ON THE INTERACTION OF UBIQUINONES WITH PHOSPHOLIPID BILAYERS

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1. Introduction

The location of coenzyme Q (ubiquinone, UQ) in the mitochondrial membrane is still a matter of discussion. An important approach to the problem involves the use of UQ analogues reconstituted in phospholipid liposome bilayers. UQ-mediated transport of protons and electrons across the membrane can easily be studied in these systems [1–3]. These, together with other functional and enzymatic studies carried out on mitochondrial membranes [4], suggest that at least the quinone ring is located in the hydrophobic membrane core. Here, we provide structural evidence, as derived from differential scanning calorimetry (DSC) and fluorescence polarisation studies, supporting the idea that the UQ molecule is located between the two half-bilayers. For comparison, a shorter chain analog, coenzyme Q_3 (UQ_3) was used in addition to the physiological UQ_{10}.

2. Materials and methods

UQ_3 and UQ_{10} were the kind gift of Drs Weber and Jenni (Hoffmann-La Roche, Basel); some experiments were also carried out with UQ_{10} from Sigma, with similar results. DPPC was obtained from Fluka, and used without further purification.

Samples were prepared as follows. Chloroform solutions containing 2 mg DPPC and the appropriate amounts of UQ were combined and evaporated to dryness under N_2 at 43°C in the dark. N_2 flushing was continued for 15 min to remove solvent traces. Water was added, and the ternary system so obtained was carefully mixed on a bench vibrator while kept above the lipid transition temperature (T_c). Alternatively, the ternary mixture was centrifuged several times through a narraw capillary while kept at above T_c [5]. Careful mixing was found to be important in order to obtain reproducible results.

Calorimetric studies were carried out on a Perkin-Elmer DSC-1B differential scanning calorimeter operating in the low-temperature mode, with liquid N_2 as the coolant, with a heating or cooling rate of 4°C/min. Peak areas were measured by weighing paper cut-outs of the peaks. Other details of the technique were as in [6]. The phospholipid contents of the pans were determined as lipid phosphorus according to [7]. The appropriate blanks for the phosphorus assay were run with empty pans and the various reagents.

Fluorescence polarisation measurements were carried out using a Perkin Elmer MPF 44B spectrofluorimeter equipped with a DCSU-2 unit which calculates the polarisation index, P. Polaroid polarisers were used. Samples were labelled by using the fluorescent probe, 1,6-diphenylhexatriene (DPH), at a probe:phospholipid molar ratio of 1:500, and incubated with the probe for 1 h at 46°C. To ensure that depolarisation due to light scattering was not occurring, the value of P was measured before and after diluting the sample. In cases where dilution gave an increase in P, the samples were diluted until the value of P had reached a maximum and was no longer concentration dependent. The use of DPH as a fluorescent probe for the hydrophobic regions of lipid bilayers has been discussed in detail [8,9].

3. Results

The calorimetric profile of the thermotropic gel-

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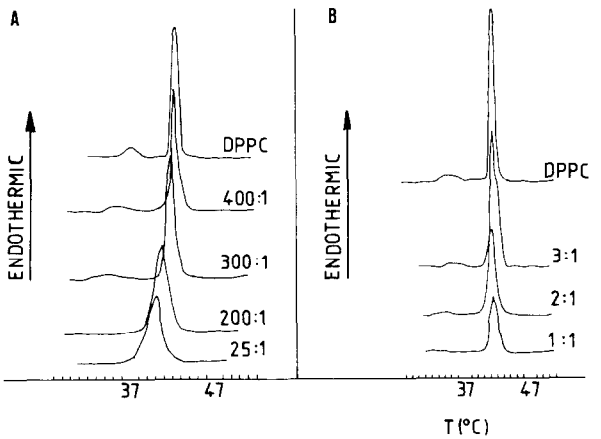

Fig.1. The DSC calorimetric heating curves for pure DPPC and DPPC:UQ systems. Molar lipid:UQ ratios are indicated on the curves. The curves have been normalized for the same amount of phospholipid in all cases: (A) UQ_3; (B) UQ_{10}.

