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A Fourier transform infrared spectroscopic study of the molecular interaction of ubiquinone-10 and ubiquinol-10 with bilayers of dipalmitoylphosphatidylcholine

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Ubiquinone-10 and ubiquinol-10 were incorporated in multibilayer vesicles made of dipalmitoylphosphatidylcholine (DPPC). The interaction between both forms of CoQ_{10} and phospholipid was followed using Fourier transform infrared spectroscopy (FT-IR), observing the temperature dependencies of the infrared spectra of pure and CoQ-containing DPPC vesicles. From the observation of the bands corresponding to the CH_2 stretching and scissoring vibrations and the C=O stretching mode of the phospholipid it was concluded that ubiquinone-10 at 5 and 25 mol% has only a small effect on the phase transition of DPPC. On the other hand, ubiquinol-10 at the same concentrations remarkably affects the phase transition of DPPC, decreasing the phase transition temperature (T_c) by several degrees and making it broader. It is concluded that ubiquinone-10 and ubiquinol-10 have different interactions with DPPC, possibly due to different localizations in the membrane.

Introduction

It is well established that ubiquinone-10 (CoQ_{10}) is an essential component of many biological electron-transfer chains and it has been suggested that it forms an ideal hydrogen-transfer component of Mitchellian protonmotive loops [1]. Although this molecule is generally regarded as a mobile electron-transport component, the mode in which it is integrated in the membrane and its mechanism of action are not fully understood. Since ubiquinone-10 in the straight-chain *trans* configuration has a length of 56 Å, i.e., greater than the thickness of a phospholipid bilayer [2], its arrangement in the membrane is a structural problem for its proposed transmembrane motion. A similar problem is found for plastoquinone-9 in chloroplasts [3].

A number of authors have tried to approach this problem by using simple models in which ubiquinone-10 and other analogues of shorter isoprenyl chains are reconstituted in phospholipid vesicles, often formed by synthetic phospholipids such as dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylcholine (DMPC). These synthetic phospholipids have a phase transition (T_c) from gel to liquid crystal at physiological

Abbreviations: CoQ_{10}, coenzyme Q_{10}; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; FT-IR, Fourier transform infrared spectroscopy; T_c, gel to liquid crystal phase transition temperature.

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temperatures, 41°C for DPPC and 23°C for DMPC. A variety of physical techniques such as differential scanning calorimetry [4–7], fluorescence probes [4,8,9], X-ray diffraction [10,11] and nuclear magnetic resonance [11–13] have been used to study the interaction between CoQs and phospholipids, monitoring the effect of the quinones on the phospholipid phase transition and also on the physical state of the phospholipid above and below the phase transition. The aim of these experiments was to infer from these interactions the localization and function of CoQ_{10} in the membrane. The general conclusion obtained from these studies is that ubiquinone-10 does not perturb the phospholipid phase transition except at very high concentrations, e.g. 2.5 : 1 DPPC : ubiquinone-10 molar ratio, and that ubiquinone-10 does not interact with phospholipids either above or below the temperature of the phase transition, so that it may be concluded that ubiquinone-10 does not readily intercalate between phospholipid molecules. Similar conclusions were derived from monomolecular film techniques [14,15].

It has been shown, however, that ubiquinone-10 can mediate transport of protons and electrons across the membrane in similar reconstituted systems [13,16,17] and that ubiquinol-10, at variance with ubiquinone-10, may perturb the phase transition of the phospholipid [7].

On the basis of all these studies, several models of CoQ_{10} organization in the membrane have been put forward, including (a) localization of ubiquinone-10 in a central part of the membrane [4,13,14] but with ubiquinol-10 interacting with the phospholipid [7], and (b), arranged as a micelle which tumbles isotropically, diffuses laterally and spans the bilayer [18].

We have undertaken in this work a study of the temperature variations of the infrared spectra of multibilayers of DPPC containing either ubiquinone-10 or ubiquinol-10, taking advantage of the high performance of Fourier transform infrared spectroscopy (FT-IR), which has been shown to be a powerful technique for the study of pure aqueous phospholipids or mixtures of phospholipids with intrinsic molecules such as cholesterol or proteins [19–22]. The results show that ubiquinol-10 perturbs the phospholipid structure considerably more than ubiquinone-10 and hence both forms of CoQ_{10} may interact differently with DPPC.

