Journal of Thermal Analysis and Calorimetry, Vol. 89 (2007) 3, 867–873

THE REDOX STATE INFLUENCES THE INTERACTION OF UBIQUINONES WITH PHOSPHOLIPID BILAYERS
A DSC study

Y. Roche[1], *P. Peretti*[2] *and Sophie Bernard*[2*]

[1]URCA-UMR CNRS 6142, Unité MéDIAN, UFR de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims Cedex, France
[2]Paris 5 – René Descartes University, Biomedical Research Center, Laboratoire de Neuro-Physique Cellulaire, EA 3817
45 rue des Saints-Pères, 75270 Paris Cedex 06, France

Differential scanning calorimetry (DSC) has been used to study the interaction of ubiquinones (UQn) and their reduced forms, ubiquinols ($UQnH_2$), with dipalmitoylphosphatidylcholine (DPPC) in multilamellar vesicles. The influence of the redox state has been investigated by comparing the effect of two ubiquinones with four (UQ4) and ten isoprene units (UQ10). In the presence of increasing amounts of UQ4 or $UQ4H_2$, concomitant shift of the gel to liquid crystalline phase transition towards lower temperatures and vanishing of the pre-transition are observed. Short-chain homologues are thus inserted parallel to phospholipid chains, their quinone ring close to the polar headgroups of the DPPC, with a larger degree of penetration for the reduced form. In addition, broadening and skewing of the main transition peak claim for a lateral self-organization in highly concentrated regions of UQ4 and $UQ4H_2$, with a redox state influence on the distribution in size and shape of lipid domains. The lipid thermotropic behavior is not affected by the presence of UQ10 which remain homogeneously dispersed within the midplane of the phospholipid bilayer, while effect of reduced $UQ10H_2$ argues for a different organization.

Keywords: *DSC, liposomes, phospholipid bilayer, ubiquinols, ubiquinones*

Introduction

Ubiquinones (UQn or coenzymes Q) [1–5] act as lipophilic electron carriers diffusing between enzymatic complexes in the respiratory chains of mitochondrial [6] and bacterial [7] membranes. They consist of a redox-active 2,3-dimethoxy-5-methylbenzoquinone ring with a hydrophobic isoprenoid chain in position 6. Ubiquinones differ from one to the other in the length of their side chain as indicated by a number (*n*) following the name which ranges between 2 and 10 isoprene units (2-methyl-2-butene) [8, 9]. They mediate the electron transfer in the mitochondrial inner membrane and also participate in the translocation of protons across the membrane [10, 11]. Ubiquinols, the two-electron reduction products of ubiquinones, are important natural antioxidants [3]: they can inhibit peroxidation by reaction with $ROO^{\bullet}$ radicals [12, 13], or regenerate vitamin E, another important liposoluble antioxidant [14].

In previous works, we studied the location and the molecular organization of a series of ubiquinones with different chain lengths in model membranes: Langmuir monolayers [15, 16] and liposomes [17]. Our conclusions pointed out that both transversal and lateral organizations of ubiquinones are strongly influenced by the isoprene chain length. Our data leaded us to establish a clear distinction between short- (UQ2, UQ4) and long-chain (UQ6, UQ10) homologues. This result is important considering the mobile carrier character of UQn. But the redox state could also be decisive in determining the localization of UQn in the membrane during the redox cycle. Several groups have investigated ubiquinol-10 organization in phospholipid bilayers. One can mention the early works of Aranda *et al*. using Fourier transform infrared spectroscopy, and calorimetry, [18–20], as well as a more recent study of this group using ^{31}P-NMR and small angle X-ray diffraction measurements on phosphatidylethanolamine bilayers [21]. The intrinsic fluorescence of prenylquinone compounds, including $UQ10H_2$, was also recently used to determine the motional properties of the molecule [22]. Another work brought some new insights on this subject with original NMR experiments [23]. Other important contributions aimed to measure the antioxidant activity of ubiquinol-10 in liposomes [12, 24, 25]. Most of the cited works propose that the redox state influences the organization of UQ10 in phospholipid bilayers, even if this question is still under debate ([23] for a discussion). However, these studies have only focused on the behavior of ubiquinol-10 and not on reduced shorter analogs, except the comparative analysis on prenylquinones by

