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A difference infrared-spectroscopic study of the interaction of ubiquinone-10 with phospholipid bilayers

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The interaction between 1,2-dipalmitoyl phosphatidylcholine and ubiquinone-10 in aqueous systems was studied by difference i.r. spectroscopy. Binary mixtures of the two lipids in proportions of 2, 5 and 15 mol% were investigated in the spectral regions reporting on the hydrocarbon chains of the phospholipid and the polar phosphate group. No spectral shifts or significant broadening of any absorbances due to the phospholipid were detected at temperatures of 20 or 54 °C. Changes in the frequency of the maximum of the CH_2 antisymmetric C–H stretching vibration with temperature indicated that the gel-to-liquid-crystalline phase-transition temperature of the phospholipid was lowered by about 2 °C in the presence of between 2 and 15 mol% ubiquinone-10. Absorbance by the benzoquinone substituent of ubiquinone-10 was detected by spectral subtraction of dispersions of phospholipid alone. Bands due to C=O stretching and ester group vibrations of ubiquinone-10 in co-dispersion with phospholipid were compared with the same spectral region when ubiquinone-10 was dissolved in solvents or as a crystalline solid. Spectral changes could be detected when ubiquinone-10 in phospholipid was compared with solution in dodecane and chloroform. These may indicate that the benzoquinone ring system is located within a hydrocarbon domain in dispersions with dipalmitoyl phosphatidylcholine. It was concluded from the study that when ubiquinone-10 is co-dispersed with dipalmitoyl phosphatidylcholine in water the two lipids phase-separate. There is no evidence that ubiquinone-10 intercalates between phospholipid molecules, which undergo a gel–liquid-crystalline phase transition in only a slightly modified form. The data suggest that the benzoquinone substituent resides in a hydrophobic domain and that aggregates spanning the bilayer are a possible arrangement of the ubiquinone in the structure.

INTRODUCTION

Ubiquinones are essential components of biological electron-transport chains and are unique in that they represent the only lipid member of these multicomponent systems (Lenaz, 1985). Being largely hydrophobic in character, it is generally assumed that they occupy a domain in the membrane common to that of the hydrocarbon chains of the membrane lipids. Since direct contact with redox centres of electron-transfer complexes of energy-transducing membranes must take place during the reduction and subsequent oxidation of ubiquinone, it follows that sites on the respective protein complexes interact in a specific manner with the coenzyme. Many elegant experiments have been reported in which these sites have been identified (Yu *et al.*, 1980*a*,*b*; Nagoaka *et al.*, 1980). Although tertiary collisional complexes between ubiquinone reductant and oxidant and the coenzyme have been proposed as a model for electron transfer in the mitochondrial membrane (Ragan & Heron, 1978; Zhu *et al.*, 1982), it is clear from the ratio of ubiquinone to other electron-transport complexes that a considerable proportion of ubiquinone must be in an unbound form. The arrangement of this pool of ubiquinone (and plastoquinone in the chloroplast thylakoid membrane) is subject to debate.

Model systems have been extensively used to examine the disposition of ubiquinones in phospholipid-bilayer membranes. Two categories can be distinguished: those that involve formation of single-bilayer vesicles on the one hand, and multilamellar dispersions of phospholipid and ubiquinone on the other. It is likely that the interpolation of ubiquinone into phospholipids that have been subjected to ultrasonication sufficient to disperse the mixture in the form of single-bilayer vesicles is different from that in coarse multilamellar dispersions. This can arise because of the constraints imposed on molecular packing in structures of low radius of curvature as well as the fact that the number of ubiquinone molecules per vesicle often amounts only to hundreds, depending on the ratio between phospholipid and ubiquinone in the dispersion.

Previous studies from this (Katsikas & Quinn, 1982*a*, 1983) and other laboratories (Alonso *et al.*, 1981) have employed multibilayer liposome structures in which packing arrangements may more closely resemble that of biological membranes. Three basic models have been considered for the arrangement of ubiquinone-10 in phospholipid bilayers. One holds that the ubiquinone diffuses freely throughout the hydrocarbon domain of the bilayer and with access to the aqueous phase on both sides of the structure (Robertson & Boardman, 1975; De Pierre & Ernster, 1977), a second is one in which the ubiquinone molecules are confined within the central plane of the phospholipid bilayer and do not penetrate extensively between the hydrocarbon chains of the phospholipids (Quinn, 1980; Katsikas & Quinn, 1981), and finally there is a micellar model in which aggregates of ubiquinone span the bilayer and either remain in a fixed orientation (Hauska, 1977; Futami *et al.*, 1977) or tumble isotropically (Quinn & Katsikas, 1985). The most consistent observation made with a variety of biophysical