Materials and Methods

L-α-Dipalmitoylphosphatidylcholine was obtained from Fluka, Busch, Switzerland, CoQ_{10} and ^2H_2O (99.8%) were obtained from Sigma, Poole, Dorset, U.K. All other chemicals were of analytical grade.

Ubiquinone-10 was reduced following the procedure of Rieske [23] by addition of dithionite to a water suspension to which ubiquinone, dissolved in acetone, had previously been added. Afterwards, ubiquinol-10 was extracted by means of cyclohexane.

The assay of ubiquinone or ubiquinol concentration in the solution used was done as described by Crane and Barr [24] by ultraviolet spectrophotometry at 275 nm, using an extinction coefficient of 14.5 $mM^{-1} \cdot cm^{-1}$, and comparing the oxidized and the reduced states. The extent of CoQ_{10} reduction was also monitored by comparing the intensities of the carbonyl stretching vibrations of the quinone ring in the infrared spectrum, so verifying that the corresponding samples actually contained totally reduced CoQ_{10}, where these bands are not seen.

The lipids mixtures for the infrared spectroscopy measurements were prepared by combination of chloroform solutions containing 10 mg DPPC and the appropriate amounts of either ubiquinone-10 or ubiquinol-10. After drying under nitrogen steam in the dark, the samples were then further dessicated under vacuum for 2–3 h to remove the last traces of solvent. After addition of 100 μl deuterated water, multilamellar liposomes were formed by careful mixing using a bench vibrator, and keeping the samples at 50–55°C. i.e., above the T_c of the pure phospholipid.

The incorporation of CoQ_{10} into liposomes was quantified with an extraction procedure using *n*-pentane, as described in Ref. 25. This organic solvent removes the CoQ_{10} which remains in the aqueous phase. The percentages of CoQ_{10} given in this work were calculated according to the actual value of CoQ_{10} incorporated into the phospholipid bilayers. Ubiquinone-10 and ubiquinol-10 gave similar levels of incorporation [7].

Infrared spectra were obtained using a Nicolet MX-1 FT-IR spectrometer, assisted by a Nicolet 1200-S computer. Samples were examined in a thermostatted Beckman FH-01 CFT cell, equipped with CaF_2 windows and using 25-μm Teflon spacers. 40 μl of sample were injected into the cell and 27 interferograms were collected for each spectrum. Underlying ^2H_2O bands were subtracted by computation prior to the measurements of frequencies and bandwidths. Temperature was controlled by means of a thermocouple inserted into the cell, and calibrated with samples of pure DMPC and DPPC. Measurements at different temperatures were always done by heating, and the samples were previously equilibrated in the cell at 19°C for at least 15 min.

Results

The following sections present the infrared spectra of DPPC multibilayers containing 5 and 25 mol% of ubiquinone-10 and ubiquinol-10, in comparison with that of pure DPPC multibilayers. The results are mainly in terms of DPPC spectra, since the CoQ spectrum is much weaker.

CH_2 stretching vibrations

Fig. 1 shows the infrared absorption spectra of DPPC multibilayers containing ubiquinone-10 (Fig. 1a) and ubiquinol-10 (Fig. 1b) at different temperatures. Samples containing DPPC and ubiquinone-10 were similar to those of pure DPPC and hence these are not shown. The strong bands at 2920 and 2851 cm^{-1} correspond, respectively, to the antisymmetric and symmetric CH_2 stretching modes of the palmitoyl chains, whereas the minor bands which appear at 2958 and 2873 cm^{-1} corresponds, respectively, to the asymmetric and symmetric CH_3 stretching modes [19,26]. Both CH_2 stretching modes show temperature-dependent variations in peak frequency, peak height and bandwidth.

