Biochimica et Biophysica Acta, 689 (1982) 363–369
Elsevier Biomedical Press

BBA 71279

THE POLYISOPRENOID CHAIN LENGTH INFLUENCES THE INTERACTION OF UBIQUINONES WITH PHOSPHOLIPID BILAYERS

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(Received January 29th, 1982)

Key words: Ubiquinone; Phospholipid; Phase transition; Coenzyme Q; Membranes

The interaction between ubiquinone homologues with polyisoprenoid chain lengths varying from 3 to 10 units and dipalmitoylphosphatidylcholine bilayers has been examined by differential scanning calorimetry and wide angle X-ray diffraction analysis. Decreasing the polyisoprenoid chain lengths of ubiquinone in mixed dispersions with phospholipid in mol ratios of about 10 mol% caused a decrease in the gel-liquid crystalline phase transition temperature of the phospholipid and a broadening of the transition. Enthalpy measurements showed that most of the phospholipid (>92%) was involved in the transition endotherm and the formation of a gel phase was also confirmed by the presence of a sharp X-ray reflection at 0.42 nm. These results are consistent with a model in which all of the ubiquinone homologous ultimately undergo a phase separation from phospholipid molecules entering a gel phase on cooling below the phase transition temperature. Reducing the length of the polyisoprenoid chain alters the amphipathic balance of the ubiquinone molecules and is reflected in the tendency of shorter chain ubiquinones to intercalate between the phospholipid molecules upon reheating through the main phase transition.

Introduction

Ubiquinone and its counterpart in chloroplast systems, plastoquinone are lipophilic redox components of electron transport chains. They are responsible for the translocation of hydrogen atoms from one side of the energy-transducing membrane to the other [1–3]. The precise mechanism by which this process is accomplished is unknown. Two mechanisms, however, have been proposed to account for the transfer of electrons and protons through the hydrocarbon domain of the membrane. One envisages that the quinone-quinol ring system flips from the lipid-water interface on one side of the lipid bilayer to the other [4,5]; the long polyisoprenoid substituent is believed to be largely oriented in the centre of the bilayer confining the coenzyme to the hydrophobic domain but permitting sufficient flexibility to allow the benzoquinone groups to penetrate to the aqueous interfaces. Variations of this model in which motion of the whole molecule is involved in hydrogen translocations at the membrane surfaces have been proposed [6,7]. An alternative mechanism that has been considered is one in which the transfer of hydrogen atoms takes place through molecular clusters of coenzyme large enough and oriented in such a way as to be accessible to the aqueous compartment at either surface of the membrane; protons and electrons in this system are translocated by a hopping process from one benzoquinone-ring substituent to another [8,9]. A third model must also be considered in the light of more recent evidence on the distribution of redox sites in the energy-transducing membrane. In this case hydrogen atoms would be taken up by the benzoquinone substituent penetrating to the membrane surface at the site of coenzyme reduction and then

transferred laterally through a quasi-crystalline domain in the centre of the bilayer where they are delivered to the site of oxidation on the opposite side of the membrane remote from where they entered the coenzyme pool. This model would be favoured for plastoquinone of the chloroplast membrane where there is strong evidence supporting the lateral segregation of the coenzyme redox centres in the plane of the membrane [10].

In all these models it is likely that the polyisoprenoid substituent plays a considerable part in providing the coenzymes with the properties required to fulfil their particular functional role. The participation of the isoprenoid chain in hydrogen atom transfer reactions, however, appears to be excluded by the fact that the saturated derivative has the same transfer rate constant and activation energy as its natural progenitor. To help establish the functional role of the polyisoprenoid substituent we have examined the interaction of ubiquinone homologous of differing polyisoprenoid chain length with pure phospholipids in bilayer configuration using differential scanning calorimetry. Detailed studies have been undertaken to compare ubiquinone-3 with ubiquinone-10 to provide data relating to short and long isoprenoid chains, respectively.

Materials and Methods

The lipid mixtures were prepared by lyophilization from a solvent of benzene/methanol (9:1, by vol.) and transferred to capillary tubes containing a small constriction. 10-fold excess by weight of water was added to the tubes which were sealed under vacuum and the lipids dispersed by sonication and centrifugation through the constriction until an apparently homogeneous dispersion was obtained. Aliquots of dispersed lipids (about 5 μl) were sealed in small aluminium pans for calorimetric studies. Heating and cooling thermograms in the temperature range 250–325 K were obtained with a reference pan containing water and a scan rate of 5 K/min. Calorimetry was performed in a Perkin Elmer DSC-2 instrument.

