# Redox State of Coenzyme $Q_{10}$ Determines Its Membrane Localization

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The interaction between oxidized (ubiquinone-10) and reduced (ubiquinol-10) coenzyme $Q_{10}$ with dimyristoylphosphatidylcholine has been examined by differential scanning microcalorimetry, X-ray diffraction, infrared spectroscopy, and $^2$H NMR. Microcalorimetry experiments showed that ubiquinol-10 perturbed considerably more the phase transition of the phospholipids than ubiquinone-10, both forms giving rise to a shoulder of the main transition peak at lower temperatures. Small angle X-ray diffraction showed an increase in *d*-spacing suggesting a thicker membrane in the presence of both ubiquinone-10 and ubiquinol-10, below the phase transition and a remarkable broadening of the peaks indicating a loss of the repetitive pattern of the lipid multilamellar vesicles. Infrared spectroscopy showed an increase in wavenumbers of the maximum of the $CH_2$ stretching vibration at temperatures below the phase transition, in the presence of ubiquinol-10, indicating an increase in the proportion of gauche isomers in the gel phase, whereas this effect was smaller for ubiquinone-10. A very small effect was observed at temperatures above the phase transition. $^2$H NMR spectroscopy of perdeuterated DMPC showed only modest changes in the spectra of the phospholipids occasioned by the presence of coenzyme $Q_{10}$. These small changes were reflected, in the presence of ubiquinol-10, by a decrease in resolution indicating that the interaction between coenzyme Q and phospholipids changed the motion of the lipids. The change was also visible in the first spectral moment ($M_1$), which is related with membrane order, which was slightly decreased at temperatures below the phase transition especially with ubiquinol-10. A slight decrease in $M_1$ values was also observed above the phase transition but only for ubiquinol-10. These results can be interpreted to indicate that most ubiquinone-10 molecules are localized in the center of the bilayer, but a considerable proportion of ubiquinol-10 molecules may span the bilayer interacting more extensively with the phospholipid acyl chains.

## Introduction

Coenzyme Q (CoQ) is an essential component of the mitochondrial respiratory chain, and it carries electrons between the NADH and succinate dehydrogenases and the cytochrome system.[1,2] The fact that CoQ is present in mitochondria in molar amounts greatly exceeding those of other respiratory chain carriers led to the concept of the pool function of ubiquinone as a redox carrier.[2,3] The knowledge of the arrangement of this pool of CoQ is important for the understanding of the molecular mechanisms of ubiquinone oxidation and reduction. The location of the oxidized (ubiquinone) and reduced (ubiquinol) forms of CoQ in the membrane and their interaction with the phospholipid bilayer is not known with certainty and is still a subject of debate.

A number of authors have extensively used model phospholipid systems together with different biophysical techniques to address the disposition of ubiquinones in the membrane. Studies based on proton magnetic resonance ($^1$H NMR),[4−7] MAS−$^{13}$C NMR,[8] differential scanning calorimetry,[9−11] infrared spectroscopy,[12,13] X-ray diffraction,[10] and neutron diffraction[14] have suggested that the quinone ring is buried in the hydrophobic core of the membrane. On the other hand, studies based on the transport of reducing equivalents through ubiquinone or ubiquinol containing bilayers,[15] fluorescence spectroscopy,[16,17] and linear dichroism[18] supported the idea that the quinone ring emerges near the lipid/water interface. The hypothesis that ubiquinone might segregate in the membrane and form aggregates has been suggested by several authors.[5,6,12,19−22]

In addition to its well-established function as a component of the mitochondrial respiratory chain, additional locations and roles have been discovered for CoQ in the membrane.[23−25] CoQ also functions in the plasma membrane electron transport system involved in activation of signaling protein kinases related to gene activation for cellular proliferation.[25] CoQ has recently acquired increasing attention with regard to its function in the reduced form (ubiquinol) as an antioxidant. Ubiquinol efficiently protects membrane phospholipids and serum low-density lipoproteins from lipid peroxidation, and it also protects mitochondrial membrane proteins and DNA from free-radical-induced oxidative damage.[23,26] Increasing importance is being given to CoQ in a number of pathological disorders as it is the case of statin-associated myopathy,[27] cerebellar ataxia,[28] neurodegenerative diseases of aging,[29] or oxidative phosphorylation diseases.[30] It has been suggested that ubiquinone supplementation will be convenient to treat some of these conditions.

Given the significance and variety of functions of CoQ in the membrane, it is important to understand CoQ−membrane lipid interaction. In addition, ubiquinone may be supplied to patients in the form of liposomes, and therefore, it is necessary to know in detail the interaction of ubiquinone with model membranes and its exact location in the lipid bilayer. Furthemore it is necessary to further clarify the influence of the redox state of CoQ on its membrane location.

