Biochimica et Biophysica Acta, 767 (1984) 423–431
Elsevier

BBA 41654

ON THE LOCALIZATION OF UBIQUINONE IN PHOSPHATIDYLCHOLINE BILAYERS

MARK A. STIDHAM [a,*], T.J. McINTOSH [b] and JAMES N. SIEDOW [a,**]

[a] *Department of Botany, Duke University, Durham, NC 27706 and* [b] *Department of Anatomy, Duke University Medical Center, Durham, NC 27710 (U.S.A.)*

(Received July 26th, 1984)

Key words: Ubiquinone; NMR; EM; X-ray diffraction; Phospholipid bilayer; Phosphatidylcholine

The location of ubiquinone-10 in phospholipid bilayers was analyzed using a variety of physical techniques. Specifically, we examined the hypothesis that ubiquinone localizes at the geometric center of phospholipid bilayers. Light microscopy of dipalmitoylphosphatidylcholine at room temperature in the presence of 0.05–0.5 mol fraction ubiquinone showed two separate phases, one birefringent lamellar phase and one phase that consisted of isotropic liquid droplets. The isotropic phase had a distinct yellow color, characteristic of melted ubiquinone. [^{13}C]NMR spectroscopy of phosphatidylcholine liposomes containing added ubiquinone indicated a marked effect on the ^{13}C-spin lattice relaxation times of the lipid hydrocarbon chain atoms near the polar head region of the bilayer, but almost no effect on those atoms nearest the center of the bilayer. X-ray diffraction experiments showed that for phosphatidylcholine bilayers, both in the gel and liquid-crystalline phases, the presence of ubiquinone did not change either the lamellar repeat period or the wide-angle reflections from the lipid hydrocarbon chains. In electron micrographs, the hydrophobic freeze-fracture surfaces of bilayers in the rippled ($P\beta'$) phase were also unmodified by the presence of ubiquinone. These results indicate that the ubiquinone which does partition into the bilayer is not localized preferentially between the monolayers, and that an appreciable fraction of the ubiquinone forms a separate phase located outside the lipid bilayer.

Introduction

According to the chemiosmotic mechanism of energy transduction in mitochondria, ubiquinone (coenzyme Q_{10}; unless otherwise specified, ubiquinone refers only to ubiquinone-10) acts as a proton pump by undergoing sequential oxidation and reduction reactions on the outer and inner surfaces of the inner mitochondrial membrane [1]. Model liposome systems consisting of phosphatidylcholine and ubiquinone have been used to demonstrate that ubiquinone can shuttle electrons across the membrane from externally-added dithionite to ferricyanide that has been trapped inside the liposome [2–5], indicating that, by analogy, a similar physical mechanism for proton translocation could operate in mitochondria.

The mechanism by which ubiquinone is reduced and oxidized in lipid bilayers cannot be determined without a detailed understanding of the physical organization and orientation of ubiquinone in the membrane. Several models have been proposed for the organization of ubiquinone in membranes, but a consensus among researchers about its location in the membrane has yet to

* Current address: American Cyanamid Company, Agricultural Research Division, Princeton, NJ 08540, U.S.A.
** To whom correspondence should be addressed.

Abbreviations: DMPC, 1,2-dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; T_1, spin-lattice relaxation time.

0005-2728/84/$03.00 © 1984 Elsevier Science Publishers B.V.

emerge. Recently, several groups have approached this problem by measuring the effects of added ubiquinone on the physical properties of phospholipid bilayers and by determining the properties of ubiquinone in these bilayers. An assumption implicit in such studies is that most of the added ubiquinone partitions into the bilayer and that measurements of the physical properties of the lipid vesicles will serve to monitor ubiquinone-phospholipid interactions. In some reports on ubiquinone organization in bilayers, mole fractions of ubiquinone as high as 0.50 have been used [6,7]. The main finding of these reports is that added ubiquinone does not greatly alter the physical properties of phospholipid bilayers [6–8]. One additional conclusion that has persisted from these studies is that ubiquinone is aggregated between opposing lipid monolayers in the bilayer [6–10]. In the present report, NMR and electron microscopic evidence is presented which is inconsistent with the localization of ubiquinone at the geometric center of the bilayer. Further, the previously reported effects of added ubiquinone on the X-ray diffraction and thermal properties of phospholipid bilayers [6–10] are reinterpreted in light of the finding that at high mole fractions of ubiquinone to phospholipid (more than 0.05), an appreciable fraction of the ubiquinone forms a separate phase outside of the bilayer.