The molar ratio of the constituents in the calorimeter pans was determined by extracting the lipids with solvent and assaying the contents. The pans were opened and the lipids dissolved in 2 ml ethanol. In some samples aliquots were taken for phosphate determination using the method of Eibl and Lands [11]. In other cases the phospholipid content was determined gravimetrically; both methods used gave almost identical results. Other aliquots were diluted in absolute ethanol and the concentrations of ubiquinone-10 and -3 were determined from A_{275} using a molar extinction coefficient of $1.45 \cdot 10^4$ which was determined separately. This value is close to the published values of $1.40 \cdot 10^4$ for ubiquinones [12]. The perturbation of the phospholipid transition was assessed by determining the slope of the peak obtained in a high-speed recording (chart speed 16 cm/min) of the thermograms. The values obtained are referred to as the melting profile index of the phospholipid transition. Dipalmitoyl- and dimyristoylphosphatidylcholines were purchased from Sigma, London. The homologous series of ubiquinones were a gift from Eisai Co. Ltd., Japan. All lipids were used without further purification.

Results

Studies of the effect of the isoprenoid chain length of ubiquinones on the thermotropic behaviour of aqueous dispersions of dipalmitoylphosphatidylcholine were undertaken by differential scanning calorimetry. Mixed dispersions of ubiquinones-3 to -10 in a ratio of approx. 0.1 mol per mol of phospholipid were prepared and heating scans were used to derive values for the melting profile index and phase transition temperatures. The results are presented in Fig. 1 and show that as the polyisoprenoid chain becomes shortened the gel-liquid crystalline phase transition temperature of the phospholipid decreases; the decrease is particularly pronounced comparing ubiquinones-9 and -10. The same pattern was also observed with the temperature of the pretransition of the phospholipid dispersed with ubiquinones-5 to -10; no pretransitions were observed in mixed dispersions of phospholipid with ubiquinones possessing polyisoprenoid chains shorter than 5 units. A plot of the melting profile index (Fig. 1) indicates that with decreasing isoprenoid chains from 9 to 3 units the ubiquinones cause an increase in the range of temperature over which the main gel-liquid crystalline phase transi-

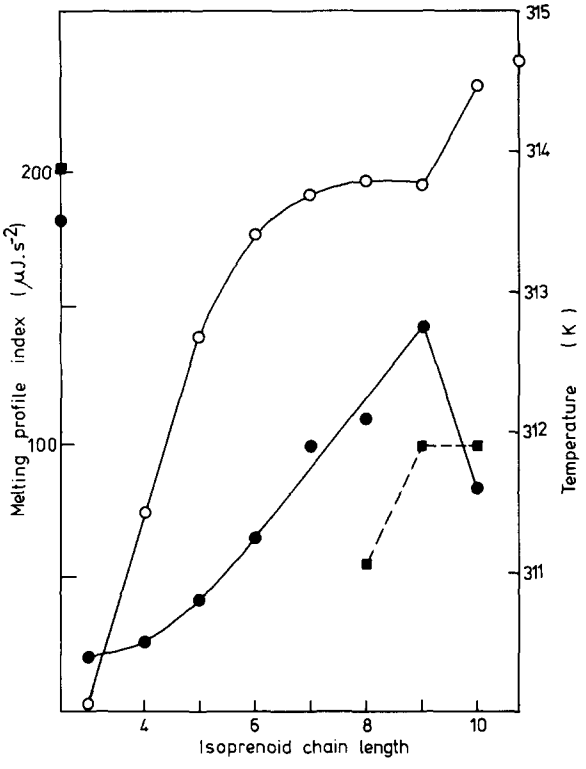

Fig. 1. The effect of ubiquinones of different isoprenoid chain length on the thermotropic properties of dipalmitoylphosphatidylcholine in aqueous dispersion. Ubiquinones were present in a proportion of about 10 mol per 100 mol of phospholipid and the temperature of the gel-liquid crystalline phase transition (○———○) and melting profile index (●———●) determined from heating thermograms of the mixed dispersions. The scans were done at a heating rate of 5 K/min at a sensitivity of 0.5 mcal·s^{-1}. The melting profile index for mixed dispersions of ubiquinones-8 to -10 with dimyristoylphosphatidylcholine is also shown (■— — —■). Symbols on the ordinates refer to pure phospholipids.

