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The interaction of ubiquinone-10 and ubiquinol-10 with phospholipid bilayers. A study using differential scanning calorimetry and turbidity measurements

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The interaction between ubiquinone-10 and ubiquinol-10 with dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine has been examined by differential scanning microcalorimetry and turbidity measurements. Microcalorimetry experiments showed that the phase transition of the phospholipids was largely unperturbed by the presence of ubiquinone-10, up to a phospholipid/CoQ molar ratio of 4:1. However, ubiquinol-10 markedly altered the calorimetric profile of both the main transition and the pretransition at molar ratios as high as 66:1, giving rise to a shoulder of the main transition peak at lower temperatures. This perturbation was larger for distearoylphosphatidylcholine than for dipalmitoylphosphatidylcholine samples. Turbidity measurements also showed that ubiquinol-10 perturbed both the pretransition and the main transition of both phospholipids. These results indicate that when the phospholipids are in the gel state, at temperatures below T_c, and at phospholipid/CoQ molar ratios higher than 4:1, ubiquinone-10 is segregated to a region of the bilayer where it does not perturb the thermotropic phase transition. However, in the same circumstances ubiquinol-10, possibly due to its more hydrophilic character, remains in a region of the phospholipid bilayer where it perturbs the phospholipid phase transition.

Introduction

Ubiquinone-10 or CoQ_{10} is a lipophilic redox component of electron-transport chains. CoQ_{10} has been implicated in the mechanism of vectorial proton translocation coupled to electron transport in mitochondria [1]. However, the precise mechanism by which this process is accomplished is still a matter of discussion.

Previous work done with differential scanning calorimetry [2–4] has established that ubiquinone-10 does not affect the pretransition or the phospholipid main phase transition of fully saturated phospholipids like dimyristoylphosphatidylcholine (DMPC) or dipalmitoylphosphatidylcholine (DPPC) except in mixtures with a very low phospholipid : ubiquinone molar ratio. It was deduced that ubiquinone-10 would not intercalate between phospholipid molecules but rather tends to be located in a separated ubiquinone-rich phase. These conclusions are also compatible with the results obtained using other techniques like kinetics measurements in vesicles containing ubiquinone-10 and trapped ferricyanide reduced by dithionite through ubiquinone [5], NMR measurements [6] and fluorescent probe experiments [2,7]. Two models have been considered for the

Abreviations: CoQ_{10}, coenzyme Q_{10}; DSC, differential scanning calorimetry; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; T_c, main phospholipid gel-to-liquid-crystalline phase transition temperature.

location of ubiquinone in phospholipid bilayers, as an aggregate spanning the bilayer and as a layer between the two half phospholipid bilayers.

However, most of the structural studies mentioned have been carried out using ubiquinone and not ubiquinol. Since both forms are present during the electron-transport process it is obviously important to study the effect of ubiquinol on the phospholipids of a bilayer and to learn from this effect something about the localization of CoQ_{10} during its functional redox cycle. This is reported in this paper, showing that the effect of ubiquinol on phospholipid bilayer phase transition is somehow different to that of ubiquinone.

Materials and Methods

Dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine were obtained respectively from Fluka, Buchs, Switzerland, and Sigma, Poole, U.K. These phospholipids were used without further purification. CoQ_{10} was obtained, as a kind gift, from Hoffman-La Roche and Co., Basle, Switzerland, and also from Sigma.

Ubiquinone-10 was reduced after the procedure of Rieske [8] by addition of dithionite to a water suspension to which ubiquinone, dissolved in acetone, had been previously added. Afterwards ubiquinol was extracted by means of cyclohexane.

The assay of ubiquinone or ubiquinol concentration in the solution used was done as described by Crane [9] 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 incorporation of CoQ between liposomes and aqueous phases was quantified with an extraction procedure using *n*-pentane, as described in Ref. 10. The phospholipid/CoQ molar ratios given in this paper are calculated according to the actual value of CoQ_{10} incorporated into the phospholipid bilayers.