Materials and Methods

Ubiquinone-10 (from yeast) was purchased from Calbiochem, while dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and egg lecithin were purchased from Avanti Biochemicals. Ubiquinone/lipid suspensions were prepared as follows. Appropriate mixtures of ubiquinone and lipid were co-dissolved in chloroform, rotary evaporated to dryness, and hydrated with doubly distilled water. The suspension was extensively vortexed under nitrogen and equilibrated above the lipid's phase-transition temperature for at least 2 h.

For light microscopy, a small drop of sample was placed on a clean microscope slide and immediately covered with a glass cover slip. The specimens were examined with a Zeiss PMI light microscope equipped with polarization optics.

For X-ray diffraction experiments, the lipid/ubiquinone suspensions were sealed in quartz capillary tubes and mounted in an X-ray camera equipped with a point collimator and a flat plate film cassette loaded with three sheets of Kodak No-Screen X-ray Film. All X-ray experiments were performed at 20°C with exposure times between 1 and 7 h. X-ray patterns were analyzed by standard techniques as described previously [11].

Freeze-fracture samples were rapidly frozen in a manner described by Costello and Corless [12]. Small samples (0.1 μl), prepared without cryoprotectants, were sandwiched between two copper strips and punged into liquid propane. The frozen specimens were inserted into a hinged double replica device adapted for use on a Balzers BA 360 freeze-fracture unit. Fracturing was done at −150°C. The fractured specimens were immediately replicated with platinum from a 45° angle and carbon from a 90° angle. The replicas were cleaned with chloroform-ethanol (1 : 1, v/v), picked up on uncoated 400-mesh electron microscope grids, and viewed with a Philips 301 electron microscope.

For the ^{13}C-NMR experiments, egg phosphatidylcholine (0.4 g) was dissolved in chloroform either in the presence or absence of 0.05 mol fraction of ubiquinone and dried under a stream of nitrogen gas. 2.3 ml $^{2}H_2O$ was added to the dried mixture and multilamellar vesicles were generated by swirling the flask. The suspension was then sonicated to clarity over nitrogen in a sonicator bath (1–2 h), and the resulting liposomes were transferred to a 10 mm NMR tube. ^{13}C-NMR spectroscopy was performed at 36°C on a JEOL FX 90Q NMR spectrometer at 22.5 MHz. The spin-lattice relaxation times (T_1) of the phosphatidylcholine carbon atoms were determined using the fast inversion recovery technique of Canet et al. [13]. The assignments of the phosphatidylcholine ^{13}C-NMR resonances were made as in Cushley and Forrest [14].

The partitioning of ubiquinone between the bilayer and aqueous phases was quantified with the rapid extraction procedure using *n*-pentane as described by Degli Esposti et al. [15].

Results

Fig. 1 shows light micrographs of a DPPC-ubiquinone dispersion with a mole fraction of ubiquinone of 0.2. Fig. 1A and C are of different fields of the same sample observed under normal bright field illumination, while Fig. 1B and D are of these same fields taken with crossed polarizers inserted. In Fig. 1A circular fluid droplets of varying diameter can be seen in water. Some of these profiles appear clear, while others, particularly the largest ones, have a distinct yellow color. When the polarizing filters are inserted (Fig. 1B), the circular objects which are clear by direct light are seen to be birefringent, many with a black cross on white background (arrow) which is characteristic of multilamellar vesicles [16]. However, the large, yellowish droplets are non-birefringent (asterisks locate the same circular object in both 1A and B). These same circular profiles are also seen in Fig. 1C, along with a large striated region at the corner right hand side of this field. This striated region is highly birefringent (Fig. 1D) and is identical in appearance to solid ubiquinone observed in ubiquinone/water mixtures at this temperature. At lower concentrations of ubiquinone (mole fraction, 0.05), smaller amounts of the yellow liquid droplets appear, but their presence is still obvious.