tion takes place. Like the effect on the phase transition temperature ubiquinone-10 appears to cause an effect which is different from the pattern observed in the remainder of the ubiquinone homologues. We checked to see if this difference was due to the fact that ubiquinones-3 to -9 are liquid while ubiquinone-10 is crystalline at the phase transition temperature of dipalmitoylphosphatidylcholine by comparing the effect of ubiquinone-9 and -10 on the melting behaviour of dimyristoylphosphatidylcholine where both ubiquinones are in a crystalline state at the temperature of the main phase transition. The pattern observed for the melting profile index of dimyristoylphosphatidylcholine codispersed with ubiquinones-8 to -10 was different from that observed for dipalmitoylphosphatidylcholine (Fig. 1). Enthalpy measurements obtained from the heating endotherms showed that on average more than 95% of the phospholipid was undergoing a gel-liquid crystalline phase transition when codispersed with about 10 mol% ubiquinone irrespective of the length of the isoprenoid chain. Enthalpy measurements of the main transition of dimyristoylphosphatidylcholine, on the other hand, showed that in mixtures containing about 10 mol% ubiquinone-10 considerably less phospholipid (about 85%) was undergoing a thermal transition.

The effect of varying proportions of ubiquinone-3 on the thermotropic behaviour of di-

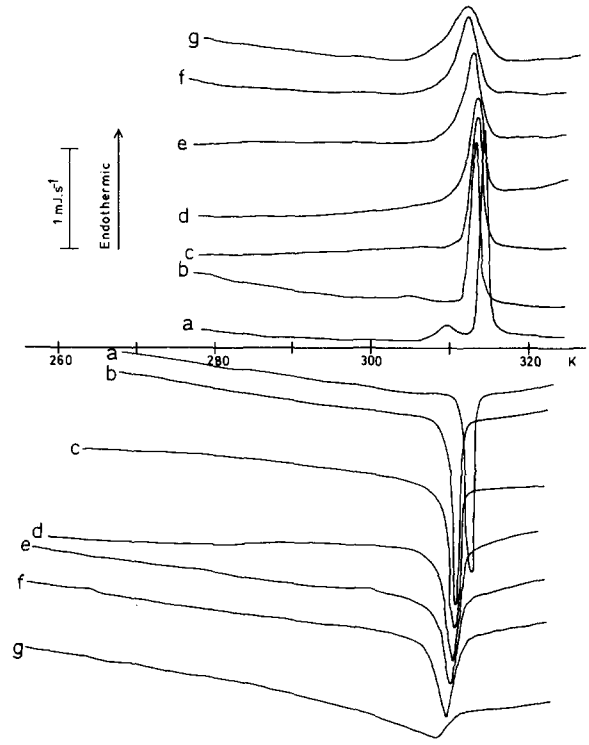

Fig. 2. Differential scanning calorimetric heating (upper) and cooling (lower) thermograms of ubiquinone-3 codispersed with dipalmitoylphosphatidylcholine in excess water. Ubiquinone-3: phospholipid ratios were: (a) 0 : 1, (b) 0.0085 : 1, (c) 0.017 : 1, (d) 0.022 : 1, (e) 0.03 : 1, (f) 0.045 : 1, (g) 0.74 : 1. The calorimeter was operated at a scan of 5 K/min and a sensitivity of 1 mcal·s^{-1}.

palmitoylphosphatidylcholine dispersions is shown in Fig. 2. This shows that the main gel-liquid crystalline phase transition becomes progressively broader and the transition temperature lowered as the proportion of ubiquinone-3 in the mixture is increased; the most pronounced effects were observed at lower mole ratios of ubiquinone-3 (< 10 mol%). Measurements of the enthalpies of the main transition showed these to be largely unaffected by the presence of ubiquinone-3 and a mean value of 31.14 ± 0.67 kJ · mol^{-1} ($n = 20$) was recorded for all of the thermograms. It can also be seen that the pretransition endotherm is markedly affected by the presence of ubiquinone-3. Thus the pretransition endotherm is considerably reduced by the presence of 0.0085 mol of ubiquinone-3 per mol of phospholipid and it is abolished by increasing the mole ratio to 0.017:1. Traces of the exotherms of these mixtures (lower part of Fig. 2) show that the crystallization of the phospholipid on cooling is perturbed to a greater extent than indicated from the profiles of the melting endotherms especially as the proportion of ubiquinone-3 is increased to greater than mol ratios of 0.15:1.