The shift in frequency of the CH_2 stretching modes which takes place during the main endothermic phase transition of DPPC has been associated to the change from *all-trans* to *gauche* conformers [19,27,28] 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 were identical to those observed for the antisymmetric stretching band. The temperature dependence of the frequencies of the CH_2 antisymmetric stretching mode in pure DPPC and in the systems that contained either ubiquinone-10 or ubiquinol-10 are shown in Fig. 2. It can be seen that the phase transition induces in pure DPPC samples a shift in frequency from 2918.3 (*all-trans*) to 2922.4 cm^{-1}

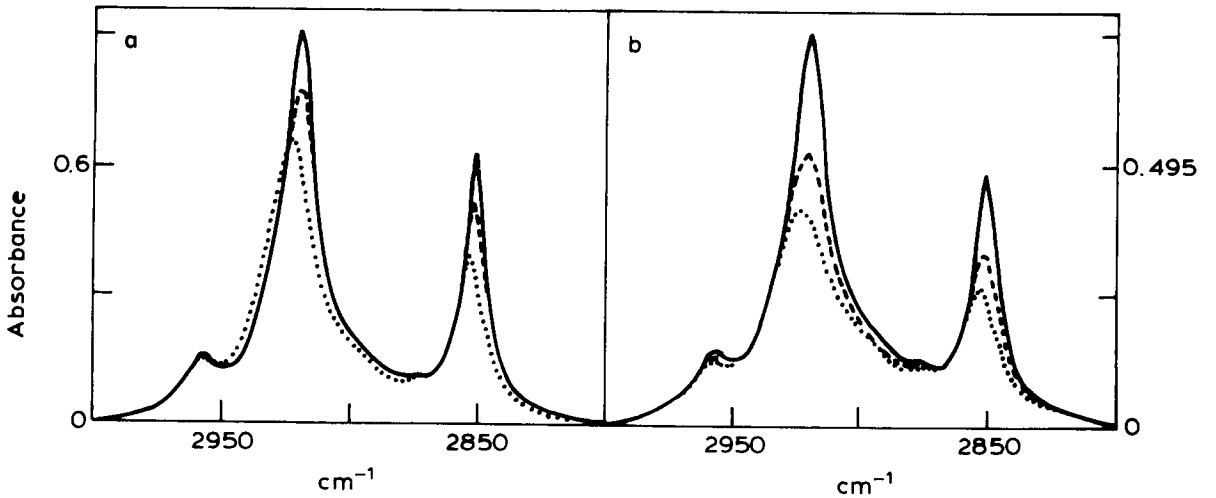

Fig. 1. Temperature dependence of the infrared spectra of the C–H stretching region of DPPC. (a) DPPC containing 5 mol% of ubiquinone-10; (b) DPPC containing 5 mol% of ubiquinol-10. ———, 25°C; - - - - - -, 38°C; · · · · · ·, 45°C.

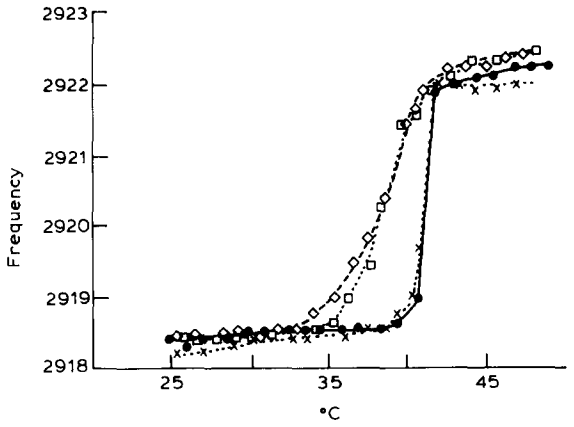

Fig. 2. Frequency of the CH_2 antisymmetric stretching mode of DPPC vs. temperature for (●———●) pure DPPC, (×····×) 25 mol% ubiquinone-10, (□····□) 5 mol% ubiquinol-10 and (◇------◇) 25 mol% ubiquinol-10.

(*gauche*), the onset of the transition being located at about 40°C. It can be seen in Fig. 2 that the phase transition is remarkably altered in the presence of ubiquinol-10. The alteration consists of a shift of the onset of the transition to lower temperatures, so that this transition begins at 34.5°C in the presence of 5 mol% and at 33°C in the presence of 25 mol% ubiquinol-10. The transition is also broader than in pure DPPC vesicles, but nevertheless the presence of ubiquinol-10 does not alter the average number of either *all-trans* conformers below the transition or the *gauche* conformers above the transition temperature. Fig. 2 shows that the effect of ubiquinone-10 on the phase transition of DPPC is much smaller, since at a concentration of 25 mol% it does not significantly alter the phase transition. The variation of bandwidth with temperature was also studied in samples of pure DPPC and DPPC in the presence of either ubiquinone-10 or ubiquinol-10 and the same trend as with frequency was found, so that ubiquinol-10 produces a shift of the phase transition towards lower temperatures and a broadening, whereas ubiquinone-10 gives a lesser effect (results not shown).