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In this study, we have used differential scanning calorimetry (DSC), infrared spectroscopy, small-angle X-ray diffraction, and $^2$H NMR to study the interaction of ubiquinone-10 and ubiquinol-10 with DMPC multilamellar vesicles, showing that ubiquinone-10 had a modest effect on the thermotropic properties of the phospholipids but, however, ubiquinol-10 had a bigger one. In addition X-ray diffraction showed that coenzyme Q widened the membrane, and all these data are interpreted as coenzyme Q occupying a position in the space between both membrane monolayers.

## Materials and Methods

**Materials.** 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl(D54)-sn-glycero-3-phosphocholine (DMPC-$d_{54}$) were purchased from Avanti Polar Lipids (Alabaster, AL), ubiquinone-10 was obtained from Fluka (Madrid, Spain), ubiquinol-10 was reduced after the procedure of Rieske,[31] deuterium-depleted water and deuterium oxide were purchased from Aldrich (Madrid, Spain). All other reagents and solvents were commercial samples of the highest purity.

**Multilamellar vesicles preparation.** Samples of pure DMPC or DMPC-$d_{54}$ (for $^2$H NMR and FTIR spectroscopy) and in the presence of different amounts of ubiquinone-10 and ubiquinol-10 (10, 20, and 30 mol%) were studied by differential scanning calorimetry (DSC), X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), and $^2$H NMR spectroscopy. The same method and buffer were used to prepare and to form the large multilamellar vesicles (MLVs) utilized in this work, only changing the amount of lipids, depending on the requirements of the technique, and using deuterium-depleted water for $^2$H NMR experiments and deuterium oxide for FTIR experiments. Appropriate amounts of DMPC, ubiquinone-10, and ubiquinol-10 were dissolved in chloroform and mixed. The chloroform was evaporated by an oxygen-free nitrogen stream and then by high vacuum for at least 2 h to remove the last trace of the organic solvent. Water was used to hydrate the mixtures and the MLVs were formed vortexing vigorously at a temperature above the transition temperature.

**DSC measurements.** A total of 2 mg of DMPC, pure and containing 10, 20, and 30 mol% of ubiquinone-10 or ubiquinol-10, was mixed and dried as previously described. MLVs were prepared adding 1 mL of water and vortexing. The samples and the water, used as reference, were degassed for 10 min before loading the calorimeter. Thermograms were recorded by using a Microcal VP Scanning Calorimeter (Microcal, Northampton, MA). The scanning of all of the samples was performed over a temperature range between 6 and 50 °C at a heating rate of 60 °C/h. Thermogram data were recorded and analyzed by using Microcal Origin 5.0 software. Baselines were created and subtracted and then the traces were normalized depending on DMPC concentration. The thermal behavior of liposomes was evaluated by determining the linear onset temperature of the pretransition and the main transition ($T_c$) and by calculating the main transition enthalpy ($\Delta H$) of DMPC molecules in the samples.

**X-ray Diffraction.** Samples for X-ray analysis were prepared, as described above, by using 10 mg of DMPC and the appropriate amount of coenzyme Q. A total of 30 $\mu$L of water was added to hydrate the dried mixture, then the samples were centrifuged at 13000$g$, and finally the pellets were placed in a steel holder with cellophane windows, which provide a good thermal contact with the Peltier heating unit. Typical exposure times were 10 min, allowing 10 min prior to the measurement for temperature equilibration. X-ray profiles were recorded at 12 and 35 °C. Small angle X-ray diffraction (SAX) measurements were carried out by means of a modified Kratky compact camera (MBraum-Graz-Optical System, Graz, Austria) and by using a linear position sensitive detector (PSD; MBraun, Garching, Germany) to monitor the s-range [$s = 2 \sin \theta/\lambda$, $2\theta$ = scattering angle, $\lambda = 1.54$ Å] between 0.0075−0.07 and 0.2−0.29 Å$^{-1}$, respectively. Nickel-filtered Cu K$\alpha$ X-rays were generated by a Philips (Eindhoven, The Netherlands) PW3830 X-ray generator operating at 50 kV and 30 mA. Calibration of the detector was carried out by using Ag-stearate (small-angle region, $d$-spacing at 48.8 Å) as reference materials.

**Infrared Spectroscopy.** Samples of DMPC-$d_{54}$ pure and in the presence of 10 and 20 mol% of ubiquinone-10 or ubiquinol-10 were analyzed by FTIR. Typically, a total of 4 mg of the phospholipid plus the appropriate amount of ubiquinone-10 and ubiquinol-10 dissolved in chloroform were mixed and dried as described above. MLVs were formed by hydrating the samples with 30 $\mu$L of deuterium oxide and vortexing vigorously. The samples were directly placed into a thermostatted Graseby Specac 20710 cell (Graseby-Specac Ltd., Orpington, Kent, U.K.) fitted with $CaF_2$ windows and 25 $\mu$m Teflon spacers. FTIR spectra were recorded with a Bruker Vector 22 Fourier transform infrared spectrometer using a liquid-nitrogen-cooled MCT detector and a normal Beer-Norton apodization function. The spectrometer was continuously purged with dry air during at least 24 h before the experiments and during data acquisition. Spectra of both deuterium oxide and samples were acquired at 2 cm$^{-1}$ resolution under the same scanning and temperature conditions. FTIR spectra were acquired using a temperature range between 14.4 and 47.9 °C, with typical 1.8 °C increments, using an external bath circulator. The actual temperature in the cell was controlled by a thermocouple placed directly over the window. A total of 128 scans were carried out for each spectrum with a nominal resolution of 2 cm$^{-1}$. A sample shuttle accessory was used to obtain the average background and sample spectra. Spectra were collected using the "Opus" software from Bruker, bands due to buffer were subtracted,[32] and the spectra were processed with the same software as described.[33,34]