methods is that the phase behaviour of the phospholipid in mixed dispersions is only marginally perturbed, leading to the conclusion that the ubiquinone is phase-separated from the phospholipid and occupies a different domain, consistent with the second and third models. The possibility that, at least at high molar ratios of ubiquinone to phospholipid, ubiquinone is not incorporated into the bilayer but exists in the aqueous phase has been considered on the basis of measurements of partition coefficients (Degli Esposti *et al.*, 1981) and confirmed directly by microscopic examination (Stidham *et al.*, 1984).

In the present experiments, coarse bilayer dispersions of phospholipids containing ubiquinone-10 in proportions up to 15 mol% were examined by difference i.r.-spectroscopic methods. Among the advantages of this method is the fact that it is non-perturbing and does not require the presence of reporter or probe molecules. Secondly, the effect of environmental factors on particular chemical groups of both molecules in the mixture can be examined independently and by one of several spectral parameters. Although extensive work has been published on the i.r.-absorption spectra of ubiquinones (Pennock *et al.*, 1962; Langemann & Isler, 1965; Quinn & Katsikas, 1985), the use of conventional spectroscopic techniques to examine ubiquinone in aqueous systems has been largely precluded by the intense water absorptions that dominate the signal (see however Asher & Levin, 1977). The development of computerized (Chapman *et al.*, 1980) and Fourier-transform (Cameron *et al.*, 1979) methods of processing i.r. spectra has successfully dealt with this problem, and a variety of studies of aqueous dispersions of phospholipids (Cameron & Mantsch, 1978; Cameron *et al.*, 1980*a*,*b*; Mantsch *et al.*, 1980; Rothschild *et al.*, 1980; Arrondo *et al.*, 1984) and biological membranes (Cortijo *et al.*, 1982) have been reported. In the present paper we describe experiments on binary mixtures of ubiquinone-10 and phospholipid in aqueous dispersions. Regions of the i.r. spectrum reporting on the hydrocarbon components and the polar head group of the molecules and the interfacial regions of the lipid structure were examined to determine the extent to which and in what manner the lipids interact.

MATERIALS AND METHODS

Preparation of lipid dispersions

Dipalmitoyl DL-α-phosphatidylcholine (99%; Sigma Chemical Co., Poole, Dorset, U.K.) and ubiquinone-10 (a gift from Eisai Co., Tokyo, Japan) were mixed in the required proportions in chloroform solution, dried under a stream of N_2 and then freeze-dried. The required amount of water or ^2H_2O (99.8%; Sigma Chemical Co.) to give 10% or 15% (w/w) phospholipid suspensions was added to the dry lipids, which were dispersed at temperatures greater than 50 °C in a vortex mixer.

I.r. spectroscopic techniques

All spectra were recorded with a microprocessor-controlled Perkin–Elmer model 298 infrared spectrometer. A Perkin–Elmer 3600 data station was used in-line with the spectrometer and was used to process the digitized data. The system was similar to that described elsewhere (Chapman *et al.*, 1980). The spectrometer was calibrated with a 50 μm-thick polystyrene film and an indene film between NaCl plates scanned under conditions identical with those used for samples.

Scans of samples in organic solvents were recorded isothermally at 20 °C in a Beckman cell equipped with CaF_2 windows providing a beam path-length of 12 or 100 μm. The absorbance spectrum in this configuration was limited at the low-frequency end to 1000 cm^{-1}. In experiments with aqueous systems and where the temperature was varied, a special thermostatically controlled cell with CaF_2 windows (Specac Ltd.) was used. The beam path-length in this cell was 6 or 12 μm. The temperature was maintained within 0.25 °C during scans by a circulating-water bath. A calibrated thermocouple placed against the cell was used to monitor the sample temperature.

Lipid dispersions or solutions in solvents were generally scanned at a rate over the i.r. region 4000 to 600 cm^{-1} of 30 min by using the medium-slit-width programme. This rate of scan showed minimum pen drift and permitted accurate difference spectra to be derived. A correction factor was applied in spectral processing to account for changes in the optical path of samples differing in composition.

RESULTS

The effect of incorporating ubiquinone-10 into bilayer dispersions of dipalmitoyl phosphatidylcholine in water was examined by i.r. spectroscopy, particular attention being devoted to regions of the spectra providing information about the acyl chain region of the phospholipid as well as regions reporting on the polar head group.

