The location of coenzyme Q10 in phospholipid membranes made of POPE:

A small-angle synchrotron X-ray diffraction study

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Abstract

The location of coenzyme Q10 inside the inner mitochondrial membrane is a topic of research aiming at a deeper understanding of the function of the mitochondrial respiratory chain. We investigated the location of Q10 inside model membranes made of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) by means of small-angle synchrotron X-ray diffraction. Q10, which stands for ubiquinone-10 (UQ) or ubihydroquinone-10 (UH), did not remarkably influence the main phase transition temperature, but significantly decreased the lamellar-inverse hexagonal phase transition temperature (T_h). The effect of UH on T_h was stronger than the effect of UQ and the effect of liquid Q10 on T_h was stronger than the effect of crystalline Q10. In presence of Q10, the lattice parameters of the lamellar phases remained unchanged whereas the $H_{||}$ lattice parameter was clearly influenced: While UQ had an increasing effect, UH had a decreasing effect. Furthermore, Q10 prevented the formation of cubic phases. The results give new evidence that the headgroup of Q10 is distant from center of the membrane, which might be important for the function of the mitochondrial respiratory chain.

Keywords: coenzyme Q10, mitochondrial respiratory chain, phosphatidylethanolamine, X-ray diffraction

1 Introduction

As an essential segment of the mitochondrial respiratory chain, Q10 transports electrons and protons between the respiratory complex I and III and between II and III (Ernster & Dallner, 1995). The antioxidant effect of Q10 counteracts the formation of free radicals during the course of the respiratory chain (Bentinger, et al., 2007).

Q10 stands for the redox pair ubiquinone-10 (UQ) and ubihydroquinone-10 (UH). Both compounds structurally differ in their headgroups. Since the partition coefficients of UQ and UH (Rich & Harper, 1990) indicate a strong hydrophobicity, Q10 is completely present inside the inner mitochondrial membrane.

The conversion of Q10 takes place at the binding sites of the respiratory complexes. The UQ binding site of respiratory complex I can only be reached by sliding “over a hydrophobic ramp” (Hunte, et al., 2010) and the UQ binding site of respiratory complex II is located in the “hydrophobic environment of the quinone-binding pocket” (Yankovskaya, et al., 2003). Both the UQ and the UH binding sites of respiratory complex III are well separated and located in a “quinone exchange cavity” that is likely to be “mostly filled with lipid acyl chains” (Cramer, et al., 2011), which also represents a hydrophobic environment. The Q10 binding sites of all respiratory complexes are spatially not close to the bilayer center but distant from it, whereas the UH binding site of respiratory complex III is more distant compared to the UQ binding site (Kurusu, et al., 2003).

The research on the location of Q10 inside a fluid phospholipid bilayer comprises the study of the aggregation, of the conformation and of the location of the headgroup and of the side chain of Q10. Proposed conformations of Q10 vary between folded and extended structures (Lenaz & Genova, 2009) and an aggregation of Q10 molecules in form of head to head aggregates is assumed (Ausili, et al., 2008; Gomez-Fernandez, et al., 1999). Proposed locations of the headgroup of Q10 vary between the bilayer center and the headgroup region of the phospholipid bilayer (Lenaz & Genova, 2009; Ausili, et al., 2008; Gomez-Fernandez, et al., 1999; Quinn, 2012; Hauss, et al., 2005), whereas the headgroup of UH is believed to be more distant from the bilayer center compared to the headgroup of UQ (Ausili, et al., 2008; Gomez-Fernandez, et al., 1999; Quinn, 2012).

A location of the whole Q10 molecule in the bilayer center speaks for the role of Q10 to be a

Abbreviations: Q10, coenzyme Q10; UQ, ubiquinone-10; UH, ubihydroquinone-10; POPE, 1-palmitoyl-2-oleoyl-phosphatidylethanolamine; L_{β} phase, lamellar gel phase; L_{α} phase, lamellar liquid phase, H_{II} phase, inverse hexagonal phase; T_m , main phase transition temperature; T_h , lamellar-inverse hexagonal phase transition temperature

fast electron and proton transporter (Hauss, et al., 2005). Furthermore, it might prevent proton leakage in order to maintain the efficiency of the mitochondrial respiratory chain. In contrast, a location of the headgroup of Q10 distant from the bilayer center plays to the Q10 binding sites of the respiratory complexes, which might be required for an efficient course of the respiratory chain. Furthermore, the antioxidant effect of Q10 requires an “extensive mobility in the membrane between the two hydrophilic surfaces” (Bentinger, et al., 2007) of the headgroup of Q10.

We present an investigation of the interaction of Q10 with fully hydrated dispersions of the phospholipid POPE using small-angle synchrotron X-ray diffraction. The location of Q10 inside the lamellar gel phase (L_β phase), lamellar liquid phase (L_α phase) and inverse hexagonal phase (H_{II} phase) of POPE will be discussed with a view to the location of the headgroup of Q10 and to the function of Q10 inside the mitochondrial respiratory chain.