to-liquid crystalline transition of DPPC, as well as that of the so-called pretransition, are unchanged by the presence of UQ_{10} up to DPPC:UQ_{10} molar ratios of 3:1, except for a slight broadening of the main peak (fig.1B). From that point, increasing concentrations of UQ_{10} in the bilayer produce blurring of the pretransition and a decreasing and further broadening of the main transition. The enthalpy variation associated with the main transition of pure DPPC was estimated to be of 8.0 kcal/mol. The corresponding values for recombinants of 6 different DPPC:UQ_{10} ratios, ranging from 200:1 to 3:1 do not show any significant difference with respect to the pure lipid, their mean value ± SD being 7.2 ± 0.34. However, ΔH of the 2:1 and 1:1 recombinants decreases to 5.6 kcal and 4.2 kcal/mol of DPPC, respectively. All the calorimetric profiles of the DPPC:UQ_{10} mixtures were perfectly symmetrical; heating and cooling curves were mirror images of each other, except for the usual hysteresis. The T_c transition temperatures of all the peaks in this series were of 41.3 ± 0.1°C for heating and 39.8 ± 0.1 for cooling experiments.

The most dilute DPPC:UQ_3 preparations (molar ratios > 300:1) do not differ in calorimetric behaviour from the pure phospholipid; however, increasing concentrations of UQ_3 have the effect of broadening the main transition peak and decreasing its height (fig.1A). The pretransition is removed as soon as the main transition is broadened appreciably. In spite of these modifications in the calorimetric profile, UQ_3 fails to induce any change in the transition enthalpy: the main value ± SD of recombinants of nine DPPC:UQ_3 molar ratios ranging from 600:1 to 15:1 is 8.1 ± 0.46 kcal/mol DPPC, similar to the 8.0 kcal/mol assigned to the pure DPPC. The T_c transition temperature of UQ_3 recombinants decreased (for heating experiments) as more quinone was being incorporated into the bilayer, from 41.3°C (pure DPPC) to 36.4°C (15:1, DPPC:UQ_3). Preparations containing >15:1 UQ_3:DPPC molar ratio gave rise to irregular and anomalous DSC patterns, and were not given further consideration in our study. The broadening of the main transition of DPPC:UQ_3 mixtures is shown in fig.2, together with the corresponding data from DPPC:UQ_{10} recombinants.

Fluorescence polarisation studies can be useful in

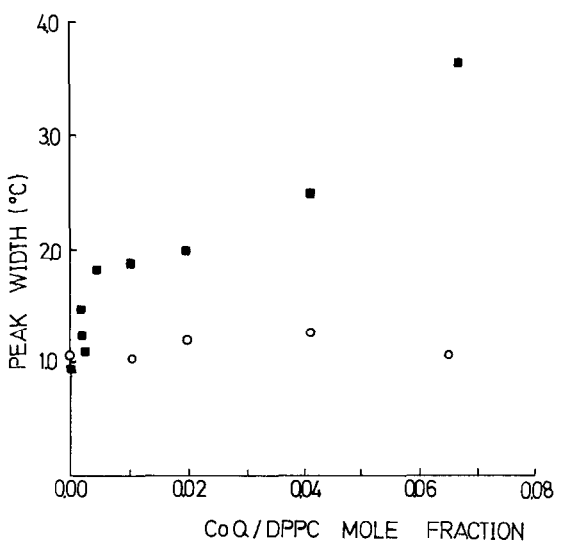

Fig.2. The width at mid-height of the DSC endotherm peaks of pure DPPC and DPPC:UQ systems: (○) UQ_{10}; (■) UQ_3.

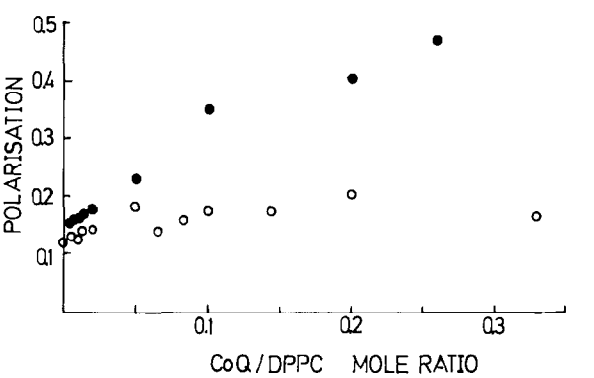

Fig.3. The polarisation index P values of DPH in pure DPPC and various DPPC:UQ mixtures at 51°C: (○) UQ_{10}; (●) UQ_3.

order to obtain information concerning the perturbation of the phospholipids in the fluid state by UQ. Fluorescence experiments carried out at 50°C, i.e., well above the main T_c transition temperature of pure DPPC, indicate that the value of the polarisation index P increases as the UQ_3 contents increases, as expected for intrinsic molecules [6]. UQ_{10} is also different in this respect. Fig.3 shows how the increase in P obtained with increasing UQ_{10}:DPPC molar ratios, is much less than in the case of UQ_3.