The lipid mixtures for the microcalorimetry measurements were prepared by combination of chloroform solutions containing appropriate amounts of phospholipid and either ubiquinone or ubiquinol giving a final volume of the chloroform solution of 50–200 μl in a small tube of 8 mm of diameter. The sample was then further desiccated under vacuum during 2–3 h to remove the last traces of solvent. After the addition of 50 μl of twice-distilled and deionized water, multilamellar liposomes were formed by carefully mixing using a bench vibrator (Super-Mixer, Lab-line Instruments Inc. Melrose Park, IL, U.S.A.) at the maximum speed, and keeping the DPPC/samples at 50–55°C and at 60–65°C the DSPC ones, i.e., above T_c of the pure phospholipid. Mixing was continued until a homogeneous and uniform suspension was obtained, usually 4–12 min. The longest periods, within this interval, were necessary for the samples which contained the highest proportion of CoQ. Finally the suspension was centrifuged several times through a narrow capillary tube while kept above T_c. 15–20-μl aliquots of these suspensions containing about 0.7 mg of phospholipid were then sealed in small aluminium pans and scanned in a Perkin-Elmer DSC-4 instrument, using a reference pan containing water. The heating rate was 5 Cdeg/min for DPPC and 4 Cdeg/min for DSPC. The range of temperature studied was from 25 to 55°C for the DPPC samples and 35 to 65°C for the DSPC ones. Samples were not cooled down below 20°C in any circumstances. Peak areas were measured by weighing paper cut-outs of the peaks. The instrument was calibrated using indium as standard.

After the scannings had been done, the pans were opened and the phospholipid was extracted with ethanol in order to measure the amount of phospholipid in the pan by means of an organic phosphorus assay [11], the redox state of the CoQ by a spectrophotometric assay [9], and the phospholipid : CoQ ratio.

Samples for turbidity measurements were prepared also as described for microcalorimetry, except that the centrifugation through the capillary constriction was omitted since, as these samples were very diluted, this step was found unnecessary to obtain good mixing. Changes in absorbance were measured spectrophotometrically at 600 nm using a Varian 635 spectrophotometer. With the sample concentrations used in these measurements, initial absorbance was around 0.2. The temperature was continuously monitored using a thermocouple inserted inside the cuvette. The use of this technique to study phase transitions in phospholipids is discussed in Ref. 12.

Results

Incorporation of CoQ into phospholipid vesicles

Using the *n*-pentane extraction procedure described by Degli-Esposti et al. [10] the amount of ubiquinone or ubiquinol not incorporated into the phospholipid vesicles was quantified for each phospholipid/CoQ molar ratio.

Some examples of the extent of incorporation of ubiquinone and ubiquinol into DPPC vesicles are shown in Table I. Very similar results were obtained for DSPC vesicles. It can be observed that using our procedure of preparation of phospholipid : CoQ mixtures, most of the ubiquinone or ubiquinol is incorporated into the phospholipid vesicles. This incorporation is almost total, i.e. around 90%, in the very concentrated samples prepared for microcalorimetry (Expts. 2 and 3 of Table I). In the most diluted samples prepared for turbidity measurements (Expt. 1 of Table I) the proportion incorporated is slightly lower. The extent of incorporation was taken into account for the calculation of the phospholipid : CoQ molar ratios. The levels of incorporation of ubiquinone and ubiquinol into the DPPC vesicles are very similar, as can be concluded from the comparison of Expts. 2 and 3 of Table I.

Samples similar to those of Expt. 1 in Table I were also observed using a light microscope equipped with a Normarski interference-contrast accessory and, although some yellow droplets were observed, which presumably correspond to non-incorporated CoQ, as described by Stidham et al. [13], they were very scarce and smaller in size than liposomes. No striated regions corresponding to solid ubiquinone of the type described in Ref. 13 were observed in any of our samples. Hence these observations confirm that under our experimental

TABLE I

INCORPORATION OF UBIQUINONE AND UBIQUINOL INTO DPPC BILAYERS

Ubiquinone or ubiquinol were mixed in chloroform with DPPC, and after evaporation of the organic solvent, multilamellar vesicles were formed after addition of water, as described in Materials and Methods.

