THE INTERACTION OF COENZYME Q WITH DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS

H. KATSIKAS and P. J. QUINN
Department of Biochemistry, Chelsea College, University of London, London, SW3 6LX, England

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1. Introduction

The operation of coenzyme Q as a mobile electron carrier between complex I and II and complex III of the mitochondrial respiratory chain [1–3], coupled with the translocation of protons out of the matrix has been suggested [4]. There have, however, been few experiments undertaken to examine the way coenzyme Q interacts with the energy transducing membrane. Some model membrane studies have been reported including the behaviour of mixed monomolecular films with phospholipids [5–7]. On the basis of these studies it was concluded that coenzyme Q_{10} interacted with the hydrocarbon chains at surface pressures $\lesssim$ 12 mN/m but became progressively squeezed out of the film at higher pressures to form a separate phase overlying a monolayer of pure phospholipid. Studies of surface pressure area isotherms of phospholipid monolayers containing coenzyme Q_4 indicated that the shorter isoprenoid chain length alters the balance of hydrophobic to polar affinities in the molecule such that it is interpolated into the film at higher surface pressures [7]. A model for the organisation of coenzyme Q_{10} in bilayers of phosphatidylcholine has been proposed [8] in which coenzyme Q_{10} is sandwiched between the two lamellae of a phospholipid bilayer.

Functional studies of coenzyme Q in phospholipid bilayer membranes [9] had earlier suggested that coenzyme Q is aggregated in the bilayer. Proton NMR studies of coenzyme Q dispersed in predeuterated dimyristoylphosphatidylcholine bilayers have, on the basis of a splitting of the resonances attributed to the $-OCH_3$ benzoquinone ring substituents, also indicated that coenzyme Q is aggregated in the bilayer [10]. Spin-label studies, on the other hand, using stearate probes with a nitroxyl group attached to either the $\omega 3$ or $\omega 14$ carbon indicated a perturbation of the phospholipid hydrocarbon in the presence of either oxidised or reduced coenzyme Q_3 or Q_9 which was interpreted in terms of an intercalation of the coenzymes between the phospholipid molecules [11].

A calorimetric study has been undertaken with mixed dispersions of coenzyme Q_{10} and coenzyme Q_3 with dipalmitoylphosphatidylcholine in water to examine the intermolecular interactions between coenzyme Q and phospholipid bilayers. The effect of varying proportions of the two components was also investigated.

2. Experimental

Pure lipids and mixtures of coenzyme Q and dipalmitoylphosphatidylcholine were lyophilized from a solvent of benzene: methanol (9:1, v/v). The dry material was transferred to capillary tubes containing a small constriction and water added in an amount equal to 10-fold the amount of phospholipid (or coenzyme Q_{10} when the phospholipid was omitted) in the preparation. The tubes were sealed under vacuum and dispersed by sonication and centrifugation through the constriction until a homogeneous dispersion was obtained. Gel filtration of dispersions of coenzyme Q and phospholipids prepared in a similar way has shown that the two components are voided from the column in the same volume and this has been used as evidence for homogeneity of the mixture [10,12]. Aliquots of the dispersion (~5 μl) were sealed in small aluminium pans for thermal analysis in a Perkin Elmer DSC-2 differential scanning calorimeter.

To establish the molar ratio of constituents in the

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pan and to calculate heats of transition, the lipids were removed from the pans and analysed. The pans were opened and the contents dissolved in 2 ml ethanol. Aliquots were taken for phosphate determination; after digestion in perchloric acid the phosphate concentration was measured as in [13]. Other aliquots were diluted in absolute ethanol and the concentration of ubiquinone determined from E_{275} using a molar extraction coefficient of 1.45×10^4 which was determined separately. The value is in close agreement with published values of 1.40×10^4 [14]. To separate the heats of the main transition of phospholipid from coenzyme Q_{10} mixtures the total enthalpy of the mixture was obtained from preparations heated from 260 K and that of the phospholipid alone was obtained by heating the preparation from 280 K; in the latter case the coenzyme Q_{10} remained in the melted form and only the thermotropic transitions of the phospholipid was observed. Similar results were obtained from measurements of enthalpy from the exotherms observed in samples cooled from 320 K; again separate exotherms of the phospholipid and the coenzyme Q_{10} were obtained. No thermal transitions in pure coenzyme Q_3 were observed over 250–330 K.

Dipalmitoylphosphatidylcholine was purchased from Sigma and coenzyme Q_{10} was a gift from Eisai Co. (Japan). Both lipids were used without further purification.