The CH_3 asymmetric stretching vibration band was also studied but it was found that the intro-

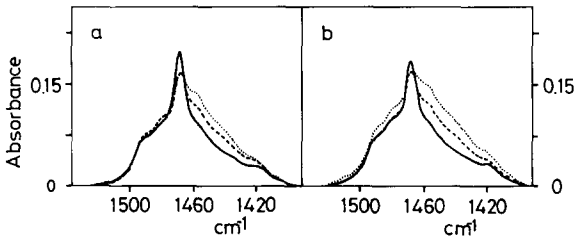

Fig. 3. Temperature dependence of the infrared spectra of the CH_2 scissoring mode of DPPC. (a) DPPC containing 5 mol% ubiquinone-10 and (b) DPPC containing 5 mol% ubiquinol-10. ———, 25°C; - - - - - -, 38°C; ·····, 45°C.

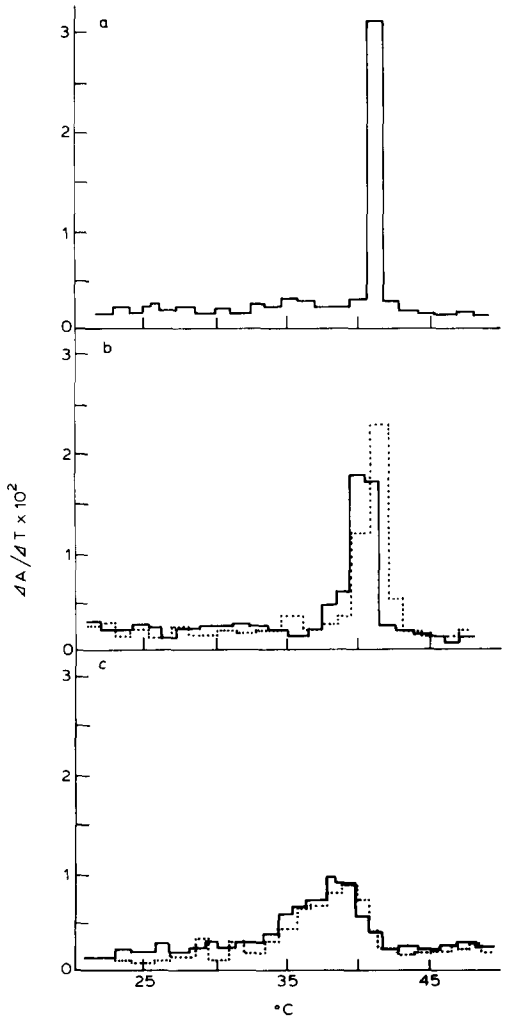

Fig. 4. Plots of $\Delta A/\Delta T$ of the CH_2 scissoring mode of DPPC vs. temperature. (a) Pure DPPC. (b) DPPC containing ubiquinone-10; ———, 5 mol%; ·····, 25 mol%. (c) DPPC containing ubiquinol-10; ———, 5 mol%; ·····, 25 mol%.

duction of either ubiquinone-10 or ubiquinol-10 did not significantly affect the pattern observed for pure DPPC and the results are not shown.

CH_2 scissoring vibrations

The CH_2 scissoring vibrations of the extended acyl chains of DPPC are located in the 1480–1460 cm^{-1} region (with minor underlying bands arising from scissoring modes of the phospholipid head group) and are characteristic of the acyl chain packing in the gel phase. Although the CH_2 scissoring mode is relatively insensitive to temperature variations in the fluid phase, it undergoes abrupt changes in intensity during the phase transition [19].