Experiment	[Phospholipid] (mM)	CoQ added (mM)	Incorporation (%)
1. DPPC/ ubiquinone	1.25	$12.5 \cdot 10^{-3}$	84.0
		$25.0 \cdot 10^{-3}$	88.8
		$125.0 \cdot 10^{-3}$	88.8
		$312.0 \cdot 10^{-3}$	67.3
2. DPPC/ ubiquinone	54.5	0.5	97.0
		5.5	92.3
		13.6	87.0
3. DPPC/ ubiquinol	54.5	0.9	94.8
		5.5	96.0
		13.6	98.0

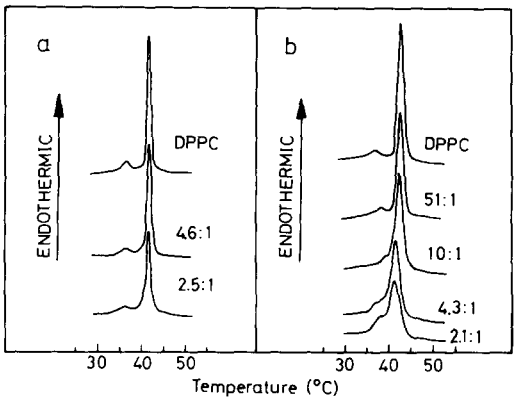

Fig. 1. The DSC calorimetric curves for pure DPPC and DPPC/CoQ_{10} systems. Molar phospholipid/CoQ_{10} ratios are indicated on the curves. The curves have been normalized for the same amount of phospholipid in each case. Panel (a), ubiquinone-10-containing samples; panel (b), ubiquinol-10-containing samples.

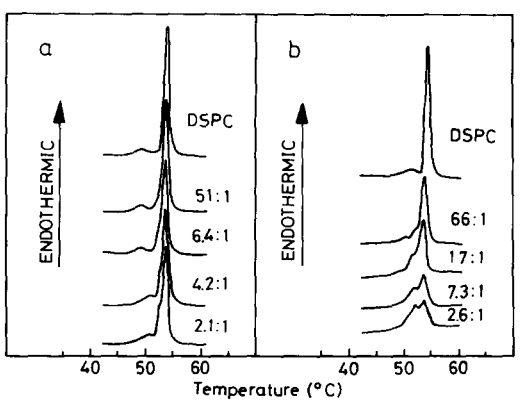

Fig. 2. The DSC calorimetric curves for pure DSPC and DSPC/CoQ_{10} systems. Molar phospholipid/CoQ_{10} ratios are indicated on the curves. The curves have been normalized for the same amount of phospholipid in each case. Panel (a), ubiquinone-10-containing samples; (b), ubiquinol-10-containing samples.

conditions most of CoQ is incorporated into liposomes.

DSC measurements

Fig. 1a shows that the calorimetric profile of the thermotropic gel-to-liquid-crystalline transition of DPPC and ubiquinone-10 up to DPPC : ubiquinone molar ratios of 4.6 : 1 and only at a molar ratio DPPC : ubiquinone of 2.5 : 1 were a decrease of the heat corresponding to the pretransition and a decrease and widening of the main transition peak observed. These observations are in agreement with our previous results [2].

However, ubiquinol-10 has a more marked effect on the calorimetric profile of DPPC phase transition. Fig. 1b shows that at DPPC : ubiquinol molar ratios as high as 51 : 1 there is a broadening of the main transition with a decrease in the height of the peak and a blurring of the pretransition, which at a ratio of 10 : 1 totally disappeared. However, the increase in the proportion of ubiquinol-10 present in the phospholipid bilayer gives rise to a shoulder of the main transition peak. This shoulder increases in size as the main peak decreases, when the proportion of ubiquinol-10 is increased. This shoulder is situated at the lower part of the main transition.

Experiments similar to those described above for DPPC : CoQ_{10} samples were done using distearoylphosphatidylcholine (DSPC) : CoQ_{10} samples. Fig. 2a shows the calorimetric profiles corresponding to the thermotropic phase transition of DSPC : ubiquinone samples. It can be seen that the calorimetric profile is not modified except for samples with a high proportion of ubiquinone (4.2 : 1 and 2.1 : 1, DSPC : ubiquinone molar ratio), where the pretransition is shifted towards higher temperatures and a small shoulder appears in the main transition peaks. As in the case of the DPPC samples, ubiquinol appears to modify the calorimetric profile more than ubiquinone. In the presence of ubiquinol a shoulder in the main transition peak is seen at a DSPC : ubiquinol molar ratio as high as 66 : 1 (Fig. 2b). In this sample the pretransition is also shifted towards a higher temperature and thus the shoulder does not arise from a shifting of the pretransition. As the concentration of ubiquinol-10 in the bilayer is increased the shoulder becomes more important and at the highest concentration of ubiquinol-10 used (DSPC : ubiquinol-10 molar ratio of 2.6 : 1) there are two peaks of similar size.