We chose 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) in order to analyze the effect of Q10 on the formation of cubic phases observed in POPE dispersions (Hickel, et al., 2008). Furthermore, only one published study (Gomez-Fernandez, et al., 1999) on the interaction of Q10 with a phosphatidylethanolamine exists so far. Phosphatidylethanolamines belong to the class of non-bilayer-forming phospholipids, which are abundant in the mitochondrial membranes (Osman, et al., 2011). They might be related to the typically curved shape of the inner mitochondrial membrane and create tension in the mitochondrial membranes, which might affect the function of the respiratory complexes. Finally, a POPE molecule contains a palmitic chain and an oleic chain, which also appear in mitochondrial membranes (Schneider, et al., 1999), such that the structure of a POPE bilayer models adequate the chain region of mitochondrial membranes.

2 Materials and Methods

POPE was purchased from Avanti Polar Lipids (Alabaster, USA). UQ and UH were a kind gift from Kaneka (Tokyo, Japan). Preweighted amounts of POPE, UQ and/or UH and deionized water were vigorously vortexed and filled into borosilicate glass capillaries.

Small-angle synchrotron X-ray diffraction measurements were carried out at the Soft Condensed Matter Beamline A2 of HASYLAB at DESY using a wavelength of 1.5 Å and a bidimensional MARCCD detector. The capillaries were stored into a sample holder with controlled temperature. Heating and cooling scans were run between 15°C and 85°C with a scan rate of 1°C/min and a waiting time of 5 minutes between the scans. Each minute, two

detector images were recorded in sequence for 10 seconds, averaged and cosmic artifacts were filtered out.

The data were transformed into reciprocal space using an in-house-program, A2tool, and Origin from OriginLab (Northampton, USA). For the tabulated values of the lattice parameters, the diffraction peaks were background-corrected using a linear baseline and fitted with a Gaussian function. The error of the calculated lattice parameters was less than 0.1 Å.

The phase transition temperatures were determined using the fraction of the phases (Rappolt, et al., 2003) during the phase transition. The mean value of the temperatures at which 50% of the evolving phase and 50% of the declining phase were formed was taken as the phase transition temperature. The error was less than 0.25°C.

3 Results

3.1 Pure Fully Hydrated POPE Dispersions

The diffraction patterns of a pure fully hydrated POPE dispersion are presented in Figure 1. We made the phase assignment of the reflections to the phases by comparison with the diffraction patterns presented in (Wang & Quinn, 2002). The lattice parameter of the L_β phase and the lattice parameter of the L_α phase d could be calculated out of the position of the (1) reflection $s(1)$ by $d = 1/s(1)$ (Klacsová, et al., 2014). The H_{II} lattice parameter a could be calculated out of the (1,0) reflection of the H_{II} phase $s(1,0)$ by $a = 2/(\sqrt{3} \cdot s(1,0))$. The lattice parameters of the L_β , L_α and H_{II} phase during the heating and the cooling scan are presented in Figure 2 and are given for selected temperatures in Table 1.

Figure 1: Diffraction patterns of a pure fully hydrated POPE dispersion during the heating scan

Figure 2: Lattice parameters d and a of the L_β , L_α and $H_{||}$ phase during the heating and cooling scan of a pure fully hydrated POPE dispersion and of fully hydrated POPE dispersions containing 10 mol%, 21 mol% or 30 mol% UQ

Table 1: Lattice parameters of the L_β , L_α and H_{II} phase for selected temperatures

T[°C]	Lattice parameters of heating [Å]						Lattice parameters of cooling [Å]					
	15	20	30	40	80	85	85	80	30	28	17	15
POPE	61.8	61.5	<i>53.4</i>	<i>52.2</i>	<i>70.3</i>	<i>69.3</i>	69.3	70.3	<i>53.4</i>	<i>53.7</i>	61.6	61.7
10%UQ	61.8	61.5	<i>53.5</i>	<i>52.3</i>	<i>72.1</i>	<i>70.8</i>	70.9	72.1	<i>53.4</i>	<i>53.7</i>	61.5	61.7
21%UQ	61.7	61.5	^{a)}	<i>52.2</i>	<i>72.6</i>	<i>71.2</i>	71.2	72.5	<i>53.5</i>	<i>53.8</i>	61.7	61.8
30%UQ	62.1	61.8	<i>53.6</i>	<i>52.4</i>	<i>73.0</i>	<i>71.6</i>	71.7	73.1	<i>53.6</i>	<i>53.8</i>	61.6	61.8
12%UH	61.8	61.5	<i>53.4</i>	<i>52.2</i>	<i>69.7</i>	<i>68.5</i>	68.5	69.3	<i>53.5</i>	<i>53.7</i>	61.7	61.8
19%UH	61.7	61.5	<i>53.5</i>	<i>52.3</i>	<i>69.1</i>	<i>68.0</i>	68.0	68.7	<i>53.8</i>	<i>53.8</i>	61.7	61.8
33%UH	62.1	61.9	<i>53.7</i>	<i>52.5</i>	<i>68.6</i>	<i>67.7</i>	67.6	68.6	<i>82.2</i>	<i>83.0</i>	62.1	62.2