Changes in the melting profile index and phase transition temperature of dipalmitoylphosphatidylcholine in mixed dispersions with varying proportions of ubiquinone-3 and ubiquinone-10 are illustrated in Figs. 3a and 3b, respectively. The range of temperatures over which the phospholipid undergoes a gel-liquid crystalline phase transition, as reflected in the melting profile index, is increased much more by ubiquinone-3 than it is by ubiquinone-10. This effect is maximised by both ubiquinones in mol ratios of less than about 0.1:1 and no further broadening is observed in the presence of higher proportions of ubiquinone. The effect of ubiquinone-3 on the main transition temperature follows a similar trend to that of the melting profile index causing a maximum decrease of about 7 K at a mol ratio of 0.3:1. The decrease in the main transition temperature caused by ubiquinone-10, although significant, is less than 1 K, but the decrease observed in the pretransition temperature is of the order of 2 K. Additional studies of the effect of ubiquinone-10 on the thermal behaviour of dimyristoylphosphatidylcholine were also undertaken (results not shown). The changes in melting profile index were about twice that for dipalmitoylphosphatidylcholine with equivalent proportions of ubiquinone-10 but the effect on the temperature of the main transition endotherm could not be determined precisely because the metastable phase transition of the ubiquinone takes place in the same temperature range as that of the main phospholipid transition.

Because the thermal transitions of dipalmitoylphosphatidylcholine were perturbed by the pres-

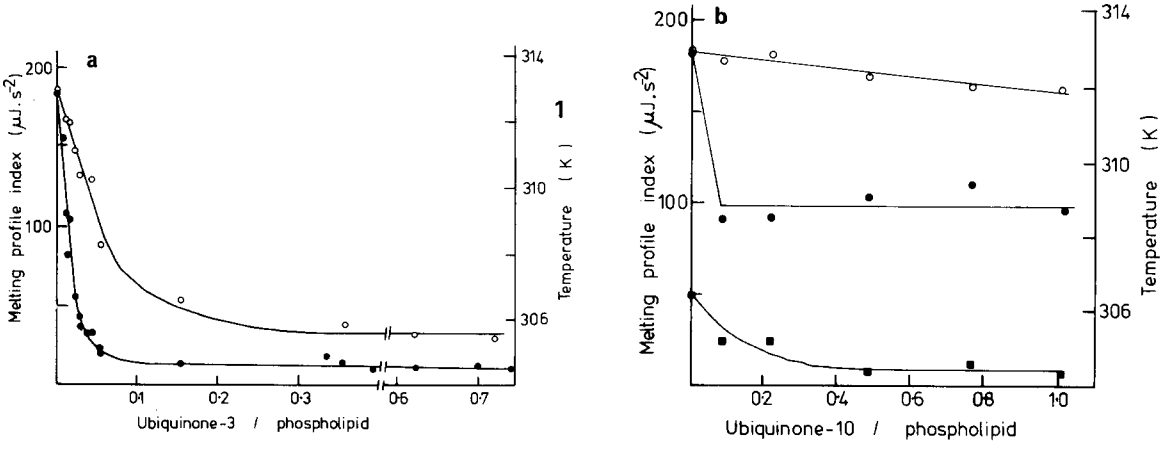

Fig. 3. The effect of differing proportions of ubiquinone-3 (a) and ubiquinone-10 (b) on the melting profile index of dipalmitoylphosphatidylcholine dispersions (●———●), gel-liquid crystalline phase transition temperature (○———○) and the pretransition temperature (■———■). The measurements were made from heating thermograms obtained at a scan rate of 5 K/min and a sensitivity of 1 mcal·s^{-1}.

