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Interaction Between Ubiquinones and Dipalmitoylphosphatidylcholine In Mixed Langmuir Monolayers

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The interaction of two ubiquinones having different chain lengths, ubiquinone-2 and ubiquinone-10 with 2 saturated or 10 unsaturated isoprenoid groups, with dipalmitoylphosphatidylcholine (DPPC) has been studied in mixed monolayers. The miscibility of the mixtures has been investigated by pressure-area measurements and simultaneous fluorescence microscopy at the air-water interface. Results were analyzed in terms of the additivity rule and the excess free energies of mixing were calculated from the compression isotherms in the full range of ubiquinone concentrations. We have also evaluated the area contribution of each ubiquinone molecule to the total molecular area of the mixed films. Stable monolayers are formed by the oxidized forms of the two ubiquinones but by increasing the lateral pressure, the ubiquinones are expelled from the films. The difference observed between the two ubiquinones in mixed monolayers are explained by a difference of localization according to their side chain lengths.

Keywords: Langmuir monolayers; mixed films; ubiquinones; DPPC; epifluorescence

INTRODUCTION

Ubiquinones (or Coenzyme Q) are known to be key components of electron transport chains of mitochondrial and bacterial membranes [1]. All compounds of this group contain a redox-active 2,3-dimethoxy-5-methylbenzoquinone nucleus with a hydrophobic prenyl side chain in the 6 position (see Figure 1). They differ from one another in the length of the isoprenoid chain and sometimes in its saturation state [2]. The major physiological form of ubiquinones in animals and humans is ubiquinone-10 (UQ-10), containing 10 monounsaturated trans-isoprenoid units.

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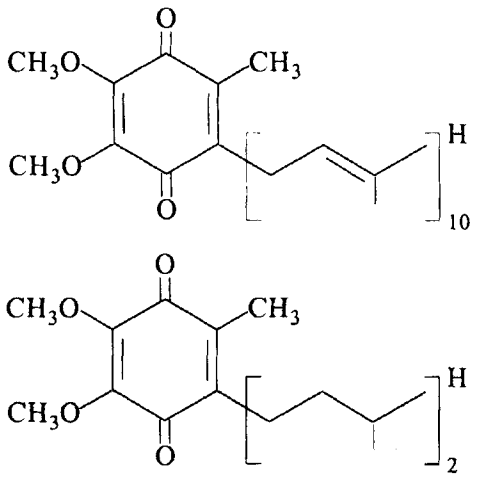

FIGURE 1 Chemical structure of Ubiquinones

The main function of ubiquinones in biology is to act as redox component of transmembrane electron transport systems. Ubiquinones have also been suggested to transport protons across the inner mitochondrial membrane by transverse diffusion, resulting in the conservation of energy ("proton-motive UQ cycle") [3, 4]. They serve as hydrogen carriers across the membrane and are responsible for the electrochemical proton gradient necessary for the production of chemical energy (ATP). It is now clear that ubiquinones serve not only as coenzymes but also, in their reduced forms (ubiquinols), as antioxidants [5]. Indeed, ubiquinols protect membrane phospholipids and serum low-density lipoprotein from lipid peroxidation and also mitochondrial membrane proteins and DNA from free-radical induced oxidative damage [6].

Determining the orientation and the localization of ubiquinones in membranes is essential for understanding their role as diffusible redox carriers. A series of reports has been published addressing that problem, using phospholipid bilayers as membrane models [7, 8, 9]. At least for UQ-10, the authors agree in that the hydrophobic part of the molecule is in the non-polar environment of the bilayers, removed from the lipid/water interface. The lack of consensus between theses studies concerns the location of the benzoquinone ring: either in the bilayer midplane or closer to the lipid headgroups.

Pure and mixed monomolecular films containing ubiquinones are interesting models to investigate their interactions with phospholipids. Indeed, the film balance technique was already successfully applied to the investigation of mixtures

of monogalactosyldiacylglycerol with plastoquinones and α-tocopherol [10]. Ubiquinone monolayers at the aqueous-air interface have not been studied to any extent, some pressure-area isotherms have been reported for UQ10 with 1,2-dimyristoyl phosphatidylcholine [11].

In the present study, we report surface pressure-area isotherms of two pure ubiquinones (UQ-10 and UQ-2, Figure 1), and of their mixing with dipalmitoylphosphatidylcholine (DPPC). The saturated dimethyl-3,7 octyl ubiquinone (UQ-2) was chosen in order to gain more information on the interaction of the benzoquinone ring of ubiquinones with phospholipids.

The interactions among molecules are analyzed by means of the mixing excess free energy and also by the expression of the area contribution of each ubiquinone molecule to the total molecular area of the mixed films [12, 13]. Using epifluorescence microscopy and image analysis, we have studied the changing sizes of condensed domains as a function of the molar fraction of ubiquinone in the mixed monolayers.