Spectral features assigned to the hydrocarbon chains

The effect of the presence of 5 mol% ubiquinone-10 on the phase properties of dipalmitoyl phosphatidylcholine bilayers is shown in Fig. 1. I.r.-absorption spectra in the region of C–H stretching vibrations are shown in which the signal from water is subtracted. Spectrum a was recorded at 20 °C, which is about 20 °C below the gel–liquid-crystalline phase-transition temperature of the phospholipid, and spectrum b was recorded at a temperature (54 °C) well above the transition temperature. Strong bands are observed at 2920 and 2851 cm^{-1}, which arise from the CH_2 antisymmetric and symmetric C–H stretching modes of the fatty acyl chains. Both these absorbances are sensitive to the change in phase of the phospholipid and become broader and weaker in intensity and shift to higher wavenumbers above the phase-transition temperature. This shift is apparent from the difference spectrum obtained by subtraction of spectrum a from spectrum b also shown in Fig. 1 (spectrum c). The thermal changes in the spectrum are believed to be due to the decrease in all-*trans* chain conformers and a corresponding increase in *gauche* rotamers with increasing temperature. The line broadening of these bands is related to the increase in rates and amplitude of motion of the fatty acyl chains with increasing temperature (Casal *et al.*, 1980). Identical spectra were obtained in dispersions of dipalmitoyl phosphatidylcholine without ubiquinone-10, suggesting that there was no significant effect of ubiquinone on the

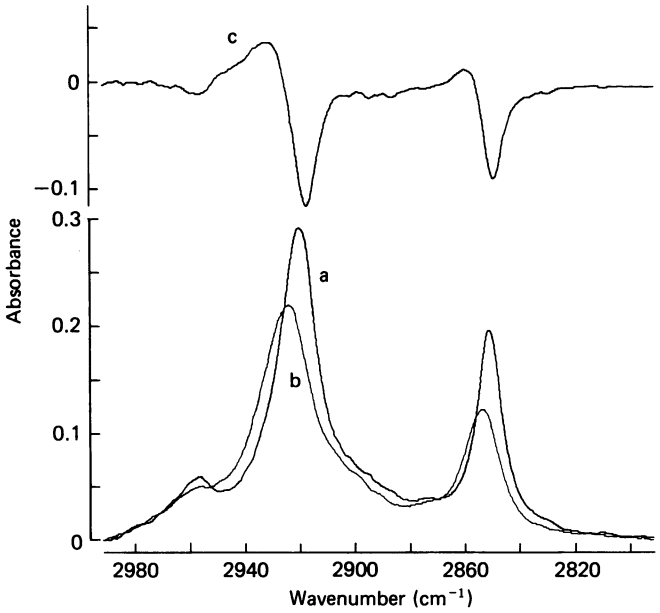

Fig. 1. I.r.-absorption spectra in the C–H stretching region of fully hydrated dipalmitoyl phosphatidylcholine co-dispersed with 5 mol% ubiquinone-10 at 20 °C (spectrum a) and 54 °C (spectrum b)

Spectrum c is a representative difference spectrum obtained by subtracting spectrum a from spectrum b.

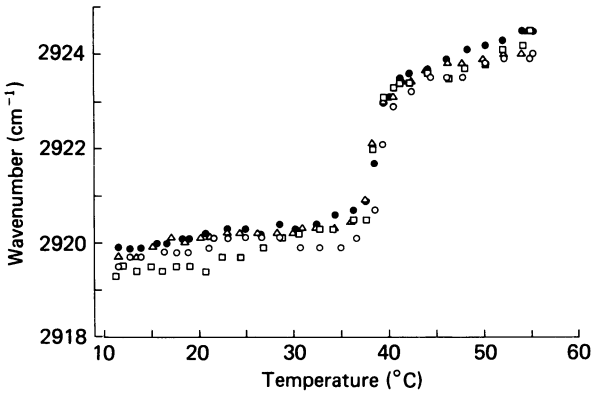

Fig. 2. Temperature-dependence of the frequency of the maximum of the CH_2 antisymmetric C–H stretching vibration of aqueous dispersions of dipalmitoyl phosphatidylcholine (○) and phospholipid co-dispersed with 2 mol% (□), 5 mol% (△) and 15 mol% (●) ubiquinone-10