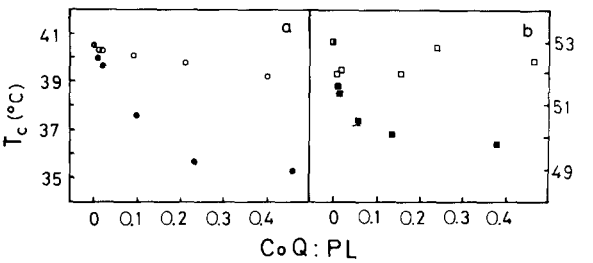

Fig. 3. Changes in T_c of systems containing different CoQ/phospholipid molar ratios. Panel (a): ○, ubiquinone-10/DPPC; ●, ubiquinol-10/DPPC. Panel (b): □, ubiquinone-10/DSPC; ■, ubiquinol-10/DSPC.

Fig. 3a shows how the T_c of DPPC samples containing ubiquinone-10 is not significantly shifted. In the sample containing a phospholipid : ubiquinone-10 molar ratio of 2.5 : 1, T_c has been shifted only 1.5 Cdeg with respect to that of the pure DPPC. However, T_c of DPPC samples containing ubiquinol-10 is shifted considerably more, and in a sample with a 2.1 : 1 molar ratio, the shift is of 5.6 Cdeg. These shifts are due to the appearance of the shoulder of the main peak at lower temperatures. Very similar results are shown in Fig. 3b for the DSPC-containing samples, although in the ubiquinone-10 samples there is a small decrease of T_c of about 1 Cdeg due to the small shoulder observed also in these samples.

Fig. 4 shows the values of ΔH versus the molar ratios of ubiquinone or ubiquinol and DPPC or DSPC. For pure DPPC ΔH was found to be 8.5 kcal/mol. The samples which contained ubiquinone with DPPC : ubiquinone molar ratios ranging between 100 : 1 and 4.6 : 1 do not show a great decrease in ΔH with respect to the pure phospholipid, i.e. just 8%. Only in the 2.5 : 1 sample does ΔH decrease notably to 6.58 kcal/mol, i.e. 22.6%. The samples containing ubiquinol are not easily quantified, since it is difficult to resolve the shoulder and the main peak. Taking together the heats corresponding to both components, only a slight decrease in ΔH is observed as the bilayer is enriched in ubiquinol-10 (Fig. 4). For example, for a molar ratio DPPC : ubiquinol-10 of 10 : 1 ΔH is 8.16 and for a ratio of 2.1 : 1 the corresponding ΔH is 7.32, i.e. 4% and 13.9% decrease with re-

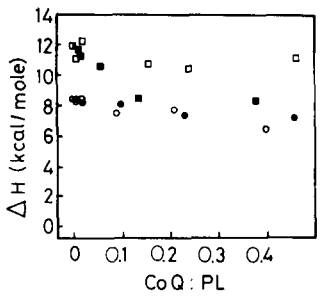

Fig. 4. ΔH of gel-to-liquid-crystalline transition of system containing different molar ratios of CoQ_{10}/phospholipid. ○, ubiquinone-10/DPPC; ●, ubiquinol-10/DPPC; □, ubiquinone-10/DSPC; ■, ubiquinol-10/DSPC.

spect to ΔH of pure DPPC, respectively.

DSPC : ubiquinone-10 samples also show a small decrease in ΔH (Fig. 4), which is, for example, 12% for a molar ratio of 4.2 : 1. However, DSPC : ubiquinol-10 samples show a bigger decrease which is 11.2% for a molar ratio of 17 : 1 and 30.2% for a 2.6 : 1 DSPC : ubiquinol-10 molar ratio. ΔH for pure DSPC was found to be 12 kcal/mol.