MATERIALS AND METHODS

Materials

Ubiquinone-2 (UQ-2) and Ubiquinone-10 (UQ-10) were purchased from Sigma and used without further purification. L-α-Dipalmitoylphosphatidylcholine (DPPC) was of the purest available quality from Sigma. The fluorescent probe 1-palmitoyl-2{6[(7-nitro-2–1,3-benzoxadizole-4-yl)amino]dodecanoyl}phosphatidylcholine (NBD-PC) was purchased from Molecular Probes Inc., USA. Chloroform was of analytical grade from Fisher Scientific Co., France. The subphase was water treated on an Elgastat UHQ 2 system (resistivity of 18 MΩcm), or water containing sodium chloride 150 mM. Stock solutions of UQ-2, UQ-10 and DPPC, 2.5 10^{-4} M, were prepared in chloroform and spread mixed or separately in the wanted molar ratios.

Methods

Monolayers

The isotherms were measured with a Krüss film balance using a pendulum 100 and they were recorded with a computer as previously described [14]. The films were spread with a Hamilton 1705 RN microsyringe and at least 20 min was allowed for solvent evaporation. The compressions were performed at a speed of

0.15 nm^2 min^{-1}. All the experiments were done at pH 5.7 and at temperature of 22°C. All the surface pressure-area isotherms presented in this work represent the average of at least three measurements.

Epifluorescence microscopy of monolayers

The epifluorescence microscopy observations were made by means of an Olympus-BX30 microscope, set on a Riegler&Kirstein Langmuir trough. An AIS (MXRi2) video camera with an image intensifier which allows for a very high sensitivity (10^{-6} Lux) enables us to visualize the film and record images. Excitation of fluorescence probe is achieved using high-pressure mercury lamp. Discrimination of excitation (470 nm) and emission (530 nm) fluorescence from the dye (NBD-PC) is regulated by dichroic mirrors and interchangeable cut-off filters.

All experiments were performed at a temperature of 22°C. Mixtures were formed from a chloroform solution of DPPC containing the desired proportions of ubiquinone and 1 mol% NBD-PC. The monolayer features were analyzed using digital image analysis software OPTIMAS 5.1 (Optimas Corporation). The percentage of the dark regions was estimated by division of the total amount of probe-excluded regions by the total area of a frame.

RESULTS AND DISCUSSION

Monolayer isotherms of UQ: DPPC mixtures

Compression isotherms of pure compounds and UQ:DPPC mixtures, performed on pure water subphase (pH 5.7), are shown in Figure 2 for the following mole fractions of ubiquinone: 0.01, 0.05, 0.1, 0.25, 0.5, 0.75. As seen from the isotherms of pure ubiquinones (Fig 2, right part), UQ-10 occupies a markedly greater molecular area than UQ-2 and the latter forms more stable monolayers at higher pressures. At surface pressures about 14 mNm^{-1} and 30 mNm^{-1} for UQ-10 and UQ-2 respectively, a characteristic plateau is observed. In both cases, the pure films could be compressed to very low limiting areas (below 0.2 nm^2) without observing a collapse in pressure. For molecular areas up to 0.9 nm^2 and 0.35 nm^2 for UQ-10 and UQ-2 respectively, the pure films can be reversibly compressed and expanded without any indication of loss of material. For lower molecular area, the expansion isotherms were shifted to lower values in area per molecule.

For mixtures up to 75 mol% for UQ-10 and 10 mol% for UQ-2, isotherms are shifted towards larger area-per-molecule values. The characteristic plateau of the

