. 2. Average area per molecule versus molar ratio of the lipids for mixed monolayers of UQ and DPPC at 10 mN/m. (The broken lines represent the additivity rule.)

lipid dispersion was then sonicated with a probe-type sonicator (Tomy Seiko Co., Ltd., Tokyo, Japan) at 50°C for 10 min.

Fluorescence spectra of pyrene embedded in DPPC liposomes were measured with a Hitachi F-4500 fluorescence spectrophotometer equipped with a temperature controlled cuvette holder. Measurements were made with an exciting wavelength of 330 nm and emission wavelengths of 373 nm and 480 nm at increasing temperature [13].

3. Results and discussion

3.1. Monolayer properties of UQ and DPPC

The behavior of UQ in mixed monolayers with DPPC was examined by determining the properties of binary mixtures of UQ with DPPC spread on subphases of water. Surface pressure-area curves for mixed monolayers are presented in Fig. 1. UQ and DPPC have a limiting area of 40 and 60 Å^2 per molecule, respectively. The average area per molecule versus the molar ratio of the lipids in the monolayer at 10 mN/m is shown in Fig. 2. The broken line between the end points in the figure represents the additivity rule of monolayer areas and of average potentials. It can be seen that at a surface pressure of 10 mN/m, the molecules occupy a volume in the film that is smaller than would be expected from the pure compounds alone. It is suggested that there is a condensing effect brought about by the interaction between the molecules of the film.

3.2. Monolayer-bilayer equilibrium of UQ/DPPC mixtures

Monolayer-bilayer equilibrium of UQ/DPPC mixtures were estimated from the measurements of the collapse and spreading pressures as shown in Fig. 3. The collapse pressure of DPPC, which is the surface pressure at transition from monolayer to bilayers, was 45.0 mN/m. The spreading pressure of DPPC, which is the surface pressure at transition from bilayers to monolayer [10], was identical to the collapse pressure. The collapse and spreading pressures of UQ, which are the surface pressures at the monolayer-solid equilibrium of this compound, were 11.0 mN/m. The collapse and spreading pressures of DPPC were also consistent with each other (45.0 mN/m), and the values agree with the reported collapse pressure of about 45.0 mN/m [14]. The collapse and spreading pressures of a lipid mixture generally have different values, and are dependent on the miscibility of the lipids in the monolayer and bulk phase (bilayer or solid) [15].

The collapse and spreading pressures of UQ/DPPC mixture obtained as a function of the composition, therefore, give a phase diagram for the monolayer-bilayer equilibrium as

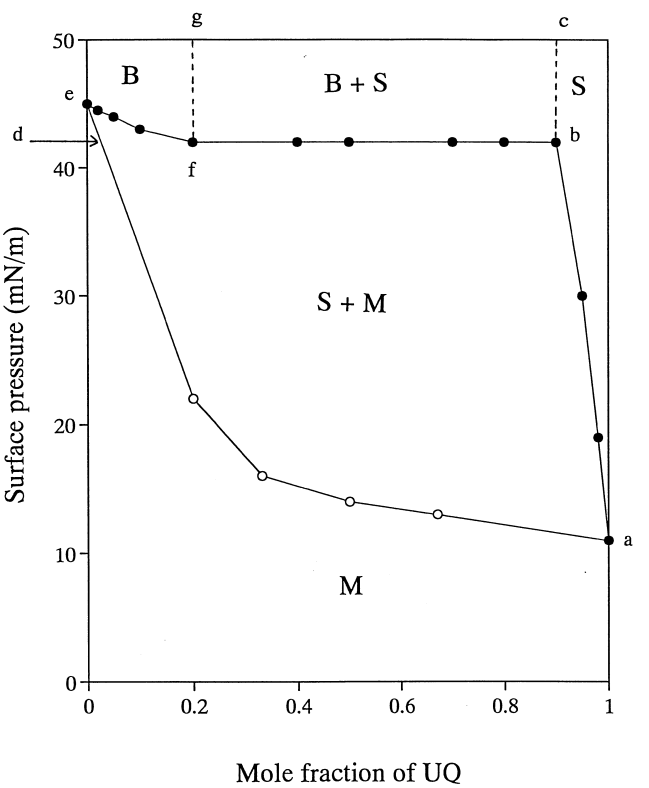

Fig. 3. Monolayer-bilayer equilibrium of the UQ/DPPC mixture in the presence of water. Spreading pressure (●), collapse pressure (○). The spreading pressure of the mixture is presented by the line abfe. The collapse pressure of the mixture is presented by the line adg.