Turbidity measurements

The results obtained with turbidity measurements support the idea obtained from those with DSC that ubiquinol has a bigger effect than ubiquinone on the thermotropic phase transition of phospholipids. Fig. 5 shows the results obtained for DPPC samples and Fig. 6 those for the DSPC

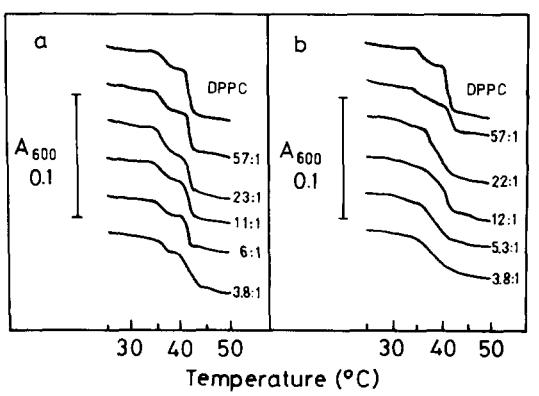

Fig. 5. Effect of increasing concentrations of CoQ_{10} on the gel-to-liquid-crystalline phase transition of DPPC (43 μg/ml) observed by changes in absorbance at 600 nm. Molar phospholipid/CoQ_{10} ratios are indicated on the curves. Panel (a), ubiquinone-10; panel (b), ubiquinol-10.

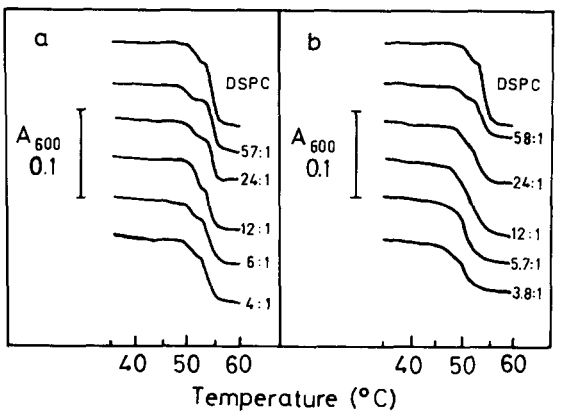

Fig. 6. Effect of increasing concentrations of CoQ_{10} on the gel-to-liquid-crystalline phase transition of DSPC (43 μg/ml) observed by changes in absorbance at 600 nm. Molar phospholipid/CoQ_{10} ratios are indicated on the curves. Panel (a), ubiquinone-10; Panel (b), ubiquinol-10.

ones. In Fig. 5a it is shown that ubiquinone did not significantly change either the pretransition or the main transition. Again, only in the sample with a molar ratio DPPC : ubiquinone 3.8 : 1 was there a blurring of the pretransition and a widening of the main transition. Something very similar was found for the DSPC-ubiquinone samples (Fig. 6a). However, Fig. 5b shows that in the presence of ubiquinol both the pretransition and the main transition were broadened even at a molar ratio DPPC : ubiquinol of 57 : 1. At 12 : 1 the pretransition completely disappeared. A similar effect can be seen for DSPC : ubiquinol samples (Fig. 6b). It is worth observing that the effect which gives rise to shoulders of the main transition peak in DSC is not detected by this technique. It has been shown [12] that the major portion of the turbidity change associated with the transition of DPPC is due to the change in refractive index of the lipid (change in phospholipid density) and the remainder corresponds to a change in optical anisotropy of the bilayer during the transition. Therefore the phospholipid which gives rise to the shoulder of the main peak in some samples has neither an altered refractive index nor a changed anisotropy with relation to the rest of the phospholipid.

Discussion

A preliminary question to be clarified about the studies of CoQ-incorporated into liposomes is the

degree of incorporation of CoQ into the bilayer. It has been recently claimed [13] that an important amount of ubiquinone does not partition from the aqueous phase into the phospholipid bilayers but exits instead as an aggregate stabilized by a small proportion of the phospholipid in the water. However, in our experimental conditions this is not the case, since, as shown in Table I, most of the ubiquinone and ubiquinol was incorporated into phospholipid vesicles. This was also confirmed by light-microscopy observation of the samples.

The observed discrepancy is perhaps due to the method used to prepare the phospholipid vesicles, since subtle factors such as the strength of the vortex mixer used to prepare the liposomes, the size of the tube, the temperature, etc., might be important.