3. Results

The thermal phase transition behaviour of binary mixtures of dipalmitoylphosphatidylcholine and coenzyme Q dispersed in excess water has been examined to establish the extent of intermolecular interactions between the two lipids. Typical thermograms of two mixtures of dipalmitoylphosphatidylcholine and coenzyme Q_{10} heated from 270–320 K are shown in fig.1 together with aqueous suspensions of dipalmitoylphosphatidylcholine and coenzyme Q_{10} separately. The temperature and heats of transition of the pure compounds and the two binary mixtures are summarised in table 1.

The most obvious feature observed in the thermograms is that the presence of coenzyme Q_{10} has very little effect on the thermal properties of the phospholipid. Thus the temperature of both the pre-transition and the main transition of dipalmitoylphosphatidylcholine are not greatly affected by the presence of up to 50 mol% coenzyme Q_{10}. Higher proportions of coenzyme Q_{10} tended to broaden the pre-transition endotherms but had no significant effect on the temperature of the main endotherm. Cooling thermograms (not shown) also indicate that the crystallization temperatures of the phospholipid is unaffected by the presence of coenzyme Q_{10}. Failure of coenzyme Q_{10} to affect the thermotropic properties of the phospholipid can also be seen from the heats of transition.

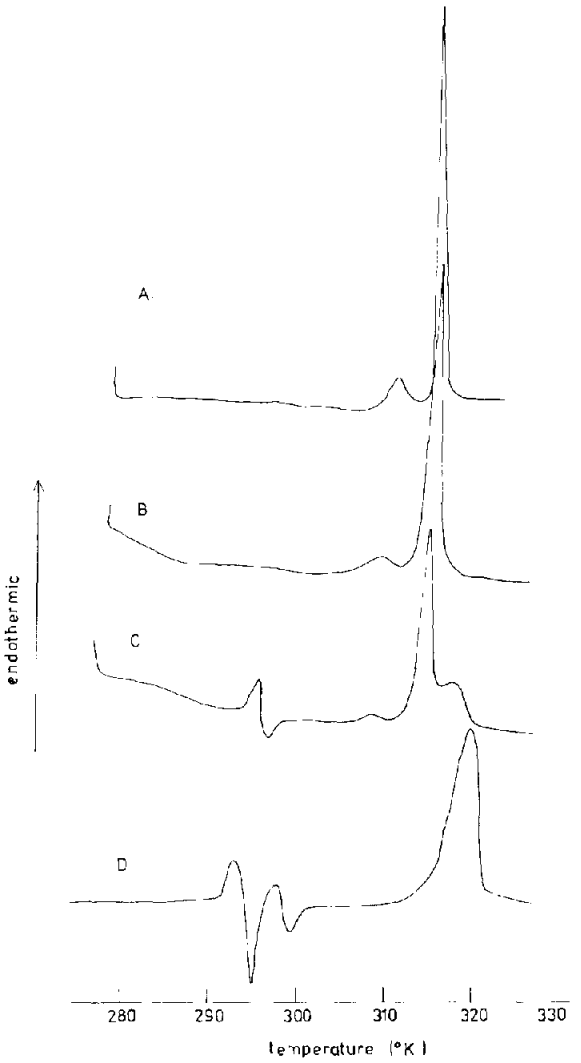

Fig.1. Differential scanning calorimetric heating curves of aqueous dispersions of: (A) dipalmitoylphosphatidylcholine; (B) coenzyme Q_{10}:dipalmitoylphosphatidylcholine, 1:4 mol ratio; (C) coenzyme Q_{10}:dipalmitoylphosphatidylcholine 1:2.2 mol ratio; (D) coenzyme Q_{10}. Scans were obtained at a heating rate of 5 K/min.

Table 1
Phase transition temperatures of binary mixtures of dipalmitoylphosphatidylcholine and coenzyme Q_{10} and heats of transition

Mole ratio Q/DPL	DPL pre-transition temp. (K)	Q_{10} first transition temp. (K)	DPL main transition temp. (K)	Q_{10} main (melting) transition temp. (K)	Heat of DPL main transition (kJ/mol)	Heat of Q_{10} main transition (kJ/mol)
0:1	307.0	–	312.7	–	33.4	–
1:4	304	–	312.4	–	31.8	–
1:2.2	304.6	292.3	311.5	313.5	32.2	38.9
1:0	–	291.7	–	315.6	–	85.7