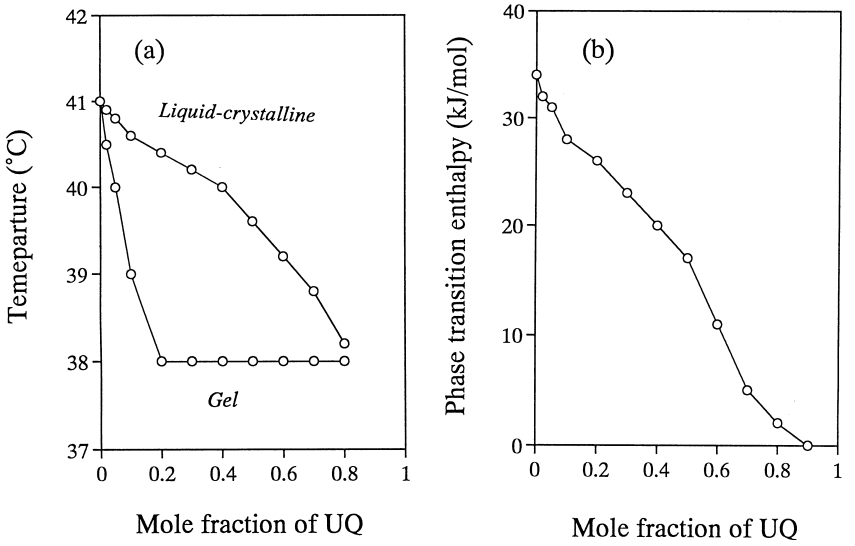

Fig. 4. a: Pseudo-binary phase diagram for UQ/DPPC mixture constructed from DSC curves. b: Phase transition enthalpy represented as a function of mole fraction of UQ (X_{UQ}) in the mixture determined by DSC.

shown in Fig. 3. The collapse pressure varied with mole fraction of UQ (X_{UQ}) in the mixed monolayer, while the spreading pressure kept constant at 42.0 mN/m in the X_{UQ} range 0.2–0.9 (line fb in Fig. 3). On the basis of the surface phase rule [15], DPPC and UQ are proved to be freely miscible in the mixed monolayer at an air/water interface (M), and to be partially miscible in bulk phase, i.e. bilayer (B) and solid phase (S). The solubility of UQ in the DPPC is about 20 mole% and presents a striking contrast to the much smaller solubility (2–5 mole%) of ubiquinone-10 [14] and triglyceride [16]. On the other hand, the solubility of DPPC in UQ is about 10 mole%.

3.3. *Thermotropic behavior of UQ/DPPC mixtures and solubility of UQ in DPPC membranes and of DPPC in UQ*

Fig. 4 represents the solubility of UQ in DPPC membranes determined by DSC. The onset and completion temperatures of the phase transition were determined (Fig. 4a) and the addition of UQ decreased the onset temperature of the phase transition, and at X_{UQ} values higher than 0.2, the phase transition temperature was constant at 38°C. The phase transition was abolished at X_{UQ}=0.9. The lowering of the phase transition temperature would suggest that UQ increases the fluidity of the hydrocarbon chain region of DPPC liposomes. This indicates that partial phase separation occurs in the gel phase bilayer and the solubility of UQ in the DPPC membrane was equivalent to a mole fraction of 0.2. This behavior is similar to that of other compounds containing a phytanoyl chain of similar length, such as vitamin K_1 [17].

The phase transition enthalpy decreased with an increase in X_{UQ}, and the phase transition was abolished at $X_{UQ} = 0.9$ (Fig. 4b). This indicates that at $X_{UQ} = 0.9$, DPPC was completely incorporated in UQ and that the solubility of UQ in the DPPC membrane was equivalent to a mole fraction of 0.1.

These findings agree with the solubility of UQ in DPPC membranes and of DPPC in UQ as determined by the monolayer-bilayer equilibrium.

The miscibility of a neutral lipid such as UQ in the phospholipid bilayer has been assumed to be dependent on the length of the hydrocarbon chains. It has been shown that fatty acid chains can be perturbed beyond C_{10} without affecting the main calorimetric transition [18]. Ubiquinone-10, because of its longer side chain, must be more lipophilic than UQ and the solubility in the phospholipid bilayer has been reported to be less than 5 mole% [19]. In contrast, the side chain of UQ is shorter than that of ubiquinone-10 and will emerge preventing the all-*trans* conformation of the carbon atoms in the region C_1–C_{10}.

On the other hand, regarding the miscibility in the mixed monolayer, it has been reported that ubiquinone-10 and egg phosphatidylcholine could be freely miscible in the monolayer [15] and we have obtained the similar results that UQ and DPPC could be freely miscible in the monolayer. Therefore, the length of the side chain of the neutral lipids will not affect the miscibility in the monolayer.