* Author for correspondence: Sophie.Bernard@univ-paris5.fr

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Jemiola-Rzeminska *et al.* [26]. In addition, attention was mainly paid to the hydroquinone ring transversal position in bilayer and not to the influence of the redox state on the lateral organization of ubiquinones. Thus, the present study aims to compare the interaction of ubiquinones in both oxidized and reduced forms with dipalmitoyl-phosphatidylcholine (DPPC) in multilamellar vesicles, using differential scanning calorimetry (DSC). Ubiquinone with four isoprene units (UQ4) was chosen as representative of short-chain homologues, while UQ10 is the longest one (ten isoprene unites, i.e. 50 carbon atoms) but the predominant form in animals and humans.

Experimental

Materials and methods

DPPC (synthetic, *L*-α type, purity grade 99%) and UQ10 (synthetic, purity grade 98%) were purchased from Sigma (France) while UQ4 (synthetic, purity grade 95%) was from Fluka (France). Organic solvents (chloroform and methanol) were of HPLC grade and were purchased from Fisher Scientific (France). Ultra-pure water was treated on an Elgastat UHQ 2 system (resistivity of 18 MΩ cm).

Liposomes preparation

The pure DPPC and mixed UQ/DPPC multilamellar vesicles with molar ratio ranging from 0.01 to 0.1 were prepared as described in the following procedure. Samples were placed under a nitrogen gas flow and simultaneously heated at 55–60°C. Once the solvent was completely evaporated, they were stored in a desiccator under vacuum at room temperature for at least 2 h in order to eliminate any traces of solvent. The formation of multilamellar vesicles was achieved by adding the appropriate volume of ultra-pure water. The samples were heated at 60°C (i.e., above the temperature of the gel to liquid crystalline phase transition of pure DPPC) and vortexed (Vortex Top-mix, Bioblock, France) several times to obtain homogeneous multilamellar suspensions.

Reduction of ubiquinones

Reduction of ubiquinones was achieved according to the protocol described by Rieske [27]. Ubiquinone (5 mL, 10^{-3} M in ethanol) was added to 15 mL of a sodium phosphate buffer (0.1 M, pH 7.4) containing 0.25 M in sucrose. A pinch of sodium dithionite (Aldrich) was added, and the mixture was shaken vigorously until it was colourless. The aqueous solution was extracted with cyclohexane and evaporated under vacuum. The remaining light-yellow sirup was then diluted in chloroform/ethanol (50:50, *v*:*v*) solution. To retard autoxidation, the solution was slightly acidified with dilute HCl.

Differential scanning calorimetry

DSC measurements were performed using a Setaram DSC 111 model microcalorimeter (France). A heating rate of 0.5°C min^{-1} in the 25–50°C temperature range was chosen. The phospholipid vesicles were prepared as stated above, so that the final DPPC concentration was 75 mM. A volume of 18 μL of multilamellar suspensions was introduced into a specific pan (Setaram). Another pan containing 18 μL of water was used as reference. The accuracy for enthalpy changes is within 5% after DSC calibration. Precision on T_m, ΔH and $\Delta T_{1/2}$ was evaluated from nine independent measurements carried out under the same conditions.

Results

Figure 1 shows the heating curves of multilamellar vesicles of pure DPPC, and of mixtures containing the following UQn/DPPC molar ratios: 0.01, 0.05 and 0.1. DPPC exhibits the two well-known endothermic transitions: a pretransition at about 35°C and a main transition at 41.1°C. The pretransition arises from the conversion of a lamellar gel phase (L'_β) to a rippled gel phase (P'_β), whereas the higher temperature transition corresponds to a conversion of a P'_β phase to a lamellar liquid crystalline (L_α) phase [28]. We determined the thermodynamic parameters related to the main transition (i.e., the temperature at which the heat-flow reaches its maximum): T_m=41.1±0.1°C, the cooperativity of the transition given by the peak width at half-height: $\Delta T_{1/2}$=0.30±0.05°C, and the enthalpy of the transition determined from the area under DSC curve: ΔH=30±1 kJ mol^{-1}. These values are in keeping with data reported in the literature [29, 30].