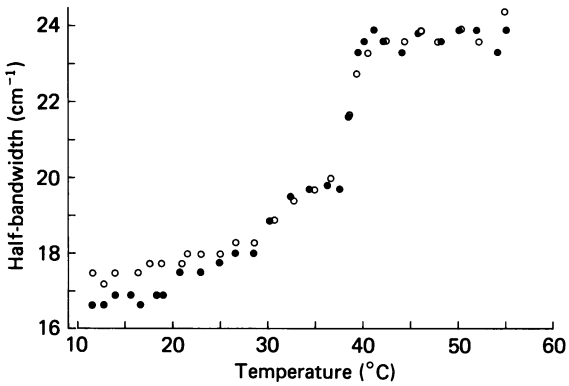

Fig. 3. Temperature-dependence of the half-bandwidth of the CH_2 antisymmetric C–H stretching vibration of aqueous dispersions of dipalmitoyl phosphatidylcholine (○) and phospholipid co-dispersed with 15 mol% (●) ubiquinone-10

acyl chains above or below the phase transition of the phospholipid.

Absorption bands at about 2956 and 2872 cm^{-1}, attributed to C–H antisymmetric and symmetric stretching vibration respectively of the terminal methyl groups of the hydrocarbon chains of the phospholipid, are also resolved in the spectra. The band at 2956 cm^{-1} is the more prominent of the two, but, as indicated from the difference spectrum (spectrum c), the intensity decreases when the dispersion is heated above the gel–liquid-crystalline phase-transition temperature. Similar difference spectra of dispersions of dipalmitoyl phosphatidylcholine or phospholipid containing 15 mol% (not shown) did not indicate that the presence of ubiquinone caused any perturbation of the hydrocarbon chains as judged by either C–H stretching of the chain methylene or terminal methyl groups. An additional broad band centred around 2900 cm^{-1} and attributed to a weak Fermi resonance interaction between the symmetric methylene stretching mode and the first overtone of the methylene scissoring mode can also be seen in the scan recorded at 54 °C. The behaviour of this absorbance also appears to be unperturbed by the presence of up to 15 mol% ubiquinone-10 in the dispersion.

To obtain more precise information about the effect of ubiquinone-10 on the pre-transition and the main lamellar gel–liquid-crystalline phase transition of the phospholipid the temperature-dependence of the frequency of the maximum absorbance of the CH_2 antisymmetric C–H stretching vibrations were determined (Fig. 2) and the half-bandwidths of these absorptions calculated (Fig. 3). The results are plotted for dipalmitoyl phosphatidylcholine and phospholipid co-dispersed with up to 15 mol% ubiquinone-10. There is a decrease in the temperature of the midpoint of the change in frequency of maximum absorbance associated with the main gel–liquid-crystalline phase transition due to the presence of ubiquinone-10 in the dispersion. The magnitude of this decrease is 1–2 °C and is the same within experimental error for ubiquinone-10 in proportions with phospholipid from between 2 and 15 mol%. No evidence for any differences is seen in the temperature range of the pre-transition of the phospholipid (34 °C). The inflexion in the spectrum seen in the pure lipid at temperatures about 20–25 °C, which correlated with a factor group splitting effect observed in the CH_2 rocking mode (Cameron *et al.*, 1980*b*), does, however, appear to be modified by the presence of ubiquinone-10 (Fig. 3).

Spectral features associated with polar groups

The carbonyl group of diacyl phospholipids gives rise to a strong C=O stretching mode located in the frequency region 1750 to 1710 cm^{-1}. The absorbance difference spectrum of an aqueous dispersion of dipalmitoyl phosphatidylcholine and phospholipid co-