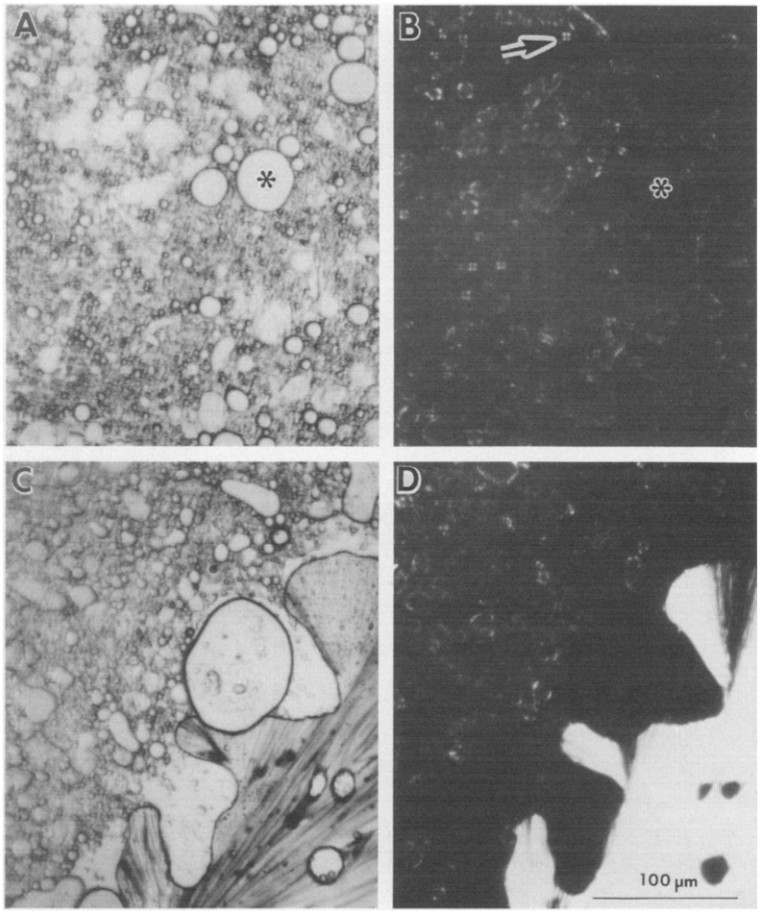

Fig. 1. Light micrographs of a DPPC-ubiquinone dispersion (mole fraction ubiquinone 0.2) with 70% water at 20°C. Figs. 1A and C were taken with normal bright field illumination, while 1B and D are of the same fields with crossed polarizers inserted. The asterisks in 1A and B identify a large, non-birefringent droplet which had a yellow color. The arrow in 1B points to a typical birefringent vesicle. All micrographs were taken at the same magnification as indicated by the scale marker in 1D.

No solid ubiquinone could be found at mole fractions below 0.05. At higher concentrations of ubiquinone (mole fractions 0.5) a larger percentage of yellow droplets and solid ubiquinone are seen relative to the lipid.

The amount of ubiquinone partitioning into the bilayer was calculated by removing the aqueous phase ubiquinone by a rapid extraction with *n*-pentane. This method was previously described by Degli Esposti et al. [15], and the results obtained with lipid preparations used in the present study appear in Table I. For ubiquinone added to egg phosphatidylcholine multilamellar vesicles, a constant fraction of ubiquinone (approx. 60%) partitioned into the bilayer over the range 0.05–0.2 mole fraction ubiquinone. Essentially the same results were obtained using DPPC in place of egg phosphatidylcholine. The apparent partition coefficient (P_{UQ}) derived from these results ranges from 64 to 73 (Table I), very similar to the value of 74 reported by Degli Esposti et al. [15] for the partitioning of added ubiquinone-10 into egg phosphatidylcholine liposomes.

If ubiquinone resides at the center of the phospholipid bilayer, changes in the mobility of the lipid acyl chain atoms near the center of the bilayer would be anticipated. Specifically, the degree of disorder in the middle of the membrane