The presence of ubiquinones induces the broadening of the DPPC main transition, which may reflect a reduced cooperativity between the acyl chains of DPPC. However, the main transition of DPPC is much more affected by the presence of UQ4 than by UQ10, whatever the redox state. Indeed, the higher the proportion of UQ4, the more the main transition peak *i*) shifts towards lower temperatures, *ii*) continuously broadens and *iii*) decreases in intensity. The reduced form, $UQ4H_2$, has a significantly more marked effect than the oxidized UQ4. In the case of UQ10, no effect on the transition is observed, while $UQ10H_2$ induces a decrease in intensity and slight shifting and broadening of the main transition peak.

The DPPC pretransition is also sensitive to the presence of ubiquinones. With UQ4, the pretransition

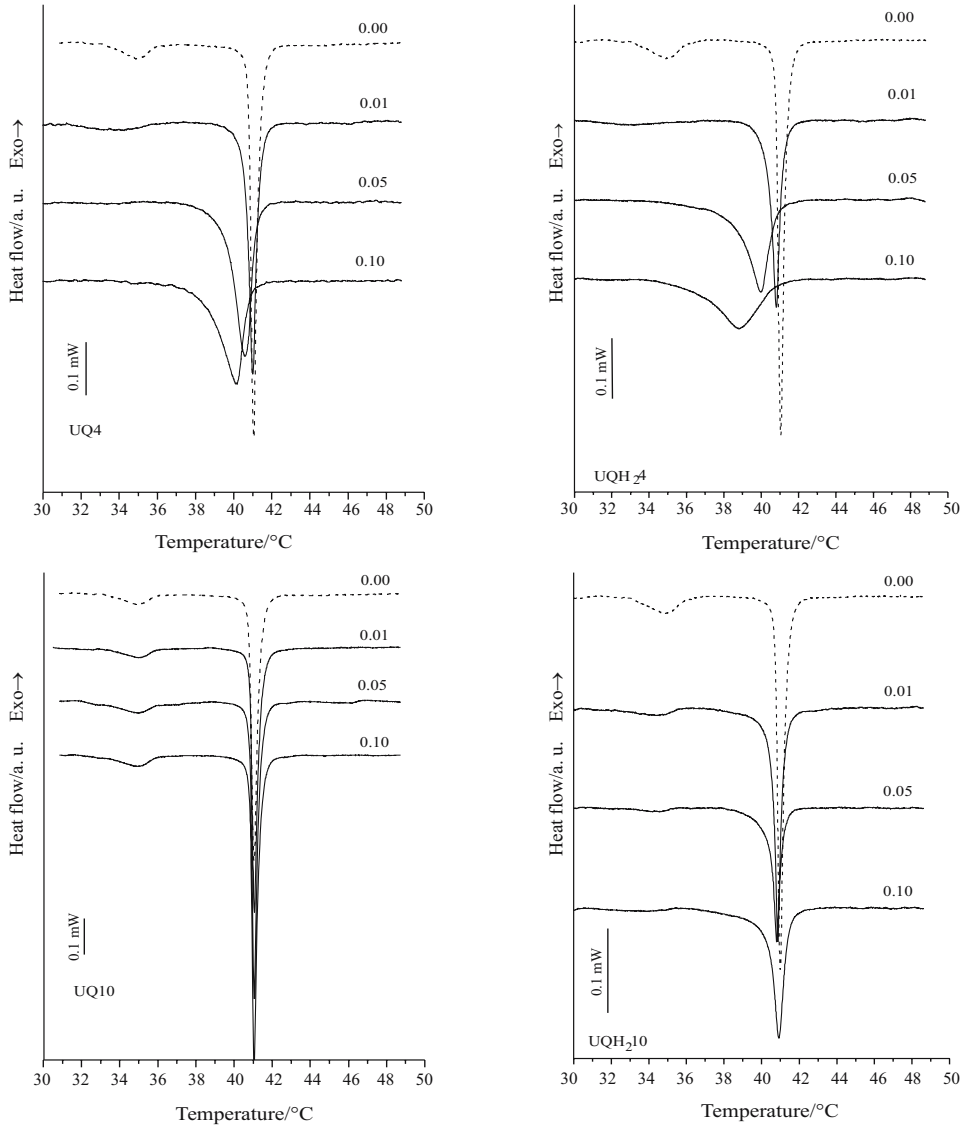

Fig. 1 DSC heating curves of multilamellar vesicles as function of the UQn/DPPC molar ratio indicated