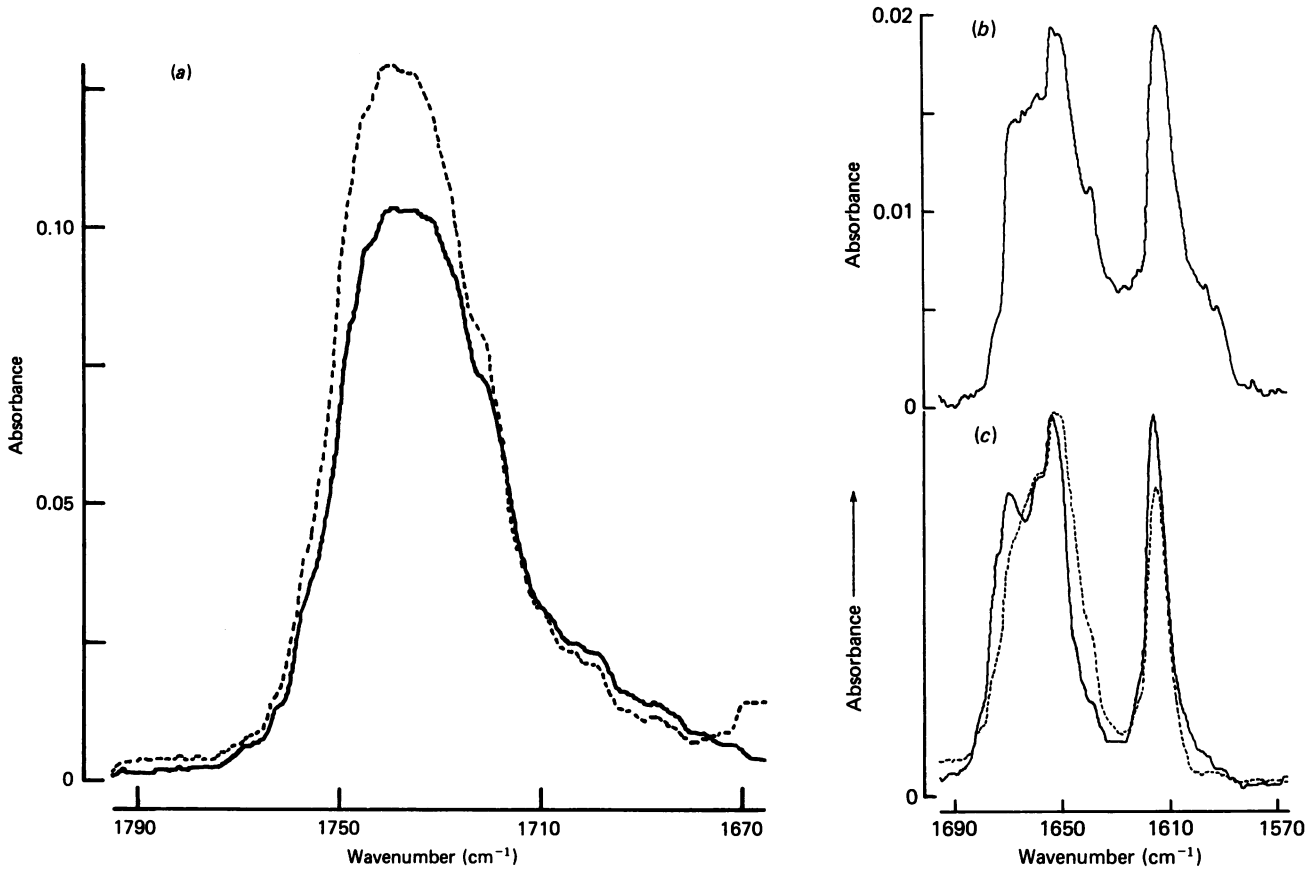

Fig. 4. I.r.-absorption difference spectra recorded at 20 °C in the C=O stretching region of fully hydrated dipalmitoyl phosphatidylcholine in the absence (——) and in the presence (– – – –) of 15 mol% ubiquinone-10 (*a*) and of ubiquinone-10 (*b* and *c*)

The spectrum shown in (*b*) was obtained by subtraction of absorbance spectrum of dispersed phospholipid shown in (*a*) from that of dispersed phospholipid containing 15 mol% ubiquinone-10. The spectra in (*c*) are difference spectra of ubiquinone-10 in dodecane (——) and chloroform (– – – –).

dispersed with 15 mol% ubiquinone-10 is shown in Fig. 4(*a*). No significant effect on these absorbances due to the presence of ubiquinone can be detected. Absorbances due to the carbonyl groups of ubiquinone are located at slightly lower frequencies and can be resolved from the spectrum by subtraction of the signal obtained from the phospholipid dispersed alone from those containing ubiquinone. A difference spectrum obtained in this way for a dispersion containing 15 mol% ubiquinone-10 is shown in Fig. 4(*b*). Two bands of approximately the same intensity are observed: one with a maximum at about 1650.3 cm^{-1} that is broad, asymmetric and multicomponent, and the other being a single sharp peak centred at about 1612.6 cm^{-1}. The broad peak is assigned to the C=O stretch vibration. No specific assignments have been made for individual components of this band, for which Fermi resonance effects are believed to be responsible (Bagli, 1961). The absorbances in this region are characterized by a splitting of the peak in the higher-intensity region into peaks at 1651.9 and 1649.5 cm^{-1}. The broad shoulder on the upfield side of the major peak contains absorbance maxima at 1657.6, 1663.4 and 1667.0 cm^{-1}.