**$^2$H NMR Spectroscopy.** A quantity of 15 mg of DMPC-$d_{54}$ and appropriate amounts of ubiquinone-10 or ubiquinol-10 to obtain molar ratios of 10 and 30% were mixed and dried directly in the bottom of the NMR glass tubes. MLVs were essentially prepared as described above and hydrating the samples with 50 $\mu$L of deuterium-depleted water. $^2$H NMR experiments were carried out on a Bruker Avance 600 instrument (Bruker, Etlingen, Germany) at 92.123 MHz using the standard quadrupole echo sequence.[35] The spectral width was 150 KHz, with a 10 $\mu$s 90° pulse, 40 $\mu$s pulse spacing, 3.35 $\mu$s dwell time, 1 s recycling time, 50 Hz line broadening, and accumulation of 15 000 transients. Spectra were acquired at temperatures ranging from 13 to 41 °C raising the temperature in 2 °C steps. The first moment, $M_1$, was calculated for each spectrum of the different samples and at each temperature using the following equation:[36]

$$M_1 = \frac{1}{A} \sum_{\omega=-x}^{x} |\omega| f(\omega)$$

where $\omega$ is the frequency shift from the central (Larmor) frequency, $f(\omega)$ is the spectral intensity, $x$ is the frequency shift range (between −60 and 60 kHz), and $A$ is defined as

$$A = \sum_{\omega=-x}^{x} f(\omega)$$

## Results

**DSC Measurements.** Figure 1A shows the calorimetric profile of the thermotropic gel-to-liquid-crystalline transition of DMPC and ubiquinone-10 up to a 30 mol% of ubiquinone-10. The onset of the pretransition of pure DMPC was at 13 °C and the onset of the main transition ($T_c$) at 23 °C. The only observed effects after the addition of 10 and 20 mol% ubiquinone-10 were a small decrease and shift toward lower temperatures of the heat corresponding to the pretransition and a decrease and widening of the main transition peak. At 30 mol% ubiquinone-10 the pretransition completely disappeared and the main transition was smeared out and it was starting now at 17 °C. In the scans corresponding to the samples containing ubiquinone-10, small peaks (at 17 °C for 10 and 20 mol%, and 15 °C for 30 mol%) are also seen which correspond to small amounts of nonincorporated crystalline ubiquinone-10.[37]

However, ubiquinol-10 has a more marked effect on the calorimetric profile of the DMPC phase transition. Figure 1B shows that at 10 mol% ubiquinol-10 there is a clear broadening of the main transition with a decrease in the height of the peak and a total absence of the pretransition. The increase in the proportion of ubiquinol-10 present in the phospholipid bilayer gave rise to a considerable blurring of the main transition peak. Small peaks were also seen in these thermograms (at 17 °C for 10 mol% ubiquinol-10 and 15 °C for 20 and 30 mol% respectively) coming from traces of nonincorporated ubiquinol-10.