shifts towards lower temperatures when molar ratio is 0.01 and completely vanishes for higher molar ratios. In the case of $UQ4H_2$, the pretransition peak is completely vanished from 0.01 molar ratio. No effect on the pretransition is observed in the presence of UQ10; for $UQ10H_2$, a shifted peak is only visible when molar ratio is 0.01.

The thermodynamic parameters of the main transition, T_m, $\Delta T_{1/2}$ and ΔH were deduced from curves, and plotted *vs*. UQn molar ratio (Figs 2 and 3, Table 1). T_m linearly decreases with the UQn molar ratio (Fig. 2). This effect is pronounced for UQ4 and $UQ4H_2$, while T_m is slightly and not affected at all by $UQ10H_2$ and UQ10, respectively. In addition, the diminution of T_m is significantly more marked for the reduced form $UQ4H_2$ than for UQ4. Indeed, ΔT_m, (i.e., the difference between T_m with and without ubiquinone), is equal to 1.0 and 2.3°C respectively in the presence of 0.10 UQ4/DPPC and $UQ4H_2$/DPPC molar ratio, while for the same proportion of UQ10 ΔT_m is null, and equal to 0.1°C in the presence of $UQ10H_2$. Thus, the influence of the redox state on T_m is only significant for UQ4. The peak width at half-height, $\Delta T_{1/2}$, is approximately linearly dependent on the UQn concentration (Fig. 3) and is overall more affected by UQ4 than by UQ10. Reduced forms cause larger $\Delta T_{1/2}$ variations than corresponding oxidized ones, with significant broadening effects in the presence of 0.05 and 0.10 $UQ4H_2$ molar ratios and for 0.10 $UQ10H_2$. Lastly, a significant decrease of ΔH with the ubiquinone molar ratio is observed only in the presence of $UQ10H_2$. Minimum values of 15 and 17 kJ mol^{-1} are reached at respectively 0.05 and 0.10 $UQ10H_2$ molar ratios. Surprisingly, a lower ΔH value was found with 0.01 $UQ4H_2$ molar ratio than with larger ubiquinone proportions.

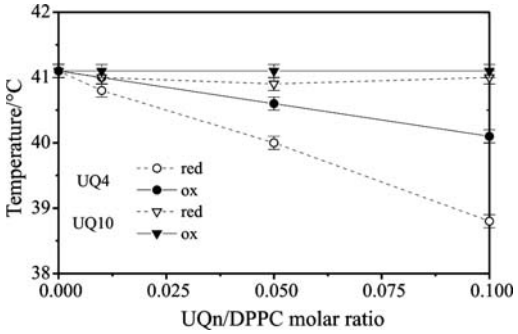

Fig. 2 Main transition temperature of mixed multilamellar vesicles as a function of the UQn/DPPC molar ratio

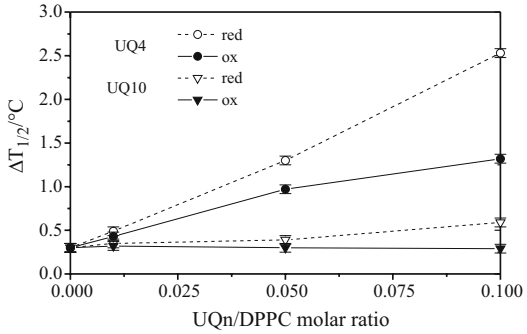

Fig. 3 Temperature width at half height of mixed multilamellar vesicles as a function of the UQn/DPPC molar ratio

However, it should be kept in mind that determined ΔH represents only an estimate of the transition enthalpy. Indeed, baseline subtraction, contribution of the wings of the transition and also accuracy on the sample mass determination, could render some apparent ΔH variations of several kJ mol^{-1} insignificant. Previously given accuracy (±1 kJ mol^{-1}) determined by the sharp main transition of DPPC liposomes should be larger in case mixed vesicles and should display a broader DPPC main transition. Consequently, only the large ΔH variations observed with $UQ10H_2$/DPPC mixtures are considered to be significant.