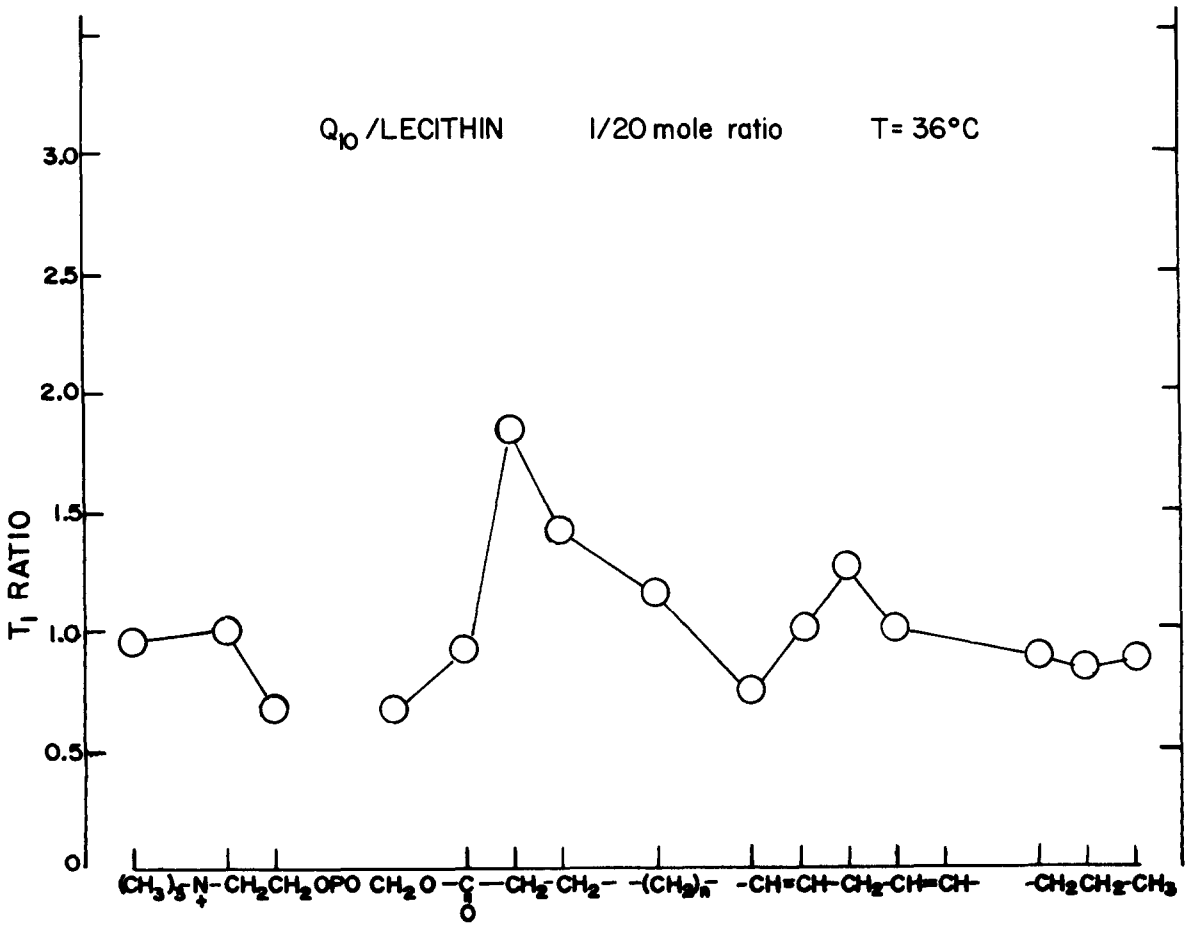

Fig. 2. The effect of ubiquinone on the mobility of egg phosphatidylcholine carbon atoms in liposomes. ^{13}C-NMR spectroscopy of egg phosphatidylcholine liposomes prepared in the presence and absence of 0.05 mole fraction of ubiquinone was performed and the spin-lattice relaxation times (T_1) of the phosphatidylcholine carbon atoms were determined using the 180-τ-90-PD inversion recovery pulse sequence as described by Canet et al. [13]. The T_1 ratio was calculated as the ratio of a given carbon atom's T_1 in the presence and absence of added ubiquinone. Other details are as described in Materials and Methods.

TABLE I

PARTITIONING OF UBIQUINONE INTO EGG PHOSPHATIDYLCHOLINE BILAYERS

The appropriate mole fraction of ubiquinone-10 was co-dissolved with 26 μmoles of egg phosphatidylcholine in chloroform. After removal of the chloroform, doubly distilled water (1.0 ml) was added and multilamellar vesicles generated by extensive vortexing under nitrogen. Liposomes were prepared by sonicating the above vesicles to clarity in a sonicator bath. Partitioning of ubiquinone into the bilayer was determined using the procedure of Degli Esposti et al. [15].