Fig. 3 shows the infrared spectra of DPPC multibilayers containing ubiquinone-10 or ubiquinol-10. A broadening of the band occurs as the temperature is increased and difference spectra can be obtained. From these difference spectra ΔA can be calculated (see Ref. 20) and in Fig. 4 $\Delta A/\Delta T$ is plotted versus temperature for several samples. Fig. 4a shows the phase transition of pure DPPC multibilayers, and Fig. 4b shows how ubiquinone-10 perturbs this transition, making it broader. Again ubiquinol-10 (Fig. 4c) alters the phase transition of the phospholipid more drastically than ubiquinone-10. It can be observed that the height of the transition is decreased in the presence of ubiquinol-10 and also that both the onset and the middle temperature of the transition are shifted towards lower temperature. At 25 mol% ubiquinol-10 the onset of the transition is located at about 33°C and the middle is located at 38.5°C, compared to 40.6 and 41.3°C, respectively, for pure DPPC.

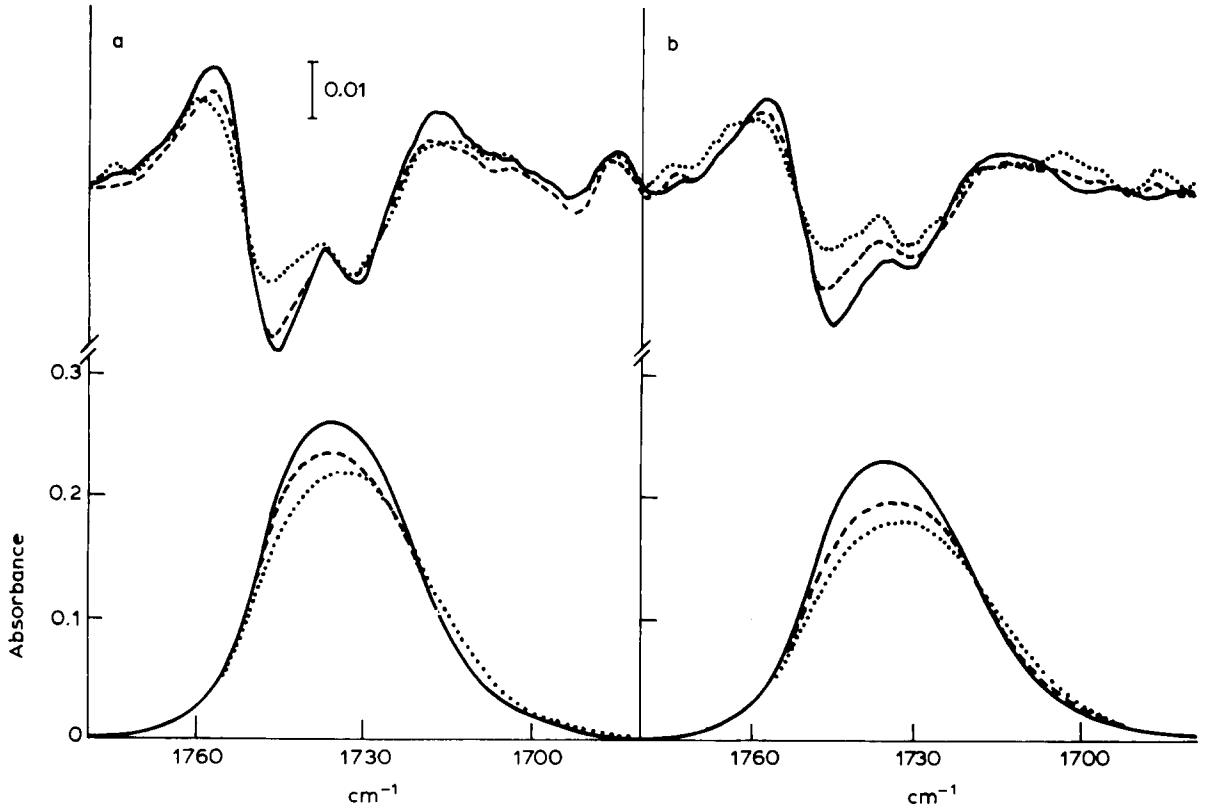

Fig. 5. Temperature dependence of the infrared spectra of the C=O stretching mode of DPPC. (a) DPPC containing 5 mol% ubiquinone-10 and (b) DPPC containing 5 mol% ubiquinol-10. The second derivative spectra is shown in the upper part of the figure, ———, 25°C; - - - - - -, 38°C; and ·····, 45°C.