To study the asymmetry changes of the main transition peak, we plotted the asymmetry index, A_s, *vs*. the UQn molar ratio. A_s is defined by the following equation [31]:

$$A_s = \frac{[(T_{onset} - T_i) / (T_{endset} - T_i)]_{sample}}{[(T_{onset} - T_i) / (T_{endset} - T_i)]_{DPPC}}$$

where T_{onset} and T_{endset} are the temperatures of the intercept between the baseline and the tangent on the peak respectively, in its increasing part (T_{onset}), in its decreasing part (T_{endset}). T_i is the interception point of these two tangents and its value is generally identical to T_m. If A_s is greater than 1, the peak is more asymmetric than for pure DPPC, while a value of A_s lower than 1 indicates than the peak is less asymmetric. This A_s parameter is significant only in the presence of a single transition peak.

Table 1 Enthalpy values of the main phase transition of UQn/DPPC vesicles

UQn/DPPC molar ratio/%	ΔH/kJ mol^{-1}			
	UQ4	$UQ4H_2$	UQ10	$UQ10H_2$
1	26	22	28	22
5	33	29	30	15
10	31	24	32	17

A_s was plotted *vs*. the UQn molar ratio (Fig. 4). First, A_s is slightly affected by UQ10 since values are included between 1 and 1.3 for the oxidized form, 2 and 2.2 in the presence of $UQ10H_2$. Second, A_s increased significantly with UQ4 amounts: its value reached 5 in the presence of 0.10 UQ4/DPPC molar ratio. This increase is slightly less marked for $UQ4H_2$, with an asymmetry index equal to 4.1 for the same ubiquinone proportion.

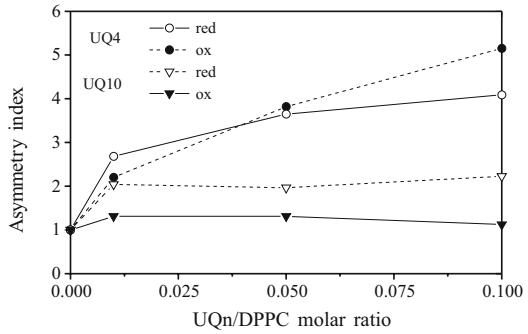

Fig. 4 Asymmetry index values of mixed multilamellar vesicles as a function of the UQn/DPPC molar ratio

Discussion

The presence of UQ4 and $UQ4H_2$ in phospholipid bilayers leads to a decrease in T_m and an increase in $\Delta T_{1/2}$. According to the classification of Jain and Wu [32, 33], both these oxidized and reduced forms would be of type A: they would be located in C_1–C_8 methylene region, parallel to the aliphatic chains of DPPC. Their polar benzoquinone rings are probably oriented towards the interfacial zone between the hydrophobic and polar parts of the bilayer. This is consistent with our previous works and other authors have drawn the same conclusion by studying the localization of short-chain UQn in phospholipid vesicles by fluorescence anisotropy [34] or by fluorescence spectroscopy and linear dichroism [35, 36]. Moreover, the pretransition is very sensitive to the presence of small quantities of UQ4 and $UQ4H_2$ since

this is no longer detected for UQn molar ratios higher than 1%. Other studies have shown [31] that the penetration of even a small amount of host substances in the hydrophobic core of bilayer could force phospholipids into a single conformational phase, probably the P'_β rippled gel phase. This confirms that short-chain ubiquinone and ubiquinol are inserted between the phospholipid aliphatic chains. Their benzo(hydro)quinone rings could interact with phospholipid polar headgroups by dipole–dipole interactions. The ubiquinols strong polar character could confer to $UQ4H_2$ a higher degree of penetration than UQ4, i.e. its hydroquinol ring would be closer to polar headgroups of DPPC. This assumption can explain why $UQ4H_2$ has a more marked effect on the lipid thermotropic phase behavior than its oxidized form. Interestingly, ΔH remains constant in the presence of increasing amounts of UQ4 and $UQ4H_2$. This could indicate that both forms are intercalated between DPPC acyl chains, hydrophobic interactions being prominent, without electrostatic interactions between ubiquinone ring and PC polar headgroups.