System	Mole fraction ubiquinone		P_{UQ}
	Added	Incorporated	
Multilamellar vesicles	0.05	0.029	73
	0.10	0.059	69
	0.20	0.110	64
Liposomes	0.05	0.046	658

should increase due to the disruptive influence of a large, branched molecule such as ubiquinone. To test this, ^{13}C-NMR spectroscopy was carried out on ubiquinone-containing phosphatidylcholine liposomes to look at the effect of added ubiquinone on the spin-lattice relaxation times (T_1) of the carbon atoms on the phospholipid hydrocarbon chains. The ubiquinone-containing liposomes were made by co-sonicating egg phosphatidylcholine and 0.05 mole fraction ubiquinone; over 90% of the added ubiquinone was incorporated into the resulting bilayer phase (Table I). Fig. 2 presents a plot of the ratio of the T_1's of the individual acyl chain carbon atoms in the presence and absence of added ubiquinone [i.e., T_1 ratio = T_1(phosphatidylcholine + ubiquinone)/T_1(phosphatidylcholine)]. There was almost no effect of added ubiquinone on the motional freedom (T_1) of the terminal three carbons of the hydrocarbon chain. In fact, a small decrease (10–15%) in T_1 appeared for these three atoms. On the other hand, significant increases in T_1 were observed for those carbon atoms of the acyl chain nearest the polar head region of the bilayer, and significant decreases in T_1 were observed for the two carbon atoms adjacent to the phosphate moiety in the polar head group (Fig. 2).

As reported previously by others, X-ray diffraction patterns of gel state DPPC consist of several lamellar orders of a periodicity of 6.4 nm and a sharp wide-angle reflection at 0.42 nm surrounded by a diffuse band at 0.41 nm, while X-ray patterns of liquid crystalline egg phosphatidylcholine contain a lamellar periodicity of 6.3 nm and a broad wide-angle band at 0.46 nm [17]. For both gel state DPPC and liquid crystalline egg PC these low-angle and wide-angle reflections are unmodified by the addition of ubiquinone (mole fractions from 0.05 to 0.7). At the higher concentration range of

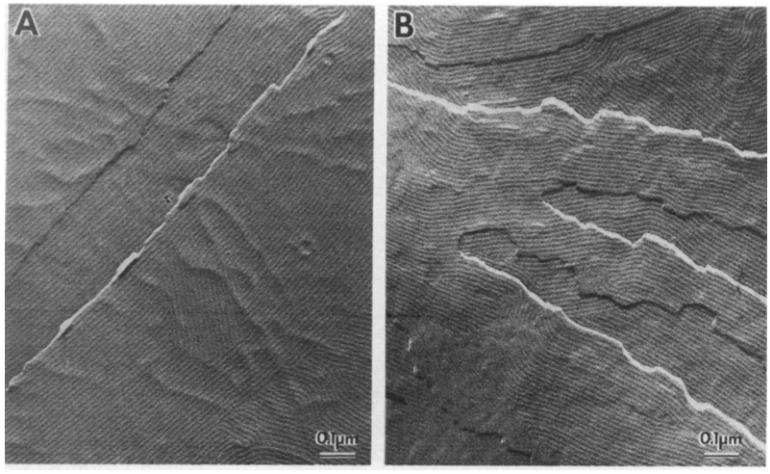

Fig. 3. Freeze-fracture electron micrographs of DMPC with 70% water quenched from 19°C in the absence (A) and presence (B) of 0.1 mole fraction ubiquinone. The ripples characteristic of the $P\beta'$ phase of DMPC can be observed in both preparations. The micrographs are mounted so that the platinum deposition direction is approximately from the bottom of the field.

ubiquinone (mole fraction 0.5–0.7), reflections characteristic of solid ubiquinone could be detected in addition to the reflections from the phospholipid.

Freeze-fracture experiments were performed because it has been suggested that ubiquinone aggregates between adjacent monolayers of the bilayer [6–9], and it is well established that freeze-fracturing exposes the hydrophobic core of the bilayer. Of particular interest was the $P\beta'$ or 'rippled' phase of phosphatidylcholine bilayers [17], because periodic ripples which are observed on the hydrophobic fracture surface would be obscured if ubiquinone formed a phase in the center of the bilayer. Fig. 3 shows DMPC frozen at 19°C in the absence (Fig. 3A) and presence (Fig. 3B) of 0.1 mole fraction added ubiquinone. There are no detectable differences in the morphology of the fracture faces. In both cases the DMPC is in the $P\beta'$ phase. Ripples are present and have the same periodicity (about 13 nm) with and without added ubiquinone.