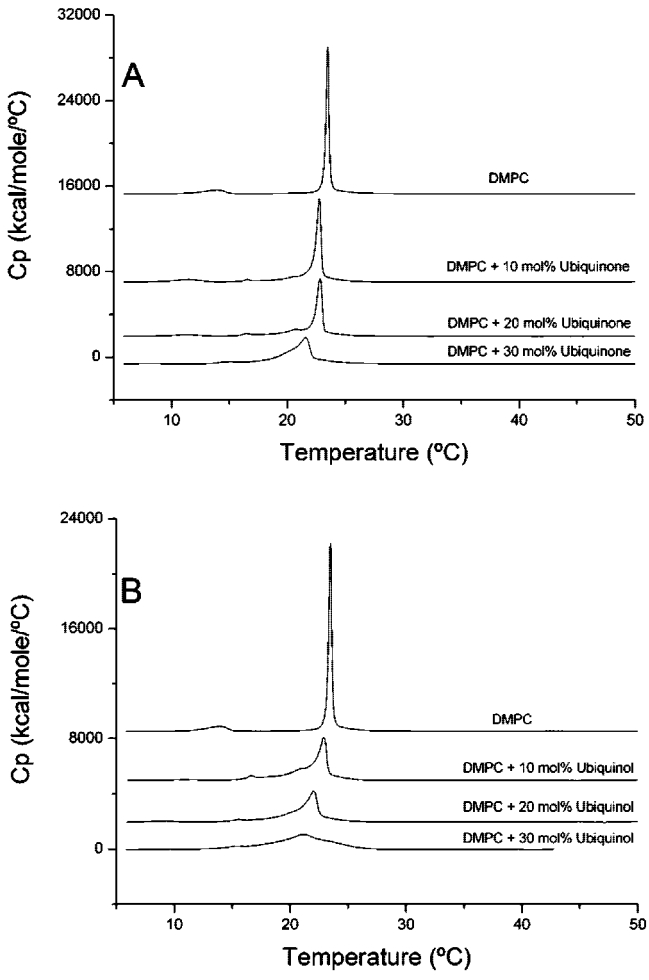


**Figure 1.** DSC heating thermograms of aqueous dispersions of pure DMPC, and in the presence of 10, 20, and 30 mol% of ubiquinone-10 (panel A) and of 10, 20, and 30 mol% of ubiquinol-10 (panel B). The concentrations of the lipid mixtures were normalized to 2.95 mM. The heating rate was 60 °C/h.

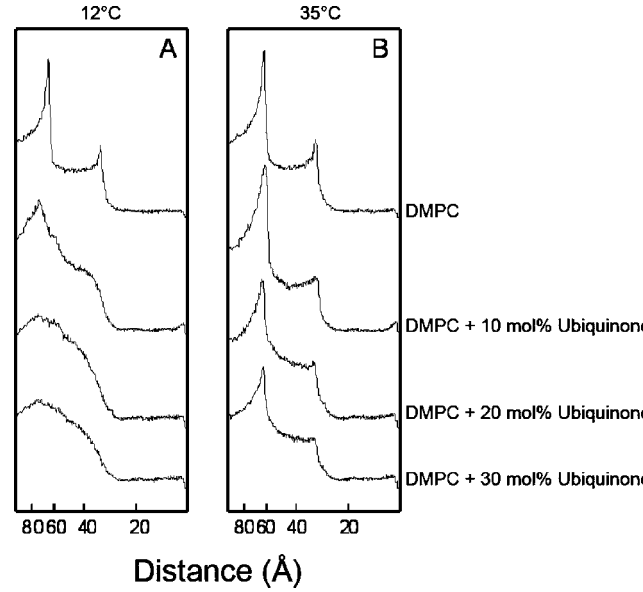


**Figure 2.** Small-angle X-ray diffraction profiles at 12 and 35 °C (panel A and B respectively) indicated temperatures of the mixtures (top to bottom) pure DMPC, and in the presence of 10, 20, and 30 mol% of ubiquinone-10.

The small peaks attributed to nonincorporated ubiquinone-10 or ubiquinol-10 observed in these experiments are found at slightly different temperatures depending on the samples. In principle, they should appear always at the same temperature since the phase transition is independent of the concentration. The observed variations are probably due to the difficulty of ascertaining $T_c$ for these peaks because they are superimposed on broad endotherms.

The values of $\Delta H$ versus the molar ratios of ubiquinone-10 or ubiquinol-10 and DMPC were determined, but only small deviations with respect to that of pure DMPC were observed (not shown), in agreement with previous observations for other phospholipids.[9]

**X-ray Diffraction.** Small angle X-ray diffraction experiments were carried out for samples containing ubiquinone-10 and ubiquinol-10. Figure 2 shows that pure DMPC at 12 °C exhibited *d*-spacings at 65 Å (first order) and at 32 Å (second order), corresponding to the lamellar gel phase, below the phase transition. The addition of increasing molar percentages of ubiquinone-10 produced a remarkable broadening of the diffraction peaks, and the first order was located now at about 73 Å (10 mol% and 20 mol%) and 74 Å (30 mol%). At 10 mol% of ubiquinone-10, a shoulder at 58 Å was observed, probably coming from traces of nonincorporated, crystalline coenzyme Q. Some small peaks were also observed at 20 mol% probably arising from neat coenzyme Q as well. These small peaks were not distinguishable at 30 mol% due to the considerable broadening of the diffraction peaks. It should be emphasized that, although due to the lack of repetitive structures it is difficult to assess that these samples are organized as lamellar structures, we assumed that this is the case, and this assumption was confirmed by the $^2$H NMR data shown below. The same applies to the ubiquinol-10 containing samples described below.