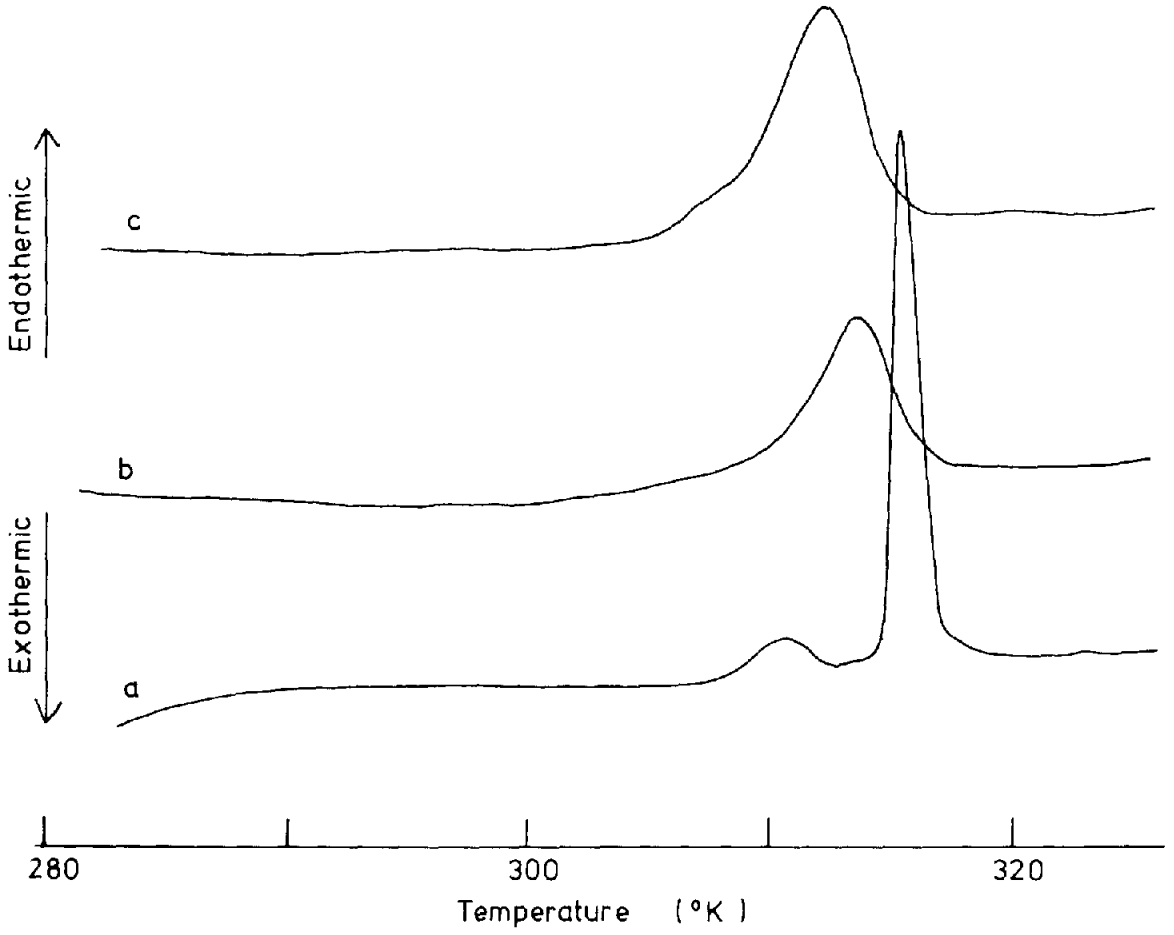

Fig.2. Differential scanning calorimetric heating curves of aqueous dispersions of: (a) dipalmitoylphosphatidylcholine; (b) coenzyme Q_3:dipalmitoylphosphatidylcholine; 1:5 mole ratio; (c) coenzyme Q_3:dipalmitoylphosphatidylcholine, 1:2.2 mol ratio. Scans were obtained at a heating rate of 5 K/min.

The enthalpy change associated with the endotherm indicates that >90% of the phospholipid molecules participate in the cooperative gel to liquid–crystalline phase transition. The enthalpy associated with the exothermic transition from liquid–crystalline to gel conformation is consistent with the observation.

It could be argued on the basis of the effects of coenzyme Q_{10} that either no coenzyme is incorporated into dipalmitoylphosphatidylcholine bilayers or that the presence of coenzyme Q_{10} in the bilayer does not perturb the thermal properties of the phospholipid. Evidence for the latter comes from the marked effect of the phospholipid on the thermal properties of coenzyme Q_{10}. Although, the temperature of onset of the first low-temperature phase transitions and main endothermic transition temperature of coenzyme Q_{10} is not significantly affected when present at ~50 mol% there is a marked decrease in molar enthalpy (table 1). Because the shape of this endotherm is not significantly different from pure coenzyme Q_{10} in water the results suggest that slightly >50% of the coenzyme has been completely removed from the cooperative phase transition whereas the remainder is indistinguishable from coenzyme Q_{10} that melts normally. Studies of the thermal properties of coenzyme Q_{10} dispersed in water or dissolved in organic solvent showed that the coenzyme only exhibited characteristic thermal behaviour when in an aggregated form.