In any case, even if the incorporation of CoQ in previous papers [2–4,7] was as low as suggested by Stidham et al. [13], i.e. 50–60%, this would not have seriously affected the main conclusions of those papers, since supposing 50% incorporation the range of phospholipid/ubiquinone molar ratios used (from 100 : 1 to 2 : 1) [2] would actually have been 200 : 1 to 4 : 1. It is obvious that the latter range would have been still adequate enough to observe the effects of ubiquinone on the phospholipid bilayers. For example, the NMR experiment, which is a crucial one in Ref. 13, was done with a molar ratio of 20 : 1. It could still be argued that the molar ratios actually present in the bilayer in previous papers [2–4,7] were not sufficient to alter the physical properties of the phospholipid bilayers, when monitored by techniques like fluorescence probe polarization or DSC. However, there are some observations which do not agree with this argument. One of them is that ubiquinone-3, which has a lower partition coefficient than CoQ_{10} [10], was reported to modify some physical properties of phospholipid vesicles, whereas ubiquinone-10 at a similar concentration was unable to do so [2]. Another observation is that ubiquinol-10 alters the phospholipid phase transition (see above) at the same nominal concentrations at which ubiquinone-10 has no effect, although both forms of CoQ_{10} have similar levels of incorporation into the bilayer (Table I).

The study of the perturbation of the thermotropic phase transition of phospholipids by the presence of other membrane components may be used to infer the type of interaction that is taking place between both membrane components. Ref. 14, for example, gives many examples of such a study. The perturbation may consist in s shifting of the critical temperature or in a broadening of the range of temperatures in which it takes place.

In the case of DSC, if the transition of the phospholipid remains unaltered by the inclusion of a molecule intrinsic to the membrane, it must be concluded that the foreign component, not having a critical size [15], is totally excluded from the gel phase of the phospholipid, and then all of the phospholipid molecules may still undergo their co-operative phase transition. Another alternative is that the fatty acid chains are perturbed beyond C_{10} and then the main calorimetric transition will not be affected [16]. Any of these possibilities may apply in the case of ubiquinone-10 after the results shown above for the DPPC samples, since only in the sample with the lowest molar ratio (2.5 : 1) is a perturbation of the phase transition observed. In the DSPC samples, however, where ubiquinone somehow modifies the symmetry of the transition peak, it can be thought that an association between oxidized CoQ and phospholipid is taking place below T_c at low DSPC : ubiquinone molar ratios. This association is very clearly seen in the DPPC : ubiquinol and specially in the DSPC : ubiquinol samples.

It was shown before [3,4,17] that ubiquinone-10 as well as other ubiquinones and ubiquinols [4] undergo upon heating some phase transitions, and a number of thermal transitions are found in the range 17–27°C, with a main endotherm at 44°C. However, to find these transitions is necessary to cool down the sample below 9°C, where an exothermic transition has been observed [4]. As in the results described here the temperature was always kept above 20°C, CoQ must be always in the fused state. Interestingly enough, it was concluded from measurements of ΔH taken from ubiquinone phase transitions [4] that a greater proportion of the coenzyme appears to be prevented from crystallizing as the phase transition of the phospholipid increases. Since in order to crystallize the CoQ must be first aggregated it could be thought that a greater proportion of CoQ would not be allowed to aggregate in DSPC than in

DPPC. This would explain why we observed that both ubiquinone and ubiquinol gave a greater perturbation of the DSPC phase transition. Another possibility is that the longer fatty acyl chains of DSPC may facilitate the interaction of the phospholipid with the long side-chain of CoQ. And it might be also that CoQ undergoes a phase transition, at a temperature intermediate between T_c of DPPC and T_c of DSPC, which is not detected by our calorimeter.

The most interesting conclusion is that ubiquinol interacts with the phospholipid in a different way than ubiquinone and it is not fully segregated from the phospholipid to the centre of the bilayer, or to any other location, below T_c of the pure phospholipid. This is what can be deduced from the shoulder which appears in the calorimetric profiles. We suggest that this shoulder arises from a mixture of phospholipid-ubiquinol which has a lower transition temperature than the pure phospholipid. The calorimetric profiles found in this paper, with a shoulder at lower temperatures but with the total enthalpy remaining constant at least for low concentrations of CoQ, is of the type B↓ after the nomenclature described in Ref. 14 and this type of profile which is given by a number of uncouplers between other types of compounds [14] is attributed to an interaction of the foreign molecule with the glycerol backbone of the phospholipid. Obviously this interaction would be more easily established with phospholipids, by ubiquinol-10 than by ubiquinone-10, given the more polar character of the first. This view is also supported by NMR measurements done with fluid membranes, from which it was concluded that ubiquinol rings are closer to the membrane surface than are ubiquinone rings [6].