Discussion

With the exceptions of the elegant NMR studies of Kingsley and Feigenson [18] and the spectral work of Degli Esposti et al. [15], most recent studies designed to localize ubiquinone in phospholipid bilayers have used some form of artificial bilayer with mole fractions of ubiquinone (to phospholipid) far in excess of the value normally associated with the mitochondrial membrane. The latter value generally falls in the range of 0.01–0.02 mole fraction of ubiquinone [19,20] while the model systems reported in the literature have rarely used mole fractions of ubiquinone-10 less than 0.1 [6–8,10]. Inherent in much of the model membrane work is the assumption that not only does added ubiquinone partition into the bilayer, but that most of the added ubiquinone partitions into the membrane phase perhaps between opposing monolayers of the bilayer [6–10]. The results of the present study show that (1) not all the added ubiquinone partitions into the bilayer phase, (2) the ubiquinone which does partition into the bilayer does not aggregate in the geometric center of the bilayer, and (3) ubiquinone in the membrane disorders the head group region of the bilayer.

Experiments like that shown in Fig. 1 indicate that at mole fractions of ubiquinone as low as 0.05, non-birefringent yellow ubiquinone-rich droplets can be clearly distinguished from birefringent lipid vesicles in the light microscope. Thus there are two phases present, a 'lipid-rich' phase and a 'ubiquinone-rich' phase. The presence of melted ubiquinone droplets in these suspensions at room temperature (Fig. 1A), well below the melting point of pure ubiquinone (49°C), indicates that some phospholipid has probably partitioned into the ubiquinone-rich droplets.

Table I shows that just over 40% of the ubiquinone is not incorporated into the bilayer when added to egg phosphatidylcholine multilamellar vesicles over the range 0.05–0.2 mole fraction ubiquinone. The latter value gives rise to an apparent ubiquinone partition coefficient of roughly 70, very similar to the value reported by Degli Esposti et al. [15] for the partitioning of ubiquinone-10 into egg phosphatidylcholine liposomes. Co-sonication of lipid and ubiquinone led to a marked increase in the incorporation of ubiquinone into the bilayer phase, again consistent with the observations of Degli Esposti et al. [15].

To date, the only work which has attempted to quantify the partitioning of ubiquinone into phospholipid bilayers is that of Degli Esposti et al. [15]. Two points should be noted here. First, the actual partitioning of ubiquinone between the aqueous and membrane phases must be weighed by the relative volumes of the two phases. Because the aqueous phase, particularly with liposomes, is generally in excess, an appreciable fraction of the added ubiquinone will remain in the aqueous phase. Second, the partition coefficient represents the equilibrium distribution between the aqueous, aggregated form of ubiquinone and the bilayer, and not the partitioning of ubiquinone monomer from an aqueous solution into the bilayer. The latter value is, no doubt, much greater than 70–74. So on thermodynamic grounds, and accepting the presence of some free energy of stabilization associated with the ubiquinone aggregates, there is good reason to expect that an appreciable amount of added ubiquinone in the model systems will not partition into the bilayer. Degli Esposti et al. also found that the uptake of ubiquinone into the lipid phase eventually saturated [15]. With ubiquinone-8

and egg phosphatidylcholine, a maximum incorporation of roughly 0.1 mole fraction was observed. No attempt was made in the present study to determine the maximum incorporation of ubiquinone.

Previous studies of ubiquinone interactions with phospholipid bilayers certainly have recognized the presence of more than one phase of ubiquinone [6,15,18], but the exact nature of these phases has yet to be well defined. Kingsley and Feigenson reported two distinct $-OCH_3$ peaks in ^{1}H-NMR spectra of co-sonicated ubiquinone/DMPC suspensions [18]. They assigned the low-field peak to ubiquinone dispersed within the bilayer and the high-field peak to a separate, 'ubiquinone-rich' phase. This second peak appeared at concentrations as low a 0.02 mole fraction ubiquinone [18]. Because either externally added or internally trapped lanthanide shift reagents affected both $-OCH_3$ resonances, this was taken to indicate that both pools of ubiquinone were present in 'vesicle-like' structures [18].

Degli Esposti et al. [15] using ultraviolet absorption spectroscopy, also demonstrated that two pools of ubiquinone existed in the presence of phospholipid vesicles at mole fractions of ubiquinone as low as 0.01. Based upon the absorption maxima of the two pools, they concluded that one represented ubiquinone in a nonpolar environment while the other corresponded to ubiquinone aggregates forming in the aqueous phase. This conclusion was supported by the observation that a brief pentane extraction could remove the aqueous, but not the membrane-bound, pool of ubiquinone. The results of Degli Esposti et al. [15] are consistent with our observations in Fig. 1. At this time, it is not obvious how the lanthanide shift results of Kingsley and Feigenson [18], which indicate a vesicle-like nature for the ubiquinone-rich phase, correlate with the ubiquinone aggregates shown in Fig. 1 and postulated by Degli Esposti et al. [15].