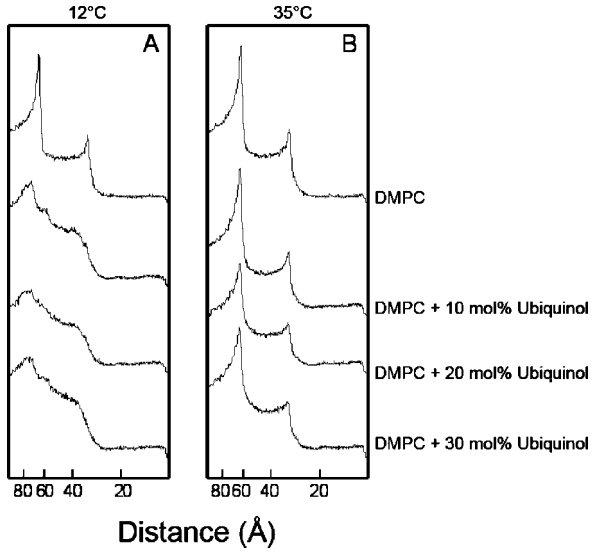


**Figure 3.** Small-angle X-ray diffraction profiles at 12 and 35 °C (panel A and B respectively) indicated temperatures of the mixtures (top to bottom) pure DMPC, and in the presence of 10, 20, and 30 mol% of ubiquinol-10.

At 35 °C, i.e., above the phase transition, the diffractogram from pure DMPC showed *d*-spacings at 62 and 31 Å, a reduction in *d*-spacings which is typical of the fluid phase (Figure 2B). Although a certain broadening of the diffraction peaks was also observed when ubiquinone-10 was added, it was much more modest than at low temperature. In addition to that, the *d*-spacings were not modified with respect to pure DMPC by the addition of either, 10, 20, or 30 mol% of ubiquinone-10. Small peaks coming from pure unincorporated ubiquinone-10 could also be observed at about 56 Å (20 mol% and 30 mol%).

When ubiquinol-10 was used, at 12 °C (Figure 3A) 10 mol% produced a broadening of the peak similar to that observed for ubiquinone-10, and also an increase in *d*-spacing up to 72 Å nm. Small peaks were also observed in this case that can be assigned to unincorporated ubiquinol-10, as 58 nm, 41 and 33 Å. At 20 mol% the first order peak appeared at 70 Å and the second at 37 Å, and at 30 mol%, the first order peak was found at 75 Å, and a small peak was also observed at 58 Å coming from unincorporated coenzyme Q. At 35 °C (Figure 3B), diffraction peaks were similar for pure DMPC and DMPC containing 10, 20, and 30 mol% of ubiquinol-10, with first spacing at 63 Å in pure DMPC and similar *d*-spacings in the other samples.

**Infrared Spectroscopy.** In order to monitor the effect of ubiquinone-10 and ubiquinol-10 on DMPC perdeurated DMPC-$d_{54}$ was used. Figure 4 shows changes in the $CH_2$ stretching symmetric vibrations modes during the main endothermic phase transition of DMPC-$d_{54}$. These changes have been associated to the change from all-trans to gauche conformers,[24,31,38] and hence, the frequencies of these bands are related to the average number of gauche conformers. The results observed for the $CH_2$ symmetric stretching band in pure DMPC-$d_{54}$ and in the systems that contained either ubiquinone-10 or ubiquinol-10 show that the phase transition induced in pure DMPC-$d_{54}$ samples a shift in wavenumbers from 2089.6 $cm^{-1}$ at 14 °C (below the phase transition; all-trans) up to 2095 $cm^{-1}$ at 45 °C, (above the phase transition, gauche).

The effect of adding 10 or 20 mol% of ubiquinone-10 to DMPC-$d_{54}$ vesicles was very small with just a small shift in the maximum of the vibration peak at temperatures below the phase transition. This shift was bigger in the case of the addition

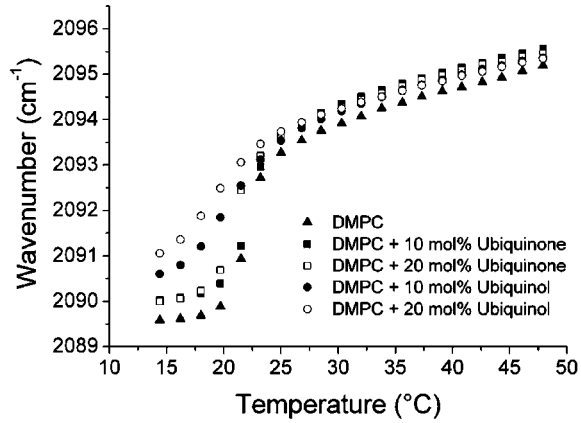


**Figure 4.** Temperature dependence of the maximum peak position of the $CH_2$ symmetric stretching band contour of FTIR spectra. The graphs of pure DMPC and in the presence of 10 and 20 mol% of ubiquinone-10 and ubiquinol-10 are reported in this figure.

of 10 and 20 mol% of ubiquinol-10, and when ubiquinol-10 was added, there was a decrease in the phase transition temperature to 17 °C (to be compared with 20 °C in pure DMPC-$d_{54}$). At 14 °C the wavenumber of the maximum for 20 mol% of ubiquinol-10 increased to 2091 $cm^{-1}$ compared to 2089.6 $cm^{-1}$, indicating a certain increase in gauche isomers induced by the presence of ubiquinol-10.