C=O absorption bands

We have studied the C=O stretching mode of DPPC and also the C=O stretching bands due to the carbonyl groups of the quinone ring of ubiquinone-10.

Fig. 5 shows a broad and intense band at 1735 cm^{-1} resulting from the C=O stretching modes of the palmitoyl ester groups of DPPC multibilayers. It has been reported that it has two main components assigned to the carbonyl stretching modes of the *sn*-1 (1743 cm^{-1}) and *sn*-2 (1729 cm^{-1}) acyl chains in DPPC [29]. These two components may be distinguished in the second derivative spectra (see Ref. 30), and they are shown in Fig. 5. As the temperature increases, the relative peak heights of these two bands change, while there are no frequency shifts of the individual components in samples of DPPC multibilayers. Exactly the same has been found after Fourier self-deconvolution of the spectra [22].

The spectra of samples containing DPPC and ubiquinone-10 (Fig. 5a) are not very different from those with pure DPPC, and hence are not shown. However, the inclusion of ubiquinol-10 modifies these spectra, mainly in the temperature interval in which both the pretransition and the main transition take place (Fig. 5b).

Our general conclusion from the results obtained from the observation of the C=O stretching band is that the effects of either form of CoQ_{10} are less important than those reported for cholesterol, which considerably perturbs the C=O groups of DPPC as seen by FT-IR studies [20]. The changes observed here might solely reveal the changes in acyl chain packing and conformational order which arise from the presence of impurities in the DPPC multibilayers, hence reflecting the noncooperative nature of the melting in the complex.

Several bands corresponding to the ubiquinone-10 molecule can be observed between 1680 and 1600 cm^{-1}, as seen in Fig. 6, where the infrared spectra of a sample of multibilayers of DPPC containing 25 mol% of ubiquinone-10 and another with 25 mol% of ubiquinol-10 are shown. The intensities of these bands, which are absent in the spectrum of ubiquinol-10, are relatively low compared with the C=O band of the phospholipid centered at 1735 cm^{-1}. The band which is observed at the highest frequency presents two components, but this pattern is also observed in a pellet of ubiquinone-10 in KBr (results not shown). We tentatively assign the bands at 1660.7 and 1648.4 cm^{-1} as arising from the C=O groups and the band at 1611.1 cm^{-1} as arising from the C=C vibration of the quinone ring of ubiquinone-10 [31].

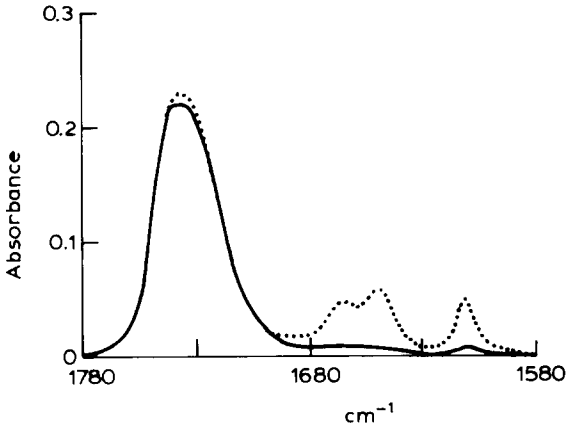

Fig. 6. Infrared spectra of the C=O and C=C stretching region of DPPC samples containing (· · · · · ·) 25 mol% ubiquinone-10 and (———) 25 mol% of ubiquinol-10. Temperature, 25°C.

No significant variations were observed in these bands on changing either ubiquinone-10 concentration or temperature. Nevertheless, these bands are useful since they allow one to confirm the redox state of the sample as they are not observed in DPPC/ubiquinol-10 samples (see Fig. 6).

Finally, other band absorptions, corresponding to the polar head group of DPPC, like the phosphate symmetric stretching at about 1080 cm^{-1} or the $C-N^+$ stretching vibration of the choline group (about 970 cm^{-1}), were also examined. Since no significant variations were observed with temperature or CoQ_{10} concentration, the results are not shown.