Some indication of the environment of the benzoquinone substituent of the ubiquinone can be obtained by a comparison of this spectrum with difference spectra of ubiquinone in solvents of n-dodecane and chloroform. These are presented in Fig. 4(*c*). The spectrum in chloroform does not show significant changes in frequency of the high- and low-wavenumber components of the C=O band, but the relative intensities show some differences. Thus high-frequency shoulders at around 1667.0 and 1663.4 cm^{-1} decrease in intensity in chloroform solution and are hardly resolved at around 1667.5 cm^{-1}. The low-wavenumber band centred at 1650.3 cm^{-1} remains unaltered except for the splitting, which becomes less apparent as the maximum at 1651.9 cm^{-1} approaches intensities similar to the absorbance at 1649.5 cm^{-1}. The difference spectrum in dodecane is characterized by a significant shift in the centre of the low-frequency C=O band at 1650.3 cm^{-1} at 1652.1 cm^{-1}. The band is also sharper because the splitting is less pronounced. There is also a relative increase in intensity of the band at 1667.0 cm^{-1}. The overall effects of solvent environment on C=O stretching indicate that in a relatively polar solvent such as chloroform the absorbance bands tend to merge into a single absorbance at relatively low frequency, whereas in the apolar solvent dodecane two components are clearly resolved. It should be noted, however, that these features may not be entirely due to the influence of solvent environment, as we have observed spectral features in crystalline ubiquinone-10 mounted on a dry KBr disc that are similar to those seen in chloroform,

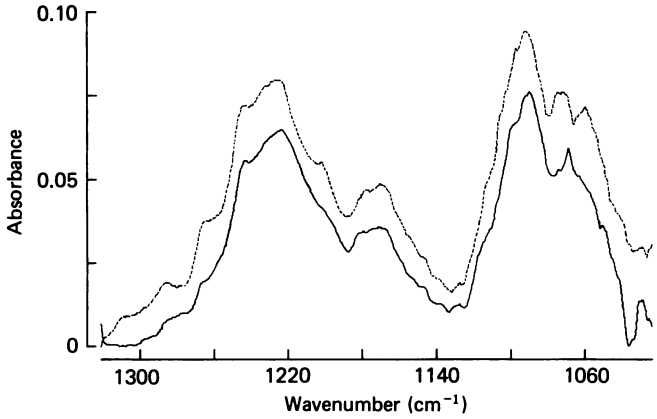

Fig. 5. I.r.-absorption spectra, recorded at 20 °C, of the ester group region of aqueous dispersions of dipalmitoyl phosphatidylcholine (——) and phospholipid co-dispersed with 15 mol% ubiquinone-10 (----)

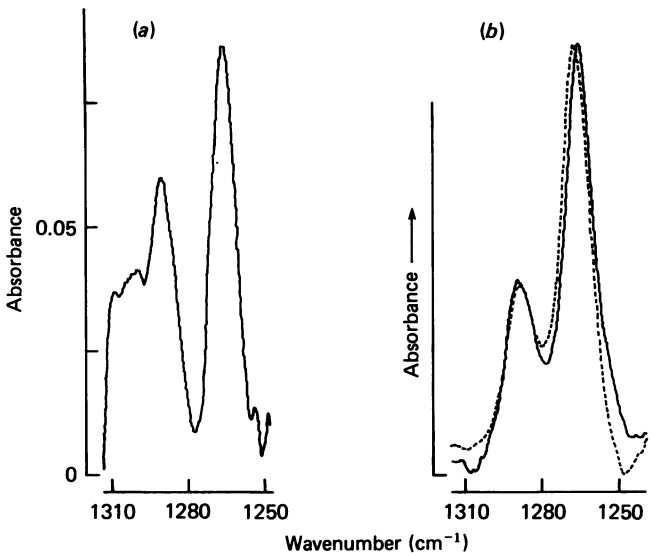

Fig. 6. I.r.-absorption difference spectra of ubiquinone-10 obtained by subtraction of spectra shown in Fig. 5 in the 1315 to 1240 cm^{-1} spectral region (*a*) and ubiquinone-10 in solvents (*b*) dodecane (——) and chloroform (----)

with an absorbance maximum about 1649.6 cm^{-1} and a high-wavelength shoulder centred at 1662.2 cm^{-1}.

Changes in the carbonyl-conjugated C=C stretch band with solvent environment do not follow the same pattern as that observed with the C=O stretch band. The frequency of maximum absorbance of this band in ubiquinone dispersed in phospholipid shifts to 1613.6 cm^{-1} when dissolved in chloroform with some decrease in intensity and to 1614.4 cm^{-1} in dodecane without loss in intensity. In crystalline ubiquinone this band is centred at 1610.6 cm^{-1}.