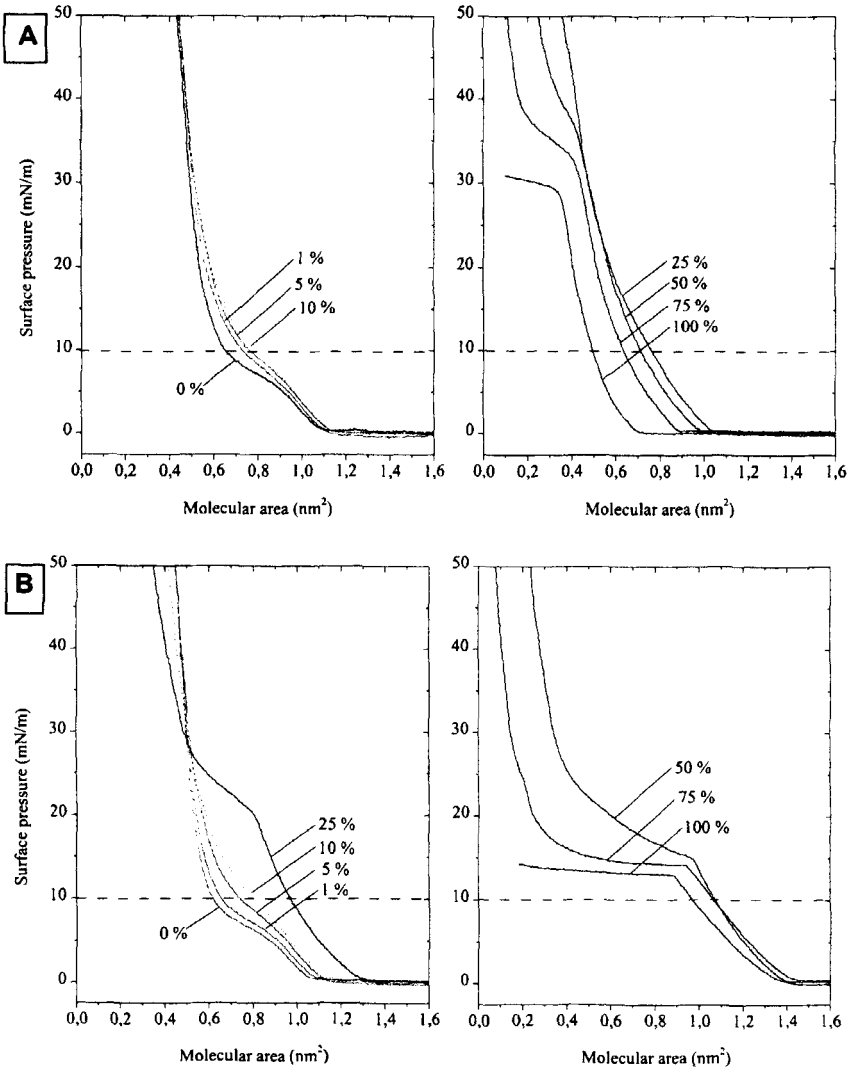

FIGURE 2 Compression isotherms of UQ:DPPC mixtures spread on water, pH 5.7, at 22°C. (A) left part 0–10 mol% UQ-2; right part 25–100 mol% UQ-2. (B) left part 0–25 mol% UQ-10; right part 50–100 mol% UQ-10. Numbers indicate mole fractions of ubiquinone. Dotted horizontal lines indicated the surface pressure at which the S_{DPPC}, S_{UQ} and $S_{DPPC+UQ}$ values have been determined

first order phase transition between liquid expanded LE and liquid condensed LC states in the isotherm of pure DPPC at a pressure of about 7 mNm^{-1} was found to vanish with increasing ubiquinone content in the mixture. At 25 mol% ubiqui-

none, the plateau has completely disappeared in both cases. Isotherms of mixtures with more than 25 mol% for UQ-10 or 50 mol% for UQ-2 showed the characteristic plateau of the ubiquinones. At high surface pressures, for 25 or more mol% ubiquinone, the area per molecule occupied was found to be slightly larger that can be expected for the minimum for DPPC alone. This difference diminishes as the mole fraction of ubiquinone increases probably with simultaneous expulsion of ubiquinone molecules since the molecular area observed at 50 mNm^{-1} with 75 mol% UQ-10 corresponds exactly to the contribution of the 25 mol% DPPC. So, evidence was obtained that UQ:DPPC mixtures with low contents of ubiquinone form stable monolayers, whereas mixtures with large amounts of ubiquinone show instability.

The presence of 150 mM NaCl in the aqueous subphase does not influence the shape of the pressure-area isotherms of the mixtures and the pure compounds at a pH of 5.7 (data not shown).

Analysis of data

An understanding of the interaction between ubiquinone and DPPC is provided by comparing the molecular areas calculated assuming ideality of the mixing through the additivity rule with the experimental areas given in Figure 3. The dashed lines were calculated assuming the additivity rule,

$$S^{ideal}_{DPPC+UQ}(\pi) = X_{DPPC}S_{DPPC} + X_{UQ}S_{UQ} \quad (1)$$

where $S^{ideal}_{DPPC+UQ}$ is the mean molecular area expected at a given surface pressure π in the two-components film, X_{DPPC} and X_{UQ} are the mole fractions of the components in the mixed monolayers, and S_{DPPC} and S_{UQ} are the molecular areas of the pure components at the same surface pressure. This equation states a linear dependence of the average area per molecule on the mole fraction of one component. This equation also holds true if both components are totally immiscible [15]. Figure 3 shows that positive deviations from ideality are observed for the two quinones at 10 mNm^{-1} independently of the nature of the subphase (pure water or NaCl 150 mM).

The additivity rule enables us to examine interactions of components in a mixed monolayer only below the collapse pressure of the component with the lower collapse pressure. Because of loss of material at pressures higher than about 15 mNm^{-1}in the case of UQ-10 mixtures, the surface pressure value of 10 $mNm^{-1}$was chosen in order to compare the behaviour of the two quinones. Moreover, the molecular area expansion is the most important at surface pressures around this value.