The effect of short-chain homologues on the broadening of the lipid main transition peak is concentration and redox state dependent and reflects diminution in the cooperativity of the transition, i.e. a decrease of the average number of molecules per cooperative unit [29]. During the main transition, the cooperative units of the minor phase of pure DPPC can be described as large, nearly circular and highly compact domains, in which all molecules melt cooperatively [32]. Then, changes in size distribution might appear in the presence of ubiquinones, as observed by Borona *et al*. with DPPC liposomes containing pthalate esters [33]. According to this model, UQ4 and $UQ4H_2$ could stabilize the gel to liquid crystalline phase domain boundaries. Consequently, domains of the minor phase become more ramified and smaller, increase in number and the non-homogeneity of the composition induces the broadening of the main transition peak [29, 31]. This effect would be more marked in the presence of $UQ4H_2$ than UQ4, with thus an influence of the ubiquinone redox state on the shape, size and number of lipid domains. In addition, comparable skewing towards lower temperatures of the transition peak in the presence of $UQ4H_2$ and UQ4 (increasing A_s), suggests that short-chain ubiquinones are preferentially soluble in the liquid crystalline phase, with similar gradient of concentrations from the surface to the inner part of the domains [33]. Consequently, we can argue that UQ4 and $UQ4H_2$ could induce such localized and enriched zones in biological membranes, even if the overall concentration is low, with a difference in the distribution in size and shape of lipid domains depending on the ubiquinone redox state. This could be of great importance for the redox functions of respiratory chains proteins that are very sensitive to their lipid environment, especially to the presence of ubiquinones [34].

The natural UQ10 and its reduced form $UQ10H_2$ exert a significantly less marked influence on thermodynamic parameters (T_m, $\Delta T_{1/2}$, ΔH, A_s). They would be of the type C according to the classification of Jain and Wu [35, 36]: rather located in the core of the bilayer, interacting with the C_9–C_{16} methylene groups. In case of UQ10, unaffected thermotropic behavior suggests that molecules lie between the two leaflets, parallel to the bilayer plan with their quinone rings interacting with one another. This assumption is in agreement with conclusions from nuclear magnetic resonance studies [37, 38]. On the other hand, some authors have proposed [39], on the basis of fluorescence anisotropy experiments, that the quinone rings of UQ10 interact with the interfacial zone, while the isoprene chains remain anchored at the center of the bilayer. However, other studies based on fluorescence spectroscopy and linear dichroism measurements [40] have indicated that, even if their rings tend to oscillate, UQ10 would statistically spend more time between the two layers, parallel to the bilayer plane.

In the presence of increasing amounts of $UQ10H_2$ significant diminutions of ΔH are observed from 0.01 mole ratio, nevertheless with small associated variations in T_m and $\Delta T_{1/2}$. This claims for a different organization compared to the oxidized form UQ10. In addition, the vanishing of pretransition observed from 0.01 mole ratio argues for the molecule to be partly inserted between the aliphatic chains, contrary to UQ10. Due to the more polar nature of $UQ10H_2$ we can propose the hydroquinone ring to be statistically more able to interact with the interfacial zone than UQ10 and explaining a partial insertion of the molecule between alkyl chains. According to Castelli *et al*. [41] diminution of enthalpy can be associated with presence of interstitial impurities, able to substitute phospholipid molecules. In the case of $UQ10H_2$ we can propose the molecules are organized themselves to substitute phospholipid molecules. A large part of an individual isoprene chain would remain anchored in the hydrophobic midplane, while polar rings would interact one another at the phospholipid alkyl chains level to minimize contacts with the hydrophobic environment. With this model, association of $UQ10H_2$ molecules through ring interactions can act as interstitial impurities and explain the observed decrease in ΔH.