Vibrational modes of the phosphate group of the phospholipid are seen in the 1300 to 1050 cm^{-1} region of the i.r. spectrum. Strong absorption bands centred at 1088.9 and 1222.8 cm^{-1} are present in the difference spectrum of dispersed dipalmitoyl phosphatidylcholine illustrated in Fig. 5. These are assigned to symmetric and antisymmetric $-PO_2-$ stretching vibrations respectively of the phospholipid polar head group. Another band at about 1066.7 cm^{-1} is believed to be due to a R–O–P–O–R′ vibration (Arrondo *et al.*, 1984). The origin of the band at 1169.8 cm^{-1} is unknown. A difference spectrum of the phospholipid containing 15 mol% ubiquinone-10 is also shown in Fig. 5, and it can be seen that neither of the two $-PO_2-$ stretch vibrations is perturbed by the presence of ubiquinone-10. There are, however, some additional features in the spectrum, namely shoulders at around 1290 and 1265 cm^{-1} and peaks centred at 1072.7 and 1057.6 cm^{-1}.

Bands at frequencies around 1262 cm^{-1} have been variously ascribed to C–O stretching of the methoxy groups of the quinone ring or to –C–O– vibrations, both associated with the quinone function (Morton *et al.*, 1958; Linn *et al.*, 1959; 1960; Shunk *et al.*, 1960). Bands in the regions 1550–1750 cm^{-1} and 1200–1350 cm^{-1} of the spectrum of quinones, and which are distinct from carbonyl bands and of higher intensity than in the corresponding spectra of related hydrocarbons, are generally attributed to coupling of the carbonyl stretching with ring skeletal or other vibrational modes in the rest of the molecule (Hadzi & Sheppard, 1951). For this reason unequivocal assignments are not possible for these absorption bands in ubiquinone, and it has been suggested that the larger contribution to the band near 1260 cm^{-1} is a quinonoid absorption that masks the methoxy band (Pennock, 1965). Other bands in this region can, however, be assigned more precisely to the $-O-CH_3$ group vibrations at around 1202, 1151 and 1101 cm^{-1} (Isler *et al.*, 1960), but they are overlapped in this spectrum by the stronger phosphate and ester modes arising from the phospholipids. The origin of the peaks observed at 1072.7 and 1057.6 cm^{-1} is unknown, and no bands in this range have been assigned in the spectrum of ubiquinone-10, although their intensity is directly related to the proportion of ubiquinone in the mixture.

The higher-frequency bands in this region from ubiquinone can be seen in Fig. 6(*a*), which shows a difference spectrum in the 1315 to 1240 cm^{-1} region obtained by subtraction of the spectrum of a dispersion of phospholipid in water from that of a mixed dispersion containing 15 mol% ubiquinone-10. The precise frequencies of these bands are 1289.5 and 1264.7 cm^{-1}. Difference spectra in this frequency range are compared in solvents of differing polarity in Fig. 6(*b*). I.r. absorption bands of polar bonds are known to be influenced by changes in the character of their environment. This is particularly the case for the carbonyl and X–H stretching vibrations, and is typified by frequency shifts and bandwidth changes (Bellamy *et al.*, 1958; Bellamy & Williams, 1959). Both bulk dielectric constant of the medium (Kirkwood, 1937; Bauer & Magat, 1938) and dipolar solute–solvent interactions (Bellamy *et al.*, 1958) have been claimed to account for these solvent effects, and their relative contributions vary depending on the nature of the chemical group and the solvent (Caldow & Thompson, 1960; Caldow *et al.*, 1960). In all environments studied in this series of experiments the higher-wavenumber band is of greater intensity than the band at lower wavenumber. The band at 1289.5 cm^{-1} shifts to higher wavenumbers in the series: crystalline ubiquinone $\leqslant$ chloroform $\leqslant$ n-hexane $<$ n-dodecane $<$ phospholipid. The shift in the band at 1264.7 cm^{-1} to higher wavenumbers is greater in

extent but in the reverse order: crystalline ubiquinone ≤ phospholipid ≤ n-dodecane < n-hexane < chloroform. The most significant shifts are for n-dodecane (0.6 cm^{-1}) and phospholipid (1.4 cm^{-1}) and for n-hexane (1.2 cm^{-1}) and chloroform (1.9 cm^{-1}) in the first and second bands respectively. The average difference in wavenumbers separating these two absorption bands increases in the order chloroform < n-hexane < crystalline ubiquinone ≤ n-dodecane < phospholipid by a maximum of 2.5 wavenumbers.