4. Discussion

The two molecules considered here, UQ_3 and UQ_{10} constitute two examples of different behaviour when incorporated to DPPC bilayers. Unless in very high (unphysiological) concentrations, UQ_{10} does not modify the calorimetric profile, whereas UQ_3 induces a broadening, i.e., a disordering of the lipids even at low concentrations. UQ_3 is also more effective than UQ_{10} in perturbing the DPH polarisation properties above T_c. This can be interpreted as an indication of the different position of both quinones in the bilayer [10,11]. UQ_{10} would most probably be located between the two half-bilayers, whereas UQ_3 would be nearer the polar phospholipid heads. Assuming that UQ_{10} is effectively incorporated into the bilayer, and all the present functional evidence confirms this point [1–3], the only possibility for a foreign molecule to be located without perturbing the lipid matrix is the one indicated above. It has been shown that the fatty acid chains can be perturbed beyond C_{10} without affecting the main calorimetric transition [12]; the region between C_{10} of one and the opposite half-bilayer is large enough to accommodate a number of UQ_{10} chains. However, when this number becomes too large (DPPC:UQ_{10} ratios $< 3:1$) other regions of the bilayer become occupied, and the corresponding disorder is reflected in the DSC curve. The possibility that the side-chain of UQ_{10} lies parallel to the phospholipid hydrocarbon chains [4] is unlikely, since the methyl substituents would seriously perturb the lipid packing.

The same conclusion, of an inner localisation of UQ_{10} in the membrane bilayer, is supported by fluorescence studies. Some different interpretations about the information obtained from steady state fluorescence anisotropy (or polarisation) using DPH as a probe have been put forward. Some authors claimed that information about lipid order can be obtained from such measurements when intrinsic molecules (e.g., proteins, cholesterol) are present in the lipid bilayers [13,14]. However it has been suggested, as well, that the information obtained from fluorescence probe measurements of P with such samples reflect mainly the hindering of motion of the probe by being adjacent to one or more intrinsic molecules [15]. Whichever is the best interpretation in the case of UQ, it is clear that the situation of both analogs, UQ_3 and UQ_{10}, in the lipid bilayer must be different. Again the data can be explained by suggesting that UQ_3 is at least partly located in the lipid palisade, whereas UQ_{10} is not interacting with the phospholipid portions closer to the aqueous phase. Steady state fluorescence anisotropy (or polarisation) depends on fluorescence lifetime. Fluorescence quenching of 12-(9-anthroyl) stearic acid by UQ_3 in DPPC bilayers was reported in [16]. Thus, in our case, the increase in P could be partially due to a decrease in the fluorescence lifetime. Obviously this effect is much less important for UQ_{10} than for UQ_3 and in any case it does not contradict our interpretation of the data.

Studies on the interaction of ubiquinones with phospholipids [17] were interpreted as the quinones having a disordering effect upon the lipid acyl chains. This is in apparent contradiction to our observations. However, those studies were carried out by ESR spectroscopy, with spin-labelled stearic acid; most probably this probe will be located itself between the half-bilayers, as ubiquinone, and not parallel to the acyl chains [11]. Consequently, the probe will sense the increase in *gauche* isomers in the region beyond C_{10}, thus confirming our interpretations.

The reason for the different location of both analog molecules may reside on their different hydrophilic–lipophilic balance: UQ_{10}, because of the longer side-chain, must be more lipophilic than UQ_3, and this more stable in the inner region of the lipid matrix. On the contrary, the side-chain of UQ_3 would not be long enough to 'anchor' the relatively polar quinone ring, and the latter will emerge preventing the all-*trans* conformation of the carbon atoms in the region C_1–C_{10}.

These results are in agreement with the present views on mitochondrial coenzyme Q oxido-reduction [18] and, if they are applicable to plastoquinones, they would also agree with their proposed function of connecting photosystems I and II [19].

Acknowledgements

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