**$^2$H NMR Spectroscopy.** Figure 5 depicts the spectra at different temperatures of the DMPC-$d_{54}$ from 13 to 41 °C. At temperatures below the transition temperature ($T_c$), the spectra were powder patterns characteristic of a gel phase.[39] At temperatures of 21 °C and higher, the spectra corresponded to a fluid-phase bilayer and were axially symmetric with some resolved quadrupole splittings arising from methylene segments in the acyl chains.

The spectra obtained after the addition to DMPC-$d_{54}$ of 10 and 30 mol% of ubiquinone-10 were very similar to those coming from pure phospholipid. However, the spectra corresponding to samples containing 10 and 30 mol% of ubiquinol-10 were changed with respect to those of pure DMPC-$d_{54}$ since a loss of resolution appeared manifested by the disappearance of the sharp edges of the peaks. This was accentuated at 30 mol% of ubiquinol-10. The decrease in resolution indicates a change in the motion of the lipids and that ubiquinol-10 may interact with the acyl chains of the phospholipids considerably more than ubiquinone-10. In addition to that, in the presence of ubiquinol-10 but not in that of ubiquinone-10, an isotropic component appeared superimposed to the spectra and the size of this component increased when the concentration of ubiquinol-10 increased. This isotropic component may indicate that the presence of ubiquinol-10 induced that the large multilamellar vesicles are fragmented into smaller size vesicles.

There was another interesting influence of coenzymes Q on the spectra. The spectra of Figure 5 corresponding to pure DMPC-$d_{54}$, obtained at high temperatures, exhibits a small deviation from a Pake line shape, which indicates a slight elongation of the phospholipids vesicles in the external magnetic field used in these experiments.[40] These spectral changes affect the distribution of spectral intensity but not the effective deuterium quadrupole splitting. In the presence of ubiquinol-10, a certain redistribution of spectral intensity at close to maximum splitting was observed while the methyl splittings remained unchanged indicating that ubiquinol-10 reduced vesicle ellipticity. This is a new indication of the effective implication of ubiquinol-10 but not of ubiquinone-10, in the molecule packing of lipidic vesicles.

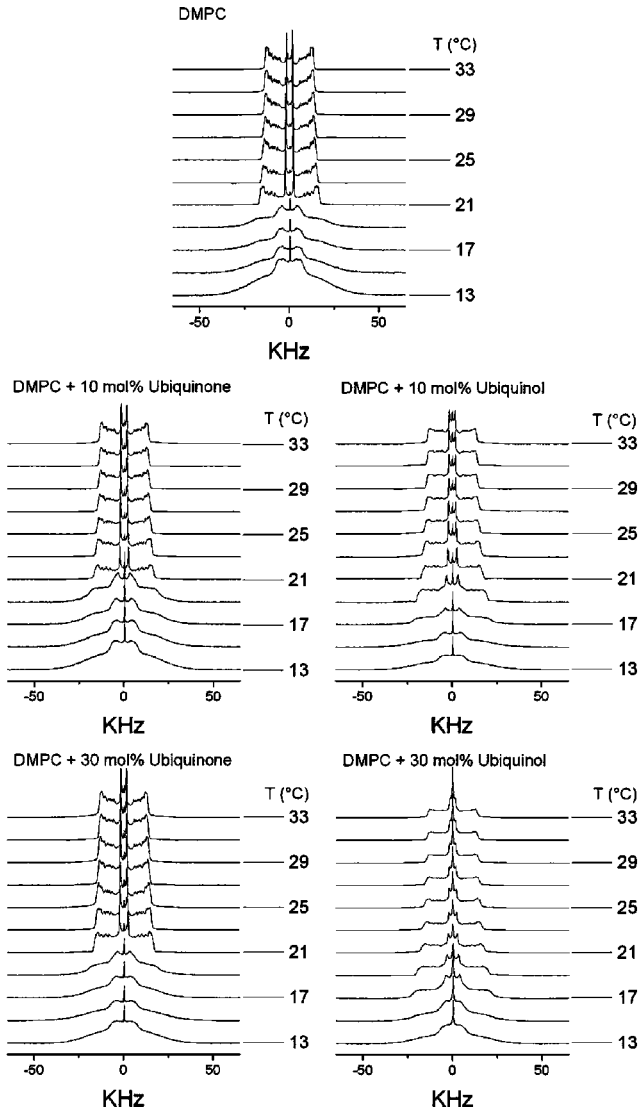


**Figure 5.** $^2$H NMR spectra of DMPC-$d_{54}$ and in the presence of 10 and 30 mol% of ubiquinone-10 and ubiquinol-10 as function of temperature (from 13 to 33 °C). The temperatures are shown on the right-hand side of each spectrum.