The turbidity measurements confirm the results obtained using DSC and make fully clear that the shoulder provoked in the main transition, as seen by DSC, has nothing to do with the pretransition. This conclusion is reached because the pretransition seen in the pure phospholipid disappears in the presence of low concentrations of ubiquinol (Figs. 5b and 6b) and the shoulder is not detected by this technique. The different nature of the shoulder and the pretransition is also clearly shown in Fig. 2b, where the 66 : 1 sample shows both of them.

It has been suggested by Stidham et al. [13] that ubiquinone present in the membrane disorders the head-group region of the bilayer. This was concluded from ^{13}C-NMR experiments. These experiments were performed with small unilamellar vesicles. Degli-Esposti et al. [10], also using small unilamellar vesicles and ultraviolet spectroscopy, concluded from the spectral characteristics of ubiquinone incorporated in the phospholipid vesicles that 'the quinone ring must be buried in the hydrophobic phase of the membrane and does not protrude toward the polar heads of the phospholipid in the water phase'. The reason for this discrepancy is not fully clear. Another reason to be cautious with the suggestion of Stidham et al. [13] is that it is well known that observations made with this type of vesicle are not necessarily comparable with those derived from larger vesicles. In particular, Kingsley and Feigenson [6] pointed out that the depth of localization of the ubiquinone ring in the bilayer may decrease as the vesicle size decreases.

In conclusion, our results are consistent with a restriction of ubiquinone-10 to a domain of the bilayer where it does not perturb the thermotropic phase transition of the phospholipid, whereas ubiquinol-10 may extend throughout the hydrocarbon phase of DPPC and specially of DSPC. This result is important considering the mobile carrier character of CoQ [18], since the redox state of the coenzyme is then decisive in determining its localization in the bilayer in a given step of its redox cycle.

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References

1 Mitchell, P. (1976) J. Theor. Biol. 62, 327–367
2 Alonso, A., Gómez-Fernández, J.C., Aranda, F.J., Belda, F.J.F. and Goñi, F.M. (1981) FEBS Lett. 132, 19–22
3 Katsikas, H. and Quinn, P.J. (1981) FEBS Lett. 133, 230–234

4 Katsikas, H. and Quinn, P.J. (1982) Eur. J. Biochem. 124, 165–169
5 Futami, A., Hunt, E. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 583–596
6 Kingsley, P.B. and Feigenson, G.W. (1981) Biochim. Biophys. Acta 635, 602–618
7 Katsikas, H. and Quinn, P.J. (1983) Eur. J. Biochem. 131, 607–612
8 Rieske, J.S. (1967) Methods Enzymol. 10, 239–245
9 Crane, F.L. and Barr, R. (1970) Methods Enzymol. 17(C), 137–164
10 Degli-Esposti, M., Bertoli, E., Parenti-Castelli, G., Fato, R., Mascarello, S. and Lenaz, G. (1981) Arch. Biochem. Biophys. 210, 21–32
11 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–471
12 Yi, P.M. and McDonald, R.C. (1973) Chem. Phys. Lipids 11, 114–134
13 Stidham, M.A., McIntosh, T.J. and Siedow, J.N. (1984) Biochim. Biophys. Acta 767, 423–431
14 Jain, M.K. and Wu, N.M. (1977) J. Membrane Biol. 34, 157–201
15 Chapman, D., Cornell, B.A. and Quinn, P.J. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E., eds.), pp. 72–85, Springer-Verlag, Berlin and Heidelberg
16 Shimshick, E.J. and McConnell, H.M. (1973) Biochemistry 12, 315–2356
17 Katsikas, H. and Quinn, P.J. (1983) J. Bioenerg. Biomembrane 15, 67–79
18 Aranda, F.J. and Gómez-Fernández, J.C. (1982) Arch. Biochem. Biophys. 218, 525–530