The freeze-fracture images (Fig. 3) give evidence that ubiquinone does not aggregate in the geometric center of the bilayer. The $P\beta'$ phase of saturated lecithins contains periodic ripples in the hydrophobic fracture faces [17]. Hydrophobic molecules, such as hexane, which accumulate in the geometric center of the bilayer produce mounds and depressions in the fracture faces which can obscure the rippled structure [21]. No mounds or depressions were observed in the fracture faces of bilayers containing ubiquinone, and ubiquinone had no effect on the appearance of the periodic ripples (Fig. 3A and B). The X-ray results also provide evidence which indicate that ubiquinone does not aggregate in the center of the bilayer, as the lamellar repeat period is not modified by up to 0.7 mole fraction added ubiquinone. Aggregations of ubiquinone between the monolayers of the bilayer would be expected to increase the thickness of the bilayer and thus increase the lamellar repeat period. Previous X-ray work with ubiquinone/phospholipid suspensions reported only wide-angle reflections and not the lamellar repeat periods [7].

Our conclusion that only limited amounts of added ubiquinone enter the bilayer, and that much of the ubiquinone is in a separate, aqueous phase, is also consistent with earlier differential scanning calorimetry and fluorescence polarization results which indicate that ubiquinone-10 does not modify the phase transition temperatures of the bilayer [8,10]. These calorimetry and fluorescence polarization results have previously been interpreted to support the idea that ubiquinone localizes between opposing monolayers of the bilayer [8,10]. A more reasonable interpretation is that there is not enough ubiquinone in the bilayer to significantly perturb the physical properties of the membrane which these techniques monitor. Likewise, if some amount of phospholipid does partition into the aggregated, aqueous pool of ubiquinone, this could well account for the observed changes in the thermal properties of ubiquinone by added phospholipids [6,8]. Previous studies of ubiquinone interactions with lipid bilayers have recognized the presence of a separate phase of ubiquinone [6], but they have failed to appreciate that this phase might (1) not be present within a bilayer structure and (2) have its own properties modified by the partitioning of phospholipids into it.

While not all the added ubiquinone partitions into the phospholipid phase in these model membrane systems, there is no question that some does. Therefore any observed effects of added ubiquinone on the physical properties of the bilayer can be attributed to the membrane-associated fraction of ubiquinone. ^{13}C-NMR spectroscopy was

used to measure the effect of added ubiquinone on the spin-lattice relaxation times (T_1) of the various lipid carbon atoms in egg phosphatidylcholine liposomes. The T_1 values of the lipid carbon atoms directly reflect the relative motion of the nuclei and it is well-established that motional freedom increases from the surface to the interior of the bilayer [22]. It was anticipated that those regions of the bilayer most affected by the incorporation of ubiquinone would show the biggest changes in T_1. Such an approach has been used previously to look at the incorporation of phytol, vitamin E and phytanic acid into phosphatidylcholine vesicles [14,23]. Spin-lattice relaxation times are particularly sensitive in those regions of the bilayer where a destabilization occurs leading to increases in measured T_1s (i.e., T_1 ratio larger than 1.0; Refs. 14 and 23).

The results in Fig. 2 indicate that added ubiquinone has a major effect on the carbon atoms of the phosphatidylcholine hydrocarbon chains nearest the polar head region and essentially no effect on the three acyl carbon atoms closest to the center of the bilayer. This observation is inconsistent with the model of Katsikas and Quinn [6–9]. It is difficult to imagine how the ubiquinone which is incorporated into the bilayer in this experiment (0.046 mole fraction) could be situated solely at the center of the bilayer and so significantly affect atoms near the head region but not affect the hydrocarbon chain packing of the terminal carbon atoms. These results are not inconsistent with the finding of Kingsley and Feigenson [18] that the quinone rings of longer-chain ubiquinones are buried deeper in the bilayer than those of shorter-chain ubiquinones, because they did not quantify how deeply any of the rings were buried. The lack of apparent disruptive effect of the branched isoprene side-chain of ubiquinone differs from the marked effects seen with the branched chains of phytol and phytanic acid [14,23]. This may reflect the lower level of incorporation of ubiquinone into the bilayer and suggests that the marked effect seen near the head region is due to interactions between the lipid hydrocarbon side chains and the bulky *p*-benzoquinone moiety of ubiquinone. In this regard, it is interesting to note that Cain et al. [24] have used X-ray diffraction to show that ubiquinone-3 resides in the bilayer between adjacent lipid molecules with the quinone moiety in the head group region and the hydrocarbon chain penetrating into the lipid hydrocarbon core. These shorter chain analogues also have been found to modify the thermotropic properties of phosphatidylcholine bilayers [7].