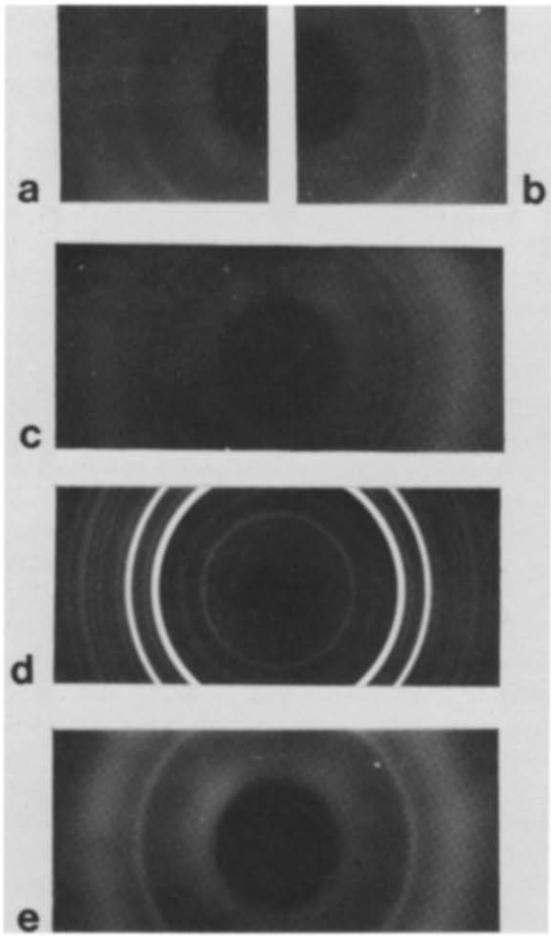

Fig. 4. X-ray diffraction patterns of ubiquinone: dipalmitoylphosphatidylcholine in mixed aqueous dispersions observed at 22°C. Ubiquinone-10: phospholipid ratios of (a) 0.25:1, (b) 0:1, (c) 0.5:1, (d) 1:0. (e) Ubiquinone-3: dipalmitoylphosphatidylcholine ratio of 0.7:1.

ence of ubiquinones in the bilayer we examined the dispersions by wide angle X-ray diffraction to determine the degree of order of the hydrocarbon chains. Fig. 4 shows X-ray diffraction patterns obtained from mixed dispersions of the phospholipid with varying proportions of ubiquinones. The diffraction pattern obtained from a dispersion containing a mol ratio of ubiquinone-10 to phospholipid of 0.25:1 (Fig. 4a) is indistinguishable from diffraction patterns obtained from the pure phospholipid dispersion (Fig. 4b). The single sharp reflection at 0.42 nm is generally assigned to hexagonally-packed hydrocarbon chains of the phospholipid. When the mol ratio is increased to 0.5:1 two additional reflections were observed at 0.38 and 0.47 nm (Fig. 4c). These two additional diffraction bands originate from crystalline ubiquinone-10 in the dispersion as can be seen from Fig. 4d which is a diffraction pattern from the pure ubiquinone-10. Ubiquinone-3 does not crystallize at 22°C, and as can be seen in Fig. 4e only reflections from the hydrocarbon chains of the phospholipid in gel configuration are seen in mixed dispersions with the shorter chain ubiquinone.

Discussion

Studies of the respiratory activity of mitochondria depleted of endogenous ubiquinone have shown that succinate oxidation can be completely restored by exogenous ubiquinones with isoprenoid chain lengths ranging from 2 to 10 [13] but NADH oxidase activity can only be fully recovered by adding long-chain homologues [14]. This, together with estimates of the rate of electron and proton transport across lipid bilayer membranes [9] and transbilayer motion of different ubiquinone and ubiquinol homologues [15], suggests that the long isoprenoid chain is required for the efficient transmembrane movement of hydrogen atoms.