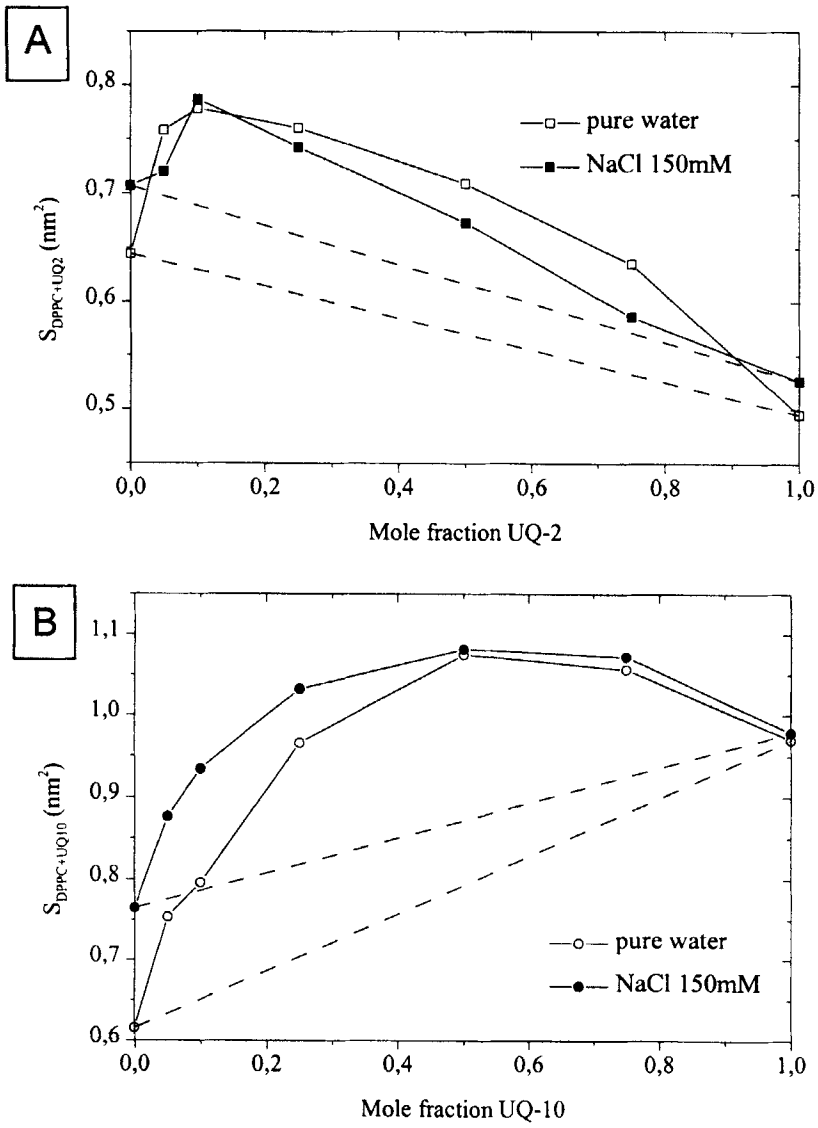

FIGURE 3 Mean molecular area of the mixed UQ-2:DPPC (A) and UQ-10:DPPC (B) as a function of the mole fraction of ubiquinone (X_{UQ}) at a surface pressure of 10 mNm^{-1}. Dashed curves, calculated results assuming the additivity rule; solid and open symbols, the experimental values. Lines between the measured points are simply a visual guide

These deviations from ideality give strong evidence for repulsive interactions between ubiquinone and DPPC and also for miscibility of the two components in the mixed monolayers [15]. In the case of UQ-10 mixtures, the maximum deviation of the experimental data from the theoretical ones for an ideal mixture is observed at 50 mol% UQ-10 corresponding to 1:1 associations. In the case of UQ-2, the deviation from ideality is maximum at only 10 mol% UQ-2 suggesting that above this content, UQ-2 molecules tend to associate themselves in order to minimize their interactions with DPPC in mixed films.

We have also chosen to express the observed effect quantitatively as the area contribution of each ubiquinone molecule S^*_{UQ} to the total molecular area of the mixed film. The films of the mixtures have been compared with that of pure DPPC at a given pressure (always 10 mNm^{-1}) at which the film undergo the same physical strain. The area S^*_{UQ} occupied by the ubiquinone molecule in the mixed film has been calculated according to:

$$S^*_{UQ}(\pi) = S_{DPPC+UQ}\left(1 + \frac{N_{DPPC}}{N_{UQ}}\right) - S_{DPPC} \cdot \frac{N_{DPPC}}{N_{UQ}} \qquad (2)$$

In this relation, N_{DPPC} and N_{UQ} are the numbers of moles of DPPC and ubiquinone, respectively in the spreading mixture. $S_{DPPC+UQ}$ is the mean molecular area of the ubiquinone/lipid mixture and S_{DPPC} the molecular area of the lipid obtained from the compression isotherm of the pure lipid, at a fixed value of the surface pressure. If we use X_{UQ} to describe the mole fraction of ubiquinone, we can further write (2) as:

$$S^*_{UQ}(\pi) = S_{DPPC+UQ}\left(\frac{1}{X_{UQ}}\right) - S_{DPPC}\left(\frac{1}{X_{UQ}} - 1\right) \qquad (3)$$

where X_{UQ} is defined by:

$$X_{UQ} = \frac{N_{UQ}}{N_{UQ} + N_{DPPC}} \qquad (4)$$

The surface pressure at which S^*_{UQ} has been determined, 10 mNm^{-1}, is indicated as dotted lines on the compression isotherms of Figure 2. Figure 4 shows the variations of S^*_{UQ} with the mole fraction of ubiquinone obtained on pure water subphase and in the presence of NaCl 150 mM. The maximum values of S^*_{UQ} are obtained at the lowest ubiquinone mole fraction, excepted for UQ-2:DPPC mixture on NaCl subphase. All curves show that S^*_{UQ} diminishes as the ubiquinone content increases and leads towards the value of S_{UQ}. However, the presence of NaCl in the subphase has a more marked effect on UQ-2:DPPC and pure UQ-2 films. There are several possible reasons for the decrease in the area occupied by the ubiquinones at the interface when the concentration increases. A part of the molecules might be squeezed out of the lipid

layer, and / or molecules might form aggregates from which the distance to the lipid is nearly the same as from the isolated ubiquinone molecule.