In summary, our data do not support the concept that ubiquinone-10 is localized at the geometric center of the bilayer in phospholipid membrane model systems. In fact, our ^{13}C-NMR data (Fig. 2) are more consistent with the quinone head group being situated near the polar end of the lipid hydrocarbon chains. The location and orientation of the isoprene side chain of ubiquinone in the bilayer remains an important topic for further investigation. The bulk of the available evidence shows that ubiquinone and phospholipids tend to form two phases in aqueous suspensions even at low ratios of ubiquinone to phospholipid. Data from such suspensions should be interpreted with the realization that limited amounts of ubiquinone will enter the bilayer, making the organization of the bilayer relatively unaffected by the presence of ubiquinone. Likewise, direct measurements made on the ubiquinone in the bulk phase will not allow much insight into the nature of the interactions between ubiquinone and the lipid within the bilayer.

Acknowledgements

We wish to thank Dr. Sidney Simon for very helpful discussions and Ms. Amy Canine for expert technical assistance. This work was supported by USDA/ARS cooperative research agreement 58-7B30-0-208 and in part by grants from The National Institutes of Health, GM27278 (to T.J.M.) and GM26095 (to J.N.S.).

References

1 Mitchell, P. (1976) J. Theor. Biol. 62, 327–367
2 Hauska, G. (1977) FEBS Lett. 79, 345–347
3 Hauska, G. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G.C. and Trebst, A., eds.), pp. 177–187, Elsevier/North-Holland, Amsterdam
4 Futami, A., Hurt, E. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 583–596
5 Futami, A. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 597–608

6 Katsikas, H. and Quinn, P.J. (1982) Eur. J. Biochem. 124, 165–169
7 Katsikas, H. and Quinn, P.J. (1982) Biochim. Biophys. Acta 689, 363–369
8 Katsikas, H. and Quinn, P.J. (1981) FEBS Lett. 133, 230–234
9 Katsikas, H. and Quinn, P.J. (1983) Eur. J. Biochem. 131, 607–612
10 Alonso, A., Gomez-Fernandez, J.C., Aranda, F.J., Belda, F.J.F. and Goni, F.M. (1981) FEBS Lett. 132, 19–22
11 McIntosh, T.J. (1980) Biophys. J. 29, 237–246
12 Costello, M.J. and Corless, J.M. (1978) J. Microsc. (Oxford) 112, 17–37
13 Canet, D., Levy, G.C. and Peat, I.R. (1975) J. Magn. Res. 18, 199–204
14 Cushley, R.J. and Forrest, B.J. (1976) Can. J. Chem. 54, 2059–2066
15 Degli Esposti, M., Bertoli, E., Parenti-Castelli, G., Fato, R., Mascarello, S. and Lenaz, G. (1981) Arch. Biochem. Biophys. 210, 21–32
16 Rosevear, F.B. (1954) J. Amer. Oil Chemist's Soc. 31, 628–639
17 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733
18 Kingsley, P.B. and Feigenson, G.W. (1981) Biochim. Biophys. Acta 635, 602–618
19 Kroger, A., Klingenberg, M. and Schweidler, S. (1973) Eur. J. Biochem. 34, 358–368
20 Capaldi, R. (1982) Biochim. Biophys. Acta 694, 291–306
21 McIntosh, T.J. and Costello, M.J. (1981) Biochim. Biophys. Acta 645, 318–326
22 Jacobs, R.E. and Oldfield, E. (1981) Prog. NMR Spectr. 14, 113–136
23 Cushley, R.J. and Forrest, B.J. (1977) Can. J. Chem. 55, 220–226
24 Cain, J., Santillan, G. and Blasie, J.K. (1972) in 'Membrane Research' (Fox, C.F., ed.), pp. 3–14, Academic Press, New York