The observed functional dicotomy implies that the ubiquinones may intercalate into membranes in a manner dependent on the length of the polyisoprenoid chain. For this reason there has been more considerable interest in the location and physical state of ubiquinones in lipid bilayers and biomembrane structures. The results of the present study are consistent with the notion that the amphipathic balance is the primary factor governing interaction of ubiquinone homologues with phospholipid bilayers and that, in the case of ubiquinone-10, additional factors including the melting point and the tendency to form aggregates are also likely to be involved in interaction with phospholipids. It is of interest that the hydrophilic affinity even of ubiquinone-3, the shortest chain homologue used in the present study, was not sufficient to prevent a phase separation of the phospholipids on cooling from the liquid-crystal-

line to the gel state. An almost complete phase separation can be deduced from enthalpy measurements of the main transition endotherm which indicates that all of the phospholipid exists in a gel state at low temperatures and no diffuse wide-angle X-ray reflection at 0.46 nm characteristic of disordered hydrocarbons is observed even in mixtures containing high proportions of ubiquinone-3. Similar conclusions were based on studies of the surface activity of ubiquinone-4 in mixed monolayers with dimyristoylphosphatidylcholine at the air-water interface [16]. An increasing tendency of ubiquinones to orient at the phospholipid/water interface of the bilayer as the polyisoprenoid chain length decreases is reflected in the extent to which the gel-liquid crystalline phase transition of the phospholipid is perturbed. This perturbation is manifest in the case of short-chain ubiquinones such as ubiquinone-3 by a broadening of the main transition and a loss of pretransition endotherm at relatively low mol ratios [5]. Contrary to other reports [17] ubiquinone-10 also significantly broadens the main transition and reduces the temperature of the pretransition as well as the main transition endotherms albeit to a less extent than shorter chain homologues. Furthermore, enthalpy measurements indicated that more than 92% of the phospholipid was involved in the main phase transition in equimolar mixtures of ubiquinone-10 and dipalmitoylphosphatidylcholine in contrast to previous studies [17] indicating that nearly half of the phospholipid was removed from the phase transition in mixed dispersions of the same composition.

The effect of ubiquinone-10 on the phase transition of dipalmitoylphosphatidylcholine appears to deviate from the pattern observed with the shorter chain ubiquinone homologues examined. The difference appears to be due in part to the physical state of the ubiquinone-10 at the phase transition temperature of the phospholipid because the anomalous behaviour was not observed in mixed dispersions with dimyristoylphosphatidylcholine, in which case ubiquinone-9 has the same effect as ubiquinone-10 on the melting profile index. The differing effects of ubiquinone on the melting behaviour of phospholipids is noteworthy in respect of the physical states of ubiquinone in bilayers. Ubiquinone-9 and -10, for example, appear to exist in two 'dispersed' forms on the basis of proton magnetic resonances of $-OCH_3$ ring protons [15] and these are believed to result from different extents of local interactions with neighbouring ubiquinone molecules. Shorter chain ubiquinones do not exhibit this behaviour. A third form of ubiquinone that cannot be detected by conventional proton magnetic resonance but which undergoes an endothermic melting process may also be present in dispersions containing physiological proportions of long-chain ubiquinones.

The effect of ubiquinones of different chain length on molecular motion and order in the hydrocarbon region, as judged by molecular probe techniques, is to some extent confused by lack of knowledge of the precise environment report by such probes. Spin-label studies of stearate probes with a nitroxyl radical attached to either carbon $\omega 3$ or $\omega 14$ indicate that the presence of ubiquinone-3 or -9 perturbs the motion of the hydrocarbon chains of phospholipids and this fluidizing effect has been interpreted as an intercalation of the ubiquinones between the phospholipid molecules [18]. The steady-state fluorescence polarization of diphenylhexatriene [17] and perylene [19], on the other hand, indicate a decreased membrane fluidity in the presence of ubiquinone-3 but no effect of ubiquinone-10. Clearly further studies are required to determine the effects of ubiquinones on molecular motion in phospholipid bilayers at temperatures above the liquid crystalline phase transition as well as the disposition and physical state of ubiquinones in such structures. The calorimetric data clearly shows that there is a progressive perturbation of the phospholipid transition as the ubiquinone becomes shorter. In the gel phase there is a complete phase separation of ubiquinones of all chain-lengths from 3 to 10 which allows all the phospholipid to participate in a gel-liquid crystalline phase transition. We conclude that the separate phase of ubiquinone is located in the centre of the phospholipid bilayer [5].

Acknowledgements

This work was assisted by a grant from the British Heart Foundation. Equipment used was purchased by grants from the Central Research Fund of London University and the Royal Society.

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