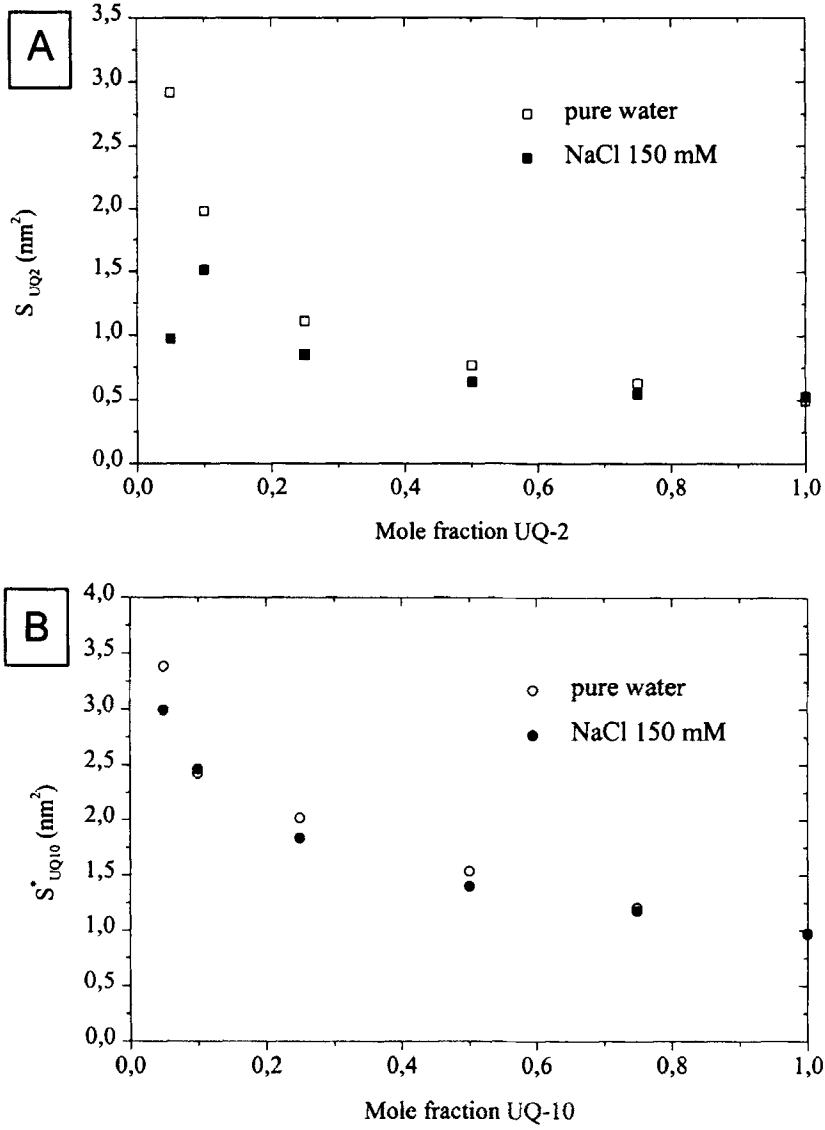

FIGURE 4 Variation of the molecular area S^*_{UQ} occupied by ubiquinone, at 10 mNm^{-1}, in the film UQ-2:DPPC (A) or UQ-10:DPPC (B) with varying molar ratios. Subphase: pure water or NaCl 150 mM, pH 5.7

In order to compare the values of S^*_{UQ} obtained for UQ-2 and UQ-10 independently of their respective molecular areas obtained from the compression isotherms of pure ubiquinones, the ratio S^*_{UQ}/S_{UQ} has been determined. These ratio values are given in Table I at the selected surface pressure of 10 mNm^{-1} for the two subphases used. As seen from the table, the ratio diminishes when the mole fraction increases, but values remain greater than unity. So, the area occupied by the ubiquinone molecule in the mixed film is in all cases greater than the molecular area of the pure quinone. On pure water subphase, UQ-2 takes up more space in the film than UQ-10. Salt addition affects strongly the ratio S^*_{UQ}/S_{UQ} in the case of UQ-2 mixtures. This effect is clearly less marked for UQ-10 mixtures, in particular at 10 mol% UQ-10 where there is no effect of salt addition on the ratio values.