DISCUSSION

The perturbation of the hydrocarbon chains of phospholipid bilayers by the presence of extraneous molecules can be detected with some precision by i.r difference spectroscopy. The C–H stretching bands of the phospholipid, for example, are sensitive to two different types of conformation within the hydrocarbon chains. Thus the C–H stretching frequency indicates the extent of static order within the lipid chains and the half-maximum bandwidth provides information about chain librational and torsional motion (Cameron *et al.*, 1980*b*). It has been shown that the presence of channel-forming peptides such as gramicidin A and alamethicin, cholesterol and intrinsic membrane proteins such as bacteriorhodopsin and Ca^{2+}-dependent ATPase caused an increase in both the proportion of *gauche* conformers and lipid chain motion, as judged by an increase in the frequency of absorbance maxima and band broadening respectively when interpolated into saturated phosphatidylcholines below the gel–liquid-crystalline phase-transition temperature (Cortijo *et al.*, 1982; Lee *et al.*, 1984). In the fluid phase, however, the effects of intrinsic proteins and peptides are much less marked, although cholesterol tends to restrict librational motion and reduce the number of *gauche* conformers in the hydrocarbon chains of phospholipids at temperatures above the P_β–L_α phase transition. In all cases where the gel phase is perturbed the sharp transition observed in the pure lipid is smeared out and the changes in spectral parameters take place over a relatively wide temperature range. The presence of ubiquinone-10 in dispersions of saturated phosphatidylcholine does not significantly perturb the fatty acyl chains either above or below the gel–liquid-crystalline phase transition, which is consistent with fluorescence-polarization measurements obtained with anthroyloxy fatty acid probes of molecular motion in this region of the structure (Katsikas & Quinn, 1983). The presence of ubiquinone, however, does lower the temperature of the gel–liquid-crystalline phase-transition temperature by 1–2 °C without markedly widening the temperature range of the transition. Moreover, spectral changes observed in the gel phase (Fig. 2) do appear to be slightly modified by the presence of ubiquinone-10, as does the L_β–P_β transition temperature as judged from differential scanning calorimetric studies (Katsikas & Quinn, 1982*b*).

All this evidence provides further support for the models in which the long-chain homologues of ubiquinone are located in a separate phase within the dispersion and do not intercalate between individual phospholipid molecules in a bilayer structure. Failure also to demonstrate significant perturbation of the spectral region reporting on the terminal methyl residues of the fatty acyl chains due to the presence of ubiquinone may also argue against the sandwich model of ubiquinone disposition. This observation is also in agreement with fluorescence quenching of probes located in the terminal methyl region of the bilayer (Katsikas & Quinn, 1983).

Vibrational modes associated with the carbonyl groups of the benzoquinone ring substituent indicate that the environment provided for ubiquinone in phospholipid/water systems is intermediate between that observed in chloroform and that in dodecane. Studies of the chemical shift of $-OCH_3$ proton resonances by n.m.r. spectroscopy supports the location of the benzoquinone moieties in close proximity to one another in phospholipid dispersions (Quinn & Katsikas, 1985). Together both types of data favour a micellar or some sort of aggregated form of ubiquinone, but the precise size or location of these aggregates within the dispersion, i.e. whether they span the phospholipid bilayers or exist predominantly in the aqueous phase, is presently unknown.

Two factors tend to favour location of the ubiquinone in a hydrocarbon domain. The first is the effect of solvent on C=O stretch and C=C absorbance associated with the benzoquinone ring in which the spectral features of these groups indicate a largely non-polar environment. Secondly, if the ubiquinone were located in the aqueous phase of the system it may be expected that the C=O stretching mode and vibrational modes of the phosphate group of the phospholipid would be modified by the presence of ubiquinone. No evidence of significant modification of these signals was obtained.

The overall conclusion from this study is that ubiquinone-10 when co-dispersed with dipalmitoyl phosphatidylcholine in water phase separates from the phospholipid. There is no evidence that ubiquinone intercalates individually between phospholipid molecules, which undergo a gel–liquid-crystalline phase transition that is only slightly modified by the presence of ubiquinone. There is an indication that at least the benzoquinone substituent is located in a hydrophobic environment, and aggregates or micelles spanning the bilayer are a plausible model for the arrangement of ubiquinone within the phospholipid structure.

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