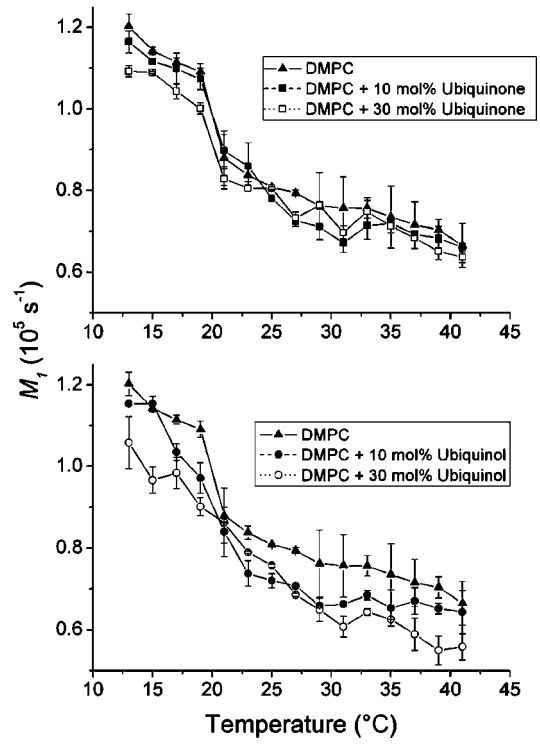


**Figure 6.** Temperature dependence of $M_1$ for pure DMPC-$d_{54}$ and DMPC-$d_{54}$ in the presence of 10 and 30 mol% of ubiquinone-10 (top panel) and 10 and 30 mol% of ubiquinol-10 (bottom panel). Mean values of three measurements are shown.

In order to better discern the effect of coenzyme Q on membranes, the first spectral moment $M_1$ was calculated for each spectrum. $M_1$ measures the average spectral width and since each phase has a distinct spectral width it will also have a characteristic $M_1$ which is proportional to the average order parameter. The variations in $M_1$ with temperature can be used to characterize membrane phase transitions and, in the liquid crystalline phase, the molecular order of a membrane. Pure DMPC-$d_{54}$ (Figure 6) showed values of $1.20 \times 10^5$ $s^{-1}$ at low temperatures (below $T_c$) and a decrease to values of $0.67 \times 10^5$ $s^{-1}$ at higher temperatures, clearly defined the phase transition taking place at about 20 °C. The addition of ubiquinone-10 (Figure 6) at 10 mol% or at 30 mol% produced only small decreases in $M_1$ values and they did not produce any significant change at temperatures above the phase transition.

The effect of ubiquinol-10 (Figure 6), was to progressively reduce the $M_1$ values as its concentration was increased at all temperatures, indicating disordering of the membrane. In this way, at 14 °C $M_1$ values were $1.12 \times 10^5$ $s^{-1}$ for pure DMPC-$d_{54}$ and $1.07 \times 10^5$ $s^{-1}$ for the sample containing 30 mol% of ubiquinol-10. At 42 °C $M_1$ for pure DMPC-$d_{54}$ was 0.67 and $0.57 \times 10^5$ $s^{-1}$ for the sample containing 30 mol% of ubiquinol-10. This indicates that ubiquinol-10 disorders the membrane hydrophobic core at all temperatures.

## Discussion

The thermotropic properties and polymorphic phase behavior of DMPC and CoQ have been studied by using a range of nonperturbing biophysical techniques to discern the interaction between these lipids and the location of CoQ in membranes.

It has been concluded from several studies that coenzyme Q is located in the membrane palisade beyond the C10 of the acyl chains.[9,37,41] Previous $^1$H NMR,[6,7] $^{13}$C-MAS NMR,[8] and neutron diffraction[14] studies have suggested a similar position in or near the center of the bilayer for coenzyme Q in phosphatidylcholine systems. A possibility to retain the relatively polar quinone ring in the hydrophobic core of the bilayer would be the formation of head to head aggregates in the middle of the membrane. $^1$H NMR spectroscopy studies support the location of the benzoquinone moieties in close proximity to one another.[20] Within these aggregates the long hydrophobic tail of ubiquinone-10 anchors the quinone ring in this central position. However, this anchoring is not totally efficient and the polar nature of the ring makes it protrude toward the terminal end of the phospholipid acyl chains.

In the case of the reduced form of CoQ (ubiquinol-10), it should be assumed that an increase in the polarity of the ring will take place, and hence, this ring has a higher tendency to protrude toward the aqueous interface of the membrane, and it could then perturb the medium to upper part of the phospholipid acyl chains. The results coming from DSC, infrared, and $^2$H NMR support this notion since a bigger effect on the thermotropic properties of DMPC was detected for ubiquinol-10, comparing with ubiquinone-10. We had previously reported a stronger effect of ubiquinol-10, when compared with ubiquinone-10, on a variety of phospholipids so that a different location

for the two redox states of CoQ can be suggested on the basis of their different hydrophilic-hydrophobic character.[9,10,12] Following this model, it is proposed that the ubiquinol rings are closer to the membrane surface than are the ubiquinone rings, and this was also previously proposed on the basis of $^1$H NMR [4,8] and $^{13}$C-MAS NMR[8] studies.