TABLE I S^*_{UQ}/S_{UQ} ratios versus mole fraction of ubiquinone at 10 mNm^{-1}

Films composition & subphase	$r = N_{UQ}/(N_{DPPC}+N_{UQ})$					S_{UQ} (nm^2)
	0.05	*0.1*	*0.25*	*0.5*	*0.75*	
UQ2 – DPPC pure water	5.9	4.0	2 24	1.56	1.28	0.49
UQ2 – DPPC NaCl 150 mM	1.85	2.87	1.61	1 22	1.04	0.53
UQ10 – DPPC pure water	3.49	2.5	2.08	1.58	1.24	0.97
UQ10 – DPPC NaCl 150 mM	3.06	2.51	1.87	1.43	1.2	0.98

Deviations from ideality can also be expressed in term of excess Gibbs energies of mixing, as in the treatment of mixing in the bulk state. The values of excess free energy of mixing ΔG_m^{XS} were calculated from the difference between areas under the isotherms of experimental and ideal films for a specified surface pressure, following the mathematical method of Simpson [16] and according to the Goodrich approach [17]:

$$\Delta G_m^{XS}(\pi) = \int_{\pi \to 0}^{\pi} (S_{DPPC+UQ} - X_{DPPC}S_{DPPC} - X_{UQ}S_{UQ})\, d\pi \qquad (5)$$

Then, the total Gibbs energies of DPPC:UQ mixing were calculated according to the following relation:

$$\Delta G_m(\pi) = \Delta G_m^{XS} + RT\,(X_{DPPC} \ln X_{DPPC} + X_{UQ} \ln X_{UQ}) \qquad (6)$$

where R is the gas constant.

The total Gibbs energies of mixing $\left(\Delta G_m^{XS} + \Delta G_m^{ideal}\right)$ were calculated at three surface pressures: 10, 25 and 40 mNm^{-1}. The results are presented in Figure 5 as a function of the mole fraction of ubiquinone with the ideal Gibbs energies of mixing for comparison. The excess free energies of DPPC:UQ mix-

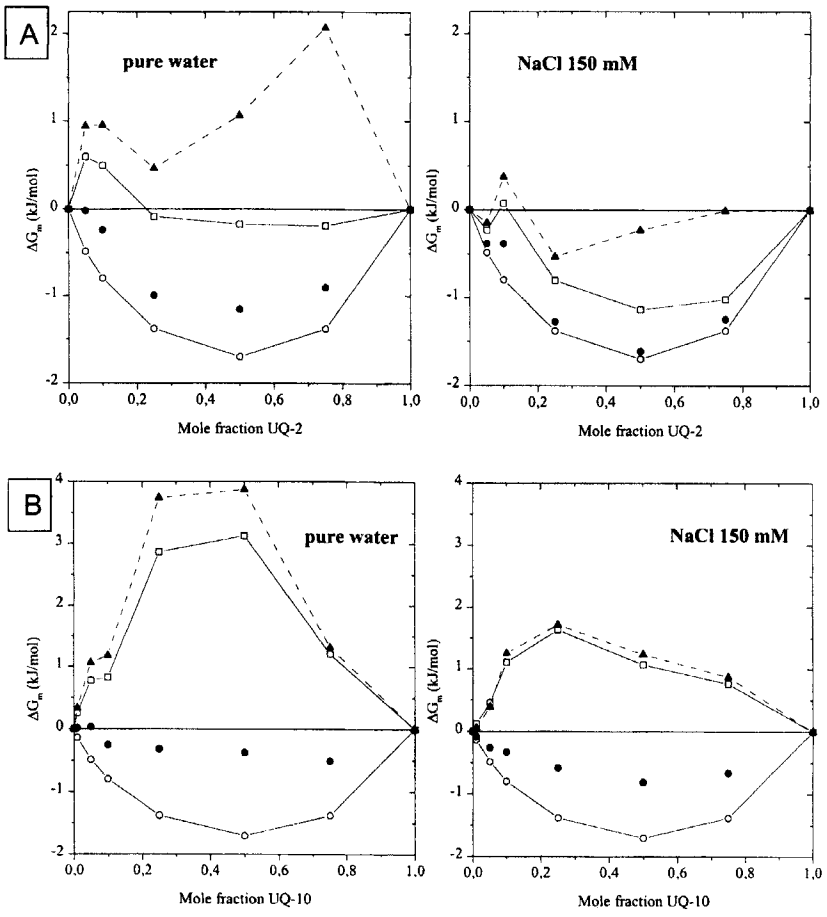

FIGURE 5 Total Gibbs free energies of mixing in UQ-2:DPPC (A) and UQ-10:DPPC (B) monolayers spread on different subphases. The surface pressures were: 10 mNm^{1} (•); 25 mNm^{-1} (□) and 40 mNm^{-1} (▲) compared with the ideal Gibbs energies of mixing (○)

ing ΔG_m^{XS} are positive at both the all the surface pressures studied and the different subphases. Their sign gives information about the stability of these mixture. The DPPC:UQ-2 mixtures appear more stable than DPPC:UQ-10 ones. The presence of NaCl stabilises the films particularly at high surface pressure even if at such a pressure a great amount of ubiquinone has been expelled from the mixed films. Moreover, at 40 mNm^{-1}, for 75 mol% ubiquinone on pure water subphase, the ΔG_m was found to be higher for UQ-2 mixture than UQ-10 one. However, as seen from the compression isotherms, the majority of UQ-10 is squeezed out of the film at this pressure whereas a more significant part of UQ-2 remains in the film.