Then, in our experiments (molar ratios below 0.10) we can argue for a homogeneous molecular dispersion of UQ10 between the two phospholipid leaflets, while $UQ10H_2$ molecules could probably orga-

nize to substitute phospholipid molecules. Such a redox specific organization of long-chain ubiquinones within bilayers has some interesting implications with regards to their biological functions, and not only for the respiratory chain role. Indeed, other long-chain natural polyisoprenes, such as dolichol, tocopherol or squalene were found to lay in or near the core of the lipidic bilayer, respectively by means of DSC, X-ray diffraction and neutron diffraction experiments on lipid bilayers ([42] and references therein). Due to their specific transversal membrane organization, polyisoprenes, as other branched lipids, were suggested to play a role in the inhibition of proton leakage across biological membranes [43]. In fact, if oxidized UQ10 are good candidates for this function in mitochondrial membranes, the proposed specific organization of reduced form would be more able to allow proton leakage.

Conclusions

To conclude, we have shown that the influence of small quantities of UQ4 and UQ10 on the thermotropic phase behavior of DPPC multilayer vesicles depends on the redox state. Both UQ4 and $UQ4H_2$ are inserted parallel to phospholipid molecules, their (hydro)quinone rings close to the polar heads of the DPPC, the reduced form $UQ4H_2$ having a higher degree of penetration than UQ4. In addition, a self-organization in highly concentrated regions localized at the boundary of lipid domains is proposed, with an influence of the redox state on the distribution in size and shape of these domains. Long-chain UQ10 and $UQ10H_2$ would be rather localized in the center of the bilayer. More specifically oxidized UQ10 remains homogeneously dispersed within the bilayer, whereas reduced forms $UQ10H_2$ are probably self-organized at the hydroquinone ring level to partially substitute DPPC molecules.

These results argue for such a redox effect to occur in biomembranes too, with related consequences for biological activities of the different UQn homologues. First of all, it is obvious from our conclusions that natural long-chain ubiquinones are more efficient to act against proton leakage across membranes when oxidized instead of reduced. On the other hand, the particular redox-dependent organization of UQ4, specially the capacity for lateral segregation, can play a role for their interactions with respiratory chain proteins during electrons and protons transport processes. Interestingly, this property can be correlated with the prominent therapeutic efficiency of short-chain exogenous ubiquinones observed in some clinical studies. Consequently, it is clear that the biological activity of ubiquinone analogues is not only modulated by their isoprene chain-length, but by their redox state as well.