Our results are mostly compatible with this model. At low concentrations of ubiquinone-10 or ubiquinol-10 (10 mol%) which are closer to physiological concentrations in mitochondrial membranes, DSC showed that the effect is relatively small, especially in the case of ubiquinone-10. Nevertheless, it is interesting that the effect of ubiquinone-10 and ubiquinol-10 on DMPC was something stronger than the effect seen on dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) studied before,[9] suggesting a relation with the acyl chain length since shorter phospholipids acyl chains will allow coenzyme Q molecules to interact with the upper part of the phospholipid molecules and this will alter more strongly the membrane structure and dynamics.

The small-angle X-ray diffraction studies presented here may be taken to also support this model, since at temperatures below the phase transition ubiquinone-10 increased *d*-spacings which is indicative of changes in interbilayer repeat. This effect is compatible with aggregates of coenzyme Q molecules residing in the center of the bilayer when the membrane is in a rigid state. The increase in *d*-spacing was observed at temperatures below the phase transition but not above. This can be explained by the better possibilities of accommodation of coenzyme Q molecules within the hydrocarbon chains of the phospholipids when they are fluid than when they are arranged in a rigid all-trans disposition.

It is difficult to apply the same type of explanation to ubiquinol-10 since ubiquinol-10 caused the *d*-spacing to increase at 12 °C, especially since the chains are shown by IR to be more disordered, which indicate that ubiquinol-10 is interacting with the hydrophobic chains of the phospholipids. An interpretation could be the hydrophobic tails of the ubiquinol-10 are also sequestered in the core in the gel phase, increasing the hydrophobic thickness. The other observation to be explained is why ubiquinol-10 did not cause the *d*-spacing to decrease at 35 °C since NMR shows definitely that the chains are more disordered. Perhaps the change in ordering translates to a very small change in hydrophobic thickness that is not large enough for X-ray to show.

X-ray diffraction also indicates that at temperatures below the phase transition there is a blurring of the peaks which indicates structural disorder producing loss of lamellar repetitive structure which is probably arising from macroscopic changes in the multilamellar vesicles.

Infrared spectroscopy showed that below the $T_c$ transition temperature the effect on membrane dynamics was bigger, with an increase of gauche isomers proportion than at temperatures above $T_c$ and ubiquinol-10 produced more important effects than ubiquinone-10. The small influence of ubiquinone-10 on the thermotropic properties of DMPC at moderate concentrations as evidenced by DSC, infrared spectroscopy, and $^2$H NMR spectroscopy may mean that this form of coenzyme Q is not influencing the packing and dynamics of the phospholipids molecules. In the lamellar gel state, the penetration of the quinone ring is only able to perturb the highly ordered DMPC molecules within the terminal C10, and this would have only a slight effect on the thermotropic properties of the phospholipids, as it is truly detected. Ubiquinol-10, however, appeared to be capable of perturbing more of the packing of the acyl chains, suggesting that at least a part of it may occupy more superficial positions in the membrane. The small effect seen above the phase transition for ubiquinone-10 may derive of its position in the center of the bilayer and the better possibilities of accommodation derived of the fluid phospholipids acyl chains. However, $^2$H NMR showed that ubiquinol-10 induced a small disordering effect of the membrane at all temperatures suggesting a bigger interaction with the acyl chains in the bilayer, this being in agreement with the results of DSC and infrared spectroscopy.

The presence of head to head aggregates of CoQ in the center of the membrane could also be important for the additional functions discovered for CoQ in the membrane, namely the antioxidant function of ubiquinol and in particular its role in maintaining tocopherol in the reduced state. We have previously shown that α-tocopherol is located in the phospholipids palisade with the phenolic hydroxyl group located near the phosphate moiety of the lipid matrix[42] and also that α-tocopherol forms enriched domains in the membrane[43] where it may efficiently prevent lipid peroxidation. The interaction between these α-tocopherol-rich domains located in the phospholipids palisade with the ubiquinol-10 molecules which emerge from the center of the bilayer when they are reduced suggests a possible mechanism for the reported regeneration of α-tocopherol by the reduced form of CoQ.[44]

In conclusion, our results are consistent with the formation of head to head aggregates of CoQ in the center of the DMPC membrane, with the ubiquinol ring more efficiently interacting with the phospholipid acyl chains. The presence of CoQ in this part of the membrane will improve the barrier properties of a mitochondrial membrane making it more impermeable to ions and in the case of the mitochondrial inner membrane to protons. This positioning will be also appropriate to stabilize the membrane, explaining recent observations on the role of CoQ on nanoliposomes.[45]

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