Fluorescence microscopy of UQ:DPPC mixtures

DPPC:UQ mixtures were investigated by epifluorescence microscopy at the air-water interface. Figure 6 shows a set of images obtained when monolayers of DPPC:NBD-PC:UQ 94:1:5 on pure water were compressed at the same rate without interruption for video recording.

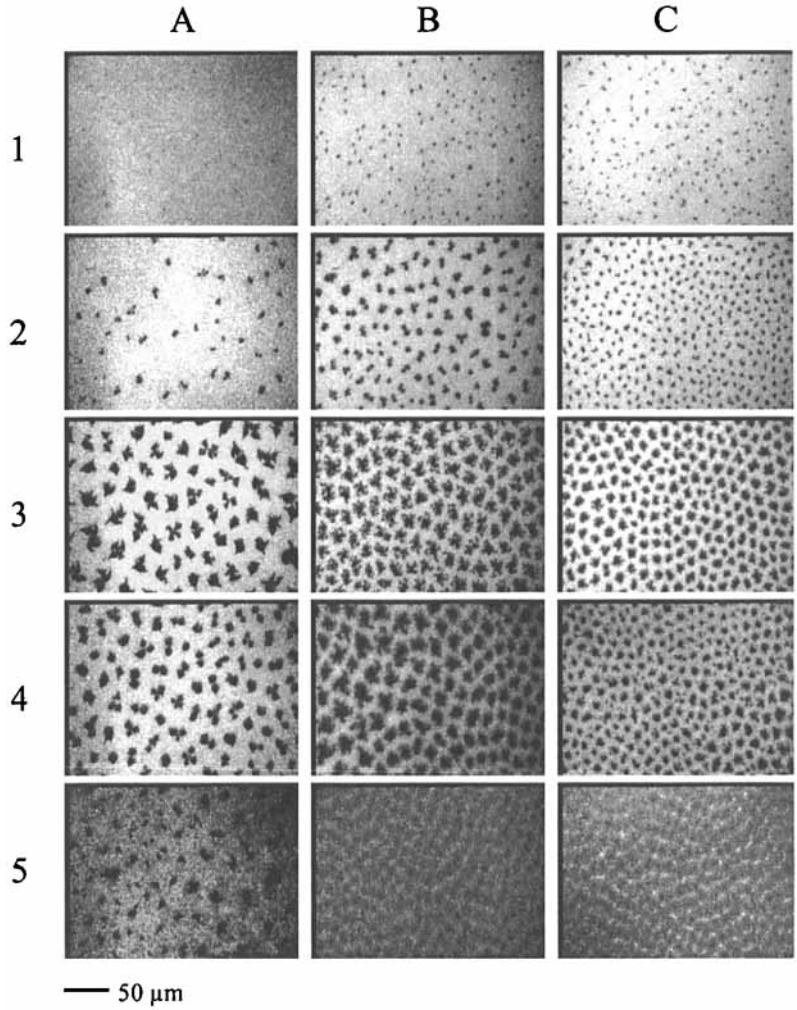

FIGURE 6 Fluorescence microscopy images of monolayers. **A : DPPC:NBD-PC (99:1)** A1 : 6,3mN/m; A2 : 6,8mN/m; A3 : 8,2mN/m; A4 : 9,5mN/m; A5 : 16,4mN/m. **B : DPPC:NBD-PC:UQ2 (94:1:5)** B1 : 8,6mN/m; B2 : 9,8mN/m; B3 : 12,1 mN/m; B4 : 20mN/m; B5 : 35mN/m. **C : DPPC:NBD-PC:UQ10 (94:1:5)** C1 : 10,7mN/m; C2 : 12mN/m; C3 : 14,8mN/m; C4 : 20,4mN/m; C5 : 38mN/m. The scale bar is 50 µm

At surface pressures near to zero, the images observed for all the monolayers consist of dark circular "bubbles" of gas in the white field of the liquid-expanded (LE) phase. At low pressures (1–5 mNm^{-1}), pure DPPC monolayers showed the homogeneous fluorescent LE phase. In mixed DPPC:UQ monolayers, the white field of the LE phase persists at higher surface pressures as the mole fraction of ubiquinone was increased. Then, as surface pressure was increased further in monolayers, transition into liquid-condensed phase occurred, indicated by the appearance of dark probe-excluded domains. These domains were relatively homogenous in shape and distributed uniformly over the visual fields. With 5 mol% ubiquinone in DPPC, the domains came in contact with one another at surface pressures about 35 mNm^{-1} as shown in Figure 6. Decreasing the DPPC content of the monolayer resulted in a decrease in the domain size at equivalent surface pressures. Similar effects have been observed in the presence of NaCl in the subphase.

Figure 7 shows a plot of percentage of condensed lipid, i.e. (total area of the condensed domains / total area of the frame) × 100, as a function of surface pressure for DPPC containing 0–10 mol% ubiquinone. Pure DPPC began to condense at ~6 mNm^{-1}, and at ~10 mNm^{-1} over 60% of the DPPC monolayer showed dark probe-excluded areas. In monolayers containing 5–10 mol% ubiquinone, dark regions were initially observed at higher surface pressures, especially in the case of UQ-10. Higher pressures were needed to reach a total percentage of dark regions similar to those obtained with pure DPPC in the presence of 5 mol% ubiquinone. Increasing the amount of ubiquinone resulted in a decreased amount of probe-excluded regions even at high surface pressures.