To examine the effect of the isoprenoid chain length of coenzyme Q on the phase transition behaviour of dipalmitoylphosphatidylcholine thermograms of the phospholipid codispersed with coenzyme Q_3 were obtained. Differential scanning calorimetric heating curves of mixtures in molar proportions up to 1:2.2 coenzyme Q_3:dipalmitoylphosphatidylcholine is shown in fig.2. In contrast to the coenzyme Q_{10} mixtures there is a marked effect on the transition temperature of the phospholipid and the temperature range over which the transition takes place. Measurements of the phase transition enthalpy, however, showed that >90% of the phospholipid undergoes a gel–liquid crystalline in the endotherm.

4. Discussion

Since almost all of the phospholipid in the dispersions containing coenzyme Q undergoes a cooperative gel–liquid crystalline phase transition with a transition enthalpy of 33 kJ/mol it may be concluded that the phospholipid is organised into a characteristic multibilayer structure. The failure of coenzyme Q_{10} to show detectable thermal transitions even when present in proportions of up to 20 mol% (fig.1B) suggests that the coenzyme is molecularly dispersed as a separate phase sandwiched between the leaflets of the bilayer. When mixed in a mole ratio of ~50 mol%, somewhat >20 mol% of the coenzyme Q_{10} may be present in this form as judged from the amount of coenzyme that does not undergo a normal melting process. A schematic diagram of the possible arrangement of the dispersed coenzyme Q_{10} in bilayers of dipalmitoylphosphatidylcholine is shown in fig.3. This model is consistent with our earlier interpretations of mixed monomolecular films of coenzyme Q_{10} and dipalmitoylphosphatidylcholine [7–8]. The arrangement of coenzyme Q_{10} as an aggregate in the bilayer as proposed in [15], at least at low mole ratios of coenzyme Q_{10}, would seem to be excluded on the grounds that it should undergo a cooperative phase transition and perturb the phase transition behaviour of phospholipids in contact with these aggregates.

It is not possible, from these experiments, to establish whether the excess coenzyme Q_{10} in mixtures with phospholipid that exhibit a normal phase

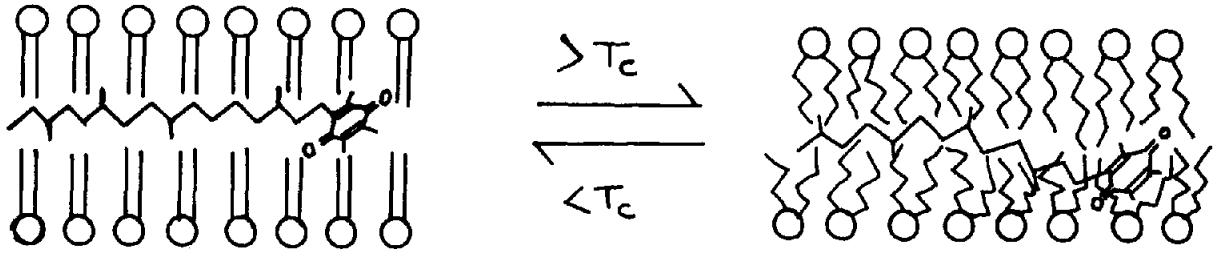

Fig.3. Schematic diagram of the disposition of coenzyme Q_{10} in dipalmitoylphosphatidylcholine bilayers above and below the phase transition temperature.

transition is in the aqueous phase or in a separate aggregated phase in the bilayer. Gel filtration studies of codispersions of coenzyme Q with phospholipids have indicated that the components are homogeneously dispersed [10,12]. Calorimetric evidence favouring its inclusion in the bilayer is the pronounced modification of the thermal events that take place between 290–300 K in the presence of the phospholipid. The state of aggregation of coenzyme Q_3 in the bilayer is unknown because no thermal events take place in the coenzyme in the temperature range studied. Because most of the phospholipid undergoes the gel–liquid crystalline phase transition, however, it is likely that coenzyme Q_3, like its Q_{10} analogue, is sandwiched between gel phase monolayers of the phospholipid at temperatures below the phase transition temperature.

The existence of lipids of low polarity sandwiched between the leaflets of a polar lipid bilayer has already been suggested from capacitance measurements of black lipid films containing alkanes [16,17] and neutron diffraction studies of deuterated hexane in bilayers of dioleoylphosphatidylcholine [18]. The phase separation of coenzyme Q into the centre of dipalmitoylphosphatidylcholine bilayers in the gel phase implies that the movement of electrons and protons can only take place across the lipid bilayer when it is in the liquid–crystalline phase.

Acknowledgements

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