Discussion

The most obvious conclusion obtained from the above results is that ubiquinone-10 and ubiquinol-10 induce a different perturbation in the gel to liquid crystal transition of DPPC multibilayers. A

similar pattern can be observed in the three bands that we have studied in detail. This consists of a broadening of the main transition accompanied by a shift of its onset towards lower temperatures when either ubiquinone-10 or ubiquinol-10 were present compared with pure DPPC, ubiquinol-10 always having a bigger effect than ubiquinone-10 when both were at the same concentration.

Since in the preceding section we have studied absorption bands corresponding to functional groups located at discrete positions in the DPPC molecule, therefore probing structural changes at different sites of the molecule, a detailed picture of the interaction between ubiquinone-10 or ubiquinol-10 and the phospholipid should be obtained.

From the study of the frequencies of the CH_2 antisymmetric stretching band of the acyl chains at different temperatures it is concluded that the transition is broadened and shifted to lower temperatures as more ubiquinone-10 or ubiquinol-10 is incorporated. The shift is similar to that found previously using differential scanning calorimetry [7] and is due to the appearance of *gauche* conformers at temperatures lower than the T_c of pure phospholipid. However, and at variance with the effect of other membrane-intrinsic molecules such as cholesterol [28,32], gramicidin or proteins [32], CoQ_{10} does not significantly influence either the frequency or the bandwidth of the CH_2 antisymmetric stretching vibrations (Fig. 2) at temperatures out of the broadened phase transition interval. It may therefore be inferred that none of the CoQ_{10} forms have the 'buffering of fluidity' characteristic of cholesterol.

Similarly, the observation of the CH_2 scissoring vibrations (Fig. 4) indicates that the packing of the DPPC palmitoyl chains occurring at the transition temperature is shifted to lower temperature by ubiquinol-10 and less so by ubiquinone-10. Finally, the C=O stretching mode band of DPPC (Fig. 5) indicates that ubiquinol-10 does not have an important interaction with the C=O groups of DPPC, in contrast to the clear effect observed previously with cholesterol [20] and also with α-tocopherol (our unpublished results) using the same FT-IR technique. We conclude that the results observed for this C=O band are mainly a consequence of the perturbation produced by ubiquinol-10 on the phase transition of pure DPPC.

Based on the FT-IR data presented here, it seems reasonable to conclude that whereas oxidized CoQ_{10} hardly perturbs at all the phase transition of DPPC bilayers the reduced form produces a significant alteration in this phase transition. It would be interesting to extract from these observations some information concerning the molecular location and organization of both forms of CoQ_{10} in DPPC bilayers. On the basis of differential scanning calorimetry and fluorescence probe studies [4,7] we have suggested a central location of ubiquinone-10 in the bilayer, since this is the lipid region in which insertion of hydrophobic compounds would least disturb the packing of the lipid molecules and so least affect the T_c transition temperature of the phospholipid bilayer [33], thus explaining the lack of effect of this compound on the phase transition of DPPC, and this observation is confirmed in this paper with new FT-IR data. On the other hand and since ubiquinol-10 is capable of perturbing the DPPC phase transition as seen with the same physical techniques, a different location in the bilayer might be suggested. It could reasonably be thought that ubiquinol-10 might interact with the polar head group of the phospholipid, say forming a hydrogen bond. However, we do not find evidence of such an interaction, since as shown above the phosphate bands are not changed by the presence of ubiquinol-10 and the perturbation of the C=O band is not large enough to infer such a conclusion. Nevertheless ubiquinol-10 molecules may certainly be located in regions of the bilayer where the phospholipid organization could be significantly perturbed, such as the beginning of the fatty acyl chains and the membrane-water interface.

The ability of long-chain ubiquinone compounds, such as ubiquinone-10, to remain in a part of the bilayer where they do not perturb the phospholipid phase transition is not shared by shorter homologues such as ubiquinone-3, as shown in differential scanning calorimetry measurements [4]. Hence the length of the phytyl chain appears as an important factor which may determine the location of CoQ_{10}, possibly through the formation of aggregates in the central part of

the bilayer for the long-chain homologues, and it may explain why they have different biological activities [34,35].

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