CONCLUSION

Pure ubiquinones and mixtures of UQ:DPPC form monolayers at the air-water interface. In particular, UQ-2 was sufficiently insoluble in the subphase to form stable films. In all cases, the ideal miscibility is not observed throughout the whole range of ratios examined. The positive deviation can be explained by the difference in the side chain lengths of both components. Ubiquinone-10 has a very long unsaturated hydrophobic tail (50 carbon atoms) and must be expelled from the monolayers at surface pressures lower than UQ-2. Indeed, the part of UQ-10 which protrudes from the monolayer can freely rotate over DPPC molecules and the rejection of UQ-10 should be seen as a way for the system to minimize its energy. In contrast, UQ-2 has a short saturated hydrophobic tail (10 carbon atoms) which allows more Van der Waals interactions with hydrocarbon chains of DPPC. The isoprenoid chain length alters the hydrophilic/hydrophobic balance of the ubiquinone so that UQ-2 is more soluble in water.

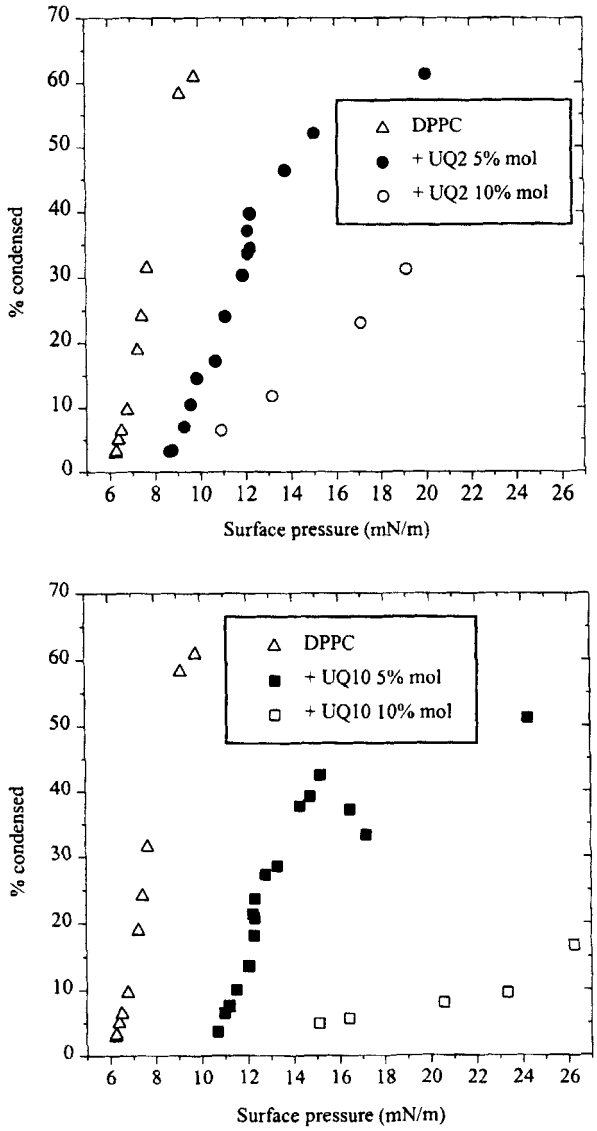

FIGURE 7 Total percentage of dark (probe-excluded) regions per frame, plotted as a function of surface pressure for DPPC:NBD-PC 99:1 (Δ), DPPC:NBD-PC:UQ2 94:1:5 (•), DPPC:NBD-PC:UQ2 89:1:10(◦), DPPC:NBD-PC:UQ10 94:1:5 (□), DPPC:NBD-PC:UQ10 89:1:10 (■)

UQ-2 seems to locate nearer the headgroup of DPPC molecules than does UQ-10, since the former appears to be more sensitive to the addition of NaCl in

the subphase than the latter does not. The values $S^*_{UQ}/S_{UQ} > 1$ at 10 mN/m must signify that repulsive forces are acting between molecules of DPPC and ubiquinone. The existence of repulsive forces is furthermore evidenced from the positive values of excess free energy of mixing ΔG^{XS}_m.

Fluorescence microscopy enabled the visualization of the fluidizing effect of ubiquinones on the LC domains of DPPC monolayers. At 10 mol% of ubiquinone in the mixed monolayers, condensed domains did form but the total amount of condensed lipid were smaller than those observed in monolayers of DPPC alone. In all cases, the condensed domains did not appear until higher surface pressures were attained than those required to produce condensed domains in monolayers of pure DPPC.

Although some concentrations of ubiquinone used in this study are higher than those in the biological systems, the above presented results can give us some information about possible localization of the ubiquinones in membranes. UQ-2 would have at natural concentrations and surface pressures their headgroups in contact with the surface of membranes and with their side chains arranged rather parallel to phospholipid acyl chains. UQ-10 would occupy a similar position but with a tendency to mainly reside in the hydrophobic part of the bilayer.

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