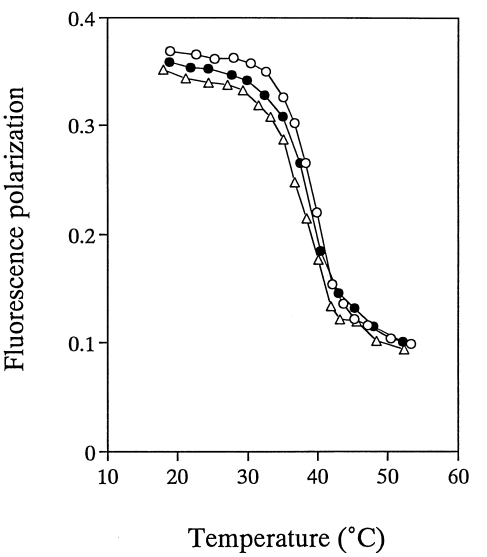

Fig. 5. Relationship between incubation temperature and fluorescence polarization using DPH as a function of the lipid mole fraction in the lipid mixtures. $X_{UQ} = 0$ (○), 0.1 (●), 0.2 (△).

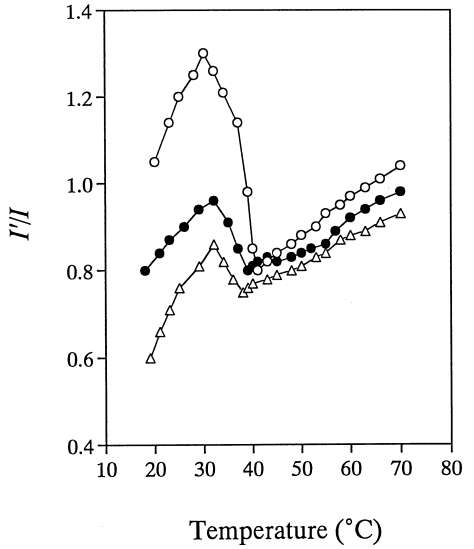

Fig. 6. Relationship between incubation temperature and I'/I for pyrene diffusion in the lipid mixtures. $X_{UQ} = 0$ (○), 0.1 (●), 0.2 (△).

3.4. Membrane fluidity

The membrane fluidity of the UQ/DPPC mixtures was evaluated using fluorescence polarization techniques (Fig. 5). The fluorescence polarization of DPH in DPPC liposomes decreased markedly around 41°C, indicating that the phase transition of the DPPC bilayer from gel to liquid crystal state occurs at this temperature. The phase transition of the lipid mixtures was dependent on X_{UQ}. At $X_{UQ} = 0$, 0.1, 0.2, the phase transition temperatures were 41, 39 and 38°C, respectively, and agreed with the results of DSC. These results indicate that with the increase in X_{UQ}, a more fluid membrane was formed and the cooperative interaction between the DPPC molecules decreased.

3.5. Pyrene diffusion

Fig. 6 shows the effect of UQ on excimer formation in DPPC membranes. The pyrene fluorescence intensity ratio I'/I, the ratio of the excimer fluorescence intensity (480 nm, I') to the monomer fluorescence intensity (373 nm, I), was plotted as a function of the temperature. With the addition of UQ, the intensity ratio I'/I decreased in the range of 15–70°C. The lipid phase transition is characterized by a sharp decrease in the intensity ratio I'/I [17]. The I'/I ratio of DPPC/UQ mixtures at $X_{UQ} = 0$, 0.1, 0.2 decreased around 41, 39, 38°C, respectively and these temperatures were suggested to be phase transition temperatures. These results are in good agreement with the results from DSC and fluorescence polarization. The change in I'/I at the phase transition decreases with decreasing pyrene concentration and correlating with diffusion coefficients (D_{diff}) for lateral diffusion of pyrene in lipid membranes [19]. The I'/I values at X_{UQ}=0, 0.1, 0.2 at 37°C were 1.14, 0.85, 0.75, indicating that as X_{UQ} is increased, D_{diff} will be decreased and there will be a reduction for the lateral diffusion.

4. Conclusions

The effects of UQ on DPPC membrane were studied using several physical techniques such as surface monolayer, DSC and fluorescence spectroscopy. DPPC and UQ are proved to be freely miscible in the mixed monolayer at an air/water interface, and to be partially miscible in bulk phase, i.e. bilayer and solid phase. There is a condensing interaction between UQ and DPPC in the UQ/DPPC mixed monolayers. The solubility of UQ in the DPPC is about 20 mole% and the solubility of DPPC in UQ is about 10 mole%. The membrane fluidity was increased by the addition of UQ and the phase transition temperature was decreased.

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