References

1 F. L. Crane, Y. Hatefi, R. L. Lester and C. Widmer, Biochim. Biophys. Acta, 25 (1957) 220.
2 R. A. Morton, G. M. Wilson, J. S. Lowe and Leat, Biochem. J., 68 (1958).
3 L. Ernster and G. Dallner, Biochim. Biophys. Acta, 1271 (1995) 195.
4 T. E. King, Bioenergetics: Molecular Biology, Biochemistry and Pathology, C. H. Kim and T. Ozawa, Eds, Plenum Press, 1990, p. 25.
5 G. Dallner and P. J. Sindelar, Free Radical Bio. Med., 29 (2000) 285.
6 I. E. Scheffler, Mitochondria, John Wiley & Sons, Inc., 1999.
7 B. Soballe and R. K. Poole, Microbiology, 145 (Pt 8) (1999) 1817.
8 M. D. Collins, Methods in Microbiology, A. Press, Ed., London 1985, p. 329.
9 G. P. Moss. Nomenclature of Quinones with Isoprenoid Side-Chains – Recommendations 1973, Vol. 2002: IUPAC-IUB Commission on Biochemical Nomenclature, 1973.
10 U. Brandt and B. Trumpower, Crit. Rev. Biochem. Mol., 29 (1994) 165.
11 H. Nohl, L. Gille and K. Staniek, Ann. NY Acad. Sci., 854 (1998) 394.
12 B. Frei, M. C. Kim and B. N. Ames, PNAS, 87 (1990) 4879.
13 E. Niki, Mol. Aspects Med., 18 (1997) s63.
14 V. E. Kagan, J. P. Fabisiak and P. J. Quinn, Protoplasma, 214 (2000) 11.
15 S. Bernard, Y. Roche, F. Etienne and P. Peretti, Mol. Cryst. Liq. Cryst., 338 (2000) 207.
16 Y. Roche, P. Peretti and S. Bernard, BBA – Biomembranes, 1758 (2006) 468.
17 Y. Roche, P. Peretti and S. Bernard, Thermochim. Acta, 447 (2006) 81.
18 F. J. Aranda and J. C. Gomez-Fernandez, BBA – Biomembranes, 820 (1985) 19.
19 F. J. Aranda and J. C. Gomez-Fernandez, Biochem. Int., 12 (1986) 137.
20 F. J. Aranda, J. Villalain and J. C. Gomez-Fernandez, BBA – Biomembranes, 861 (1986) 25.
21 J. C. Gomez-Fernandez, M. A. Llamas and F. J. Aranda, Eur. J. Biochem., 259 (1999) 739.
22 M. Jemiola-Rzeminska, J. Kruk and K. Strzalka, Chem. Phys. Lipids, 123 (2003) 233.
23 M. Afri, B. Ehrenberg, Y. Talmon, J. Schmidt, Y. Cohen and A. A. Frimer, Chem. Phys. Lipids, 131 (2004) 107.
24 M. Cipollone, D. Fiorentini, M. C. Galli, A. M. Sechi and L. Landi, Chem. Phys. Lipids, 69 (1994) 87.
25 J. Kruk, M. Jemiola-Rzeminska and K. Strzalka, Chem. Phys. Lipids, 87 (1997) 73.
26 M. Jemiola-Rzeminska, B. Mysliwa-Kurdziel and K. Strzalka, Chem. Phys. Lipids, 114 (2002) 169.
27 J. S. Rieske, Methods Enzymology, 10 (1997) 67.

28 K. M. G. Taylor and R. M. Morris, Thermochim. Acta, 248 (1995) 289.
29 R. L. Biltonen and D. Lichtenberg, Chem. Phys. Lipids, 64 (1993) 129.
30 A. Tahir, C. Grabielle-Madelmont, C. Betrencourt, M. Ollivon and P. Peretti, Chem. Phys. Lipids, 103 (1999) 57.
31 S. Bonora, L. Ercoli, A. Torreggiani and G. Fini, Thermochim. Acta, 385 (2002) 51.
32 W. W. van Osdol, Q. Ye, M. L. Johnson and R. L. Biltonen, Biophys. J., 63 (1992) 1011.
33 S. Bonora, G. Fini and B. Piccirilli, J. Therm. Anal. Cal., 61 (2000) 731.
34 G. Lenaz, FEBS Letters, 509 (2001) 151.
35 M. K. Jain and N.-Y. Wu, J. Membrane Biol., 34 (1977) 157.
36 R. N. McElhaney, Chem. Phys. Lipids, 30 (1982) 229.
37 E. L. Ulrich, M. E. Girvin, W. A. Cramer and J. L. Markley, Biochemistry, 24 (1985) 2501.
38 G. Metz, K. P. Howard, W. B. S. van Liemt, J. H. Prestegard, J. Lugtenburg and S. O. Smith, J. Am. Chem. Soc., 117 (1995) 564.
39 M. Jemiola-Rzeminska, J. Kruk, M. Skowronek and K. Strzalka, Chem. Phys. Lipids, 79 (1996) 55.
40 B. Samori, G. Lenaz, M. Battino, G. Marconi and I. Domini, J. Membrane Biol., 128 (1992) 193.
41 F. Castelli, V. Librando and M. G. Sarpietro, Thermochim. Acta, 373 (2001) 133.
42 T. Hauss, S. Dante, N. A. Dencher and T. H. Haines, BBA – Bioenergetics, 1556 (2002) 149.
43 T. H. Haines, Prog. Lipid Res., 40 (2001) 299.

Received: August 4, 2006
Accepted: February 6, 2007

DOI: 10.1007/s10973-006-7916-4