In standard script: L_β phase, *in italics*: L_α phase, underlined: H_{II} phase, ^{a)} Beam loss

The main phase transition (L_β - L_α phase transition) occurred at the temperature T_m of 26.5°C and the L_α - H_{II} phase transition at a temperature T_h of 73.5°C (Table 2). For the heating scan rate of 1°C/min, the values were in accordance with published data on pure fully hydrated POPE dispersions measured using small-angle X-ray diffraction (Wang & Quinn, 2002): T_m =25°C, T_h =71°C for a heating scan rate of 2°C/min, (Rappolt, et al., 2003): T_h =74.8°C for a heating scan rate of 0.5°C/min). Both transitions showed a small hysteresis in the cooling scan. The difference of the values of T_m and T_h from the heating and cooling scan was 1°C and 5.7°C, respectively.

Table 2: Onset temperature T_{on} , completion temperature T_{off} and phase transition temperature of the L_{β} - L_{α} phase transition and of the L_{α} - H_{II} phase transition during the heating and the cooling scan

	Phase transition parameters during the heating scan [°C]						Phase transition parameters during the cooling scan [°C]					
	L_{β} - L_{α}			L_{α} - H_{II}			H_{II} - L_{α}			L_{α} - L_{β}		
	T_{on}	T_{off}	T_m	T_{on}	T_{off}	T_h	T_{on}	T_{off}	T_h	T_{on}	T_{off}	T_m
POPE	24	27	26.5	72	78	73.5	70	66	67.8	26	21	25.5
10%UQ	21	28	25.9	50	67	55.8	46	24	40.5	26	24	25.7
21%UQ	a)	a)	a)	49	73	53.5	61	20	39.5	a)	24	a)
30%UQ	21	27	25.7	49	59	51.9	42	24	40.6	26	23	25.9
12%UH	23	26	25.9	45	61	49.2	43	a)		24	25	
19%UH	24	27	b)	45	53	46.7	29	20		25	23	
33%UH	25	27	26.3	45	51	46.0	c)	a)		23	c)	

The onset temperature of a phase transition is the temperature of the first diffraction pattern at which a signal of the evolving phase appears. The completion temperature of a phase transition is the temperature of the last diffraction pattern at which a signal of the declining phase can be observed.

Since the H_{II} phase was present during the main phase transition for the UH samples during the cooling scan, T_m and T_h could not be determined precisely enough.

a) Beam loss, b) Signals too instable to determine T_m properly, c) No L_{α} phase present

3.2 Fully Hydrated POPE Dispersions Containing UQ

We investigated fully hydrated POPE dispersions containing 10 mol%, 21 mol% or 30 mol% UQ. The lattice parameters of the L_{β} , L_{α} and H_{II} phase during the heating and the cooling scan are presented in Figure 2 and are given for selected temperatures in Table 1. The temperature parameters of the phase transitions are given in Table 2.

UQ did not influence the lattice parameters of the lamellar phases. T_m was slightly decreased during the heating scan and slightly increased during the cooling scan. The characteristics of the main phase transition seemed to be affected. The onset temperatures during the heating scan and the offset temperatures during the cooling scan varied more than the offset temperatures during the heating scan and the onset temperatures during the cooling scan compared to the pure POPE sample.

The values of the H_{II} lattice parameter were strikingly increased by UQ. The higher the UQ concentration was, the bigger was the H_{II} lattice parameter. For the samples containing 10 mol%, 21 mol% and 30 mol% UQ, an increase of 1.5 Å, 1.9 Å and 2.3 Å, respectively, was observed at 85°C compared to the pure POPE sample.

The L_{α} - H_{II} phase transition showed a significant, concentration dependent decrease of T_h and was clearly broadened. The higher the UQ concentration was, the lower was T_h . For the samples containing 10 mol%, 21 mol% and 30 mol% UQ, a decrease of 17.7°C, 20.0°C and 21.6°C, respectively, compared to the pure POPE sample was measured. However, the H_{II} onset temperature was concentration independent and had a value of about 49°C. During the cooling scan, the onset, the transition and the completion temperatures of the H_{II} - L_{α} phase transition appeared for all samples containing UQ at lower temperatures compared to the heating scan. The difference of the T_h values from the heating and cooling scan for the samples containing 10 mol%, 21 mol% and 30 mol% UQ was 15.3°C, 14.0°C and 11.3°C, respectively, which shows that the hysteresis behavior was more pronounced compared to the pure POPE sample.

The sample containing 30 mol% UQ was temperature cycled several times and showed in every cycle a similar phase behavior, with the exception of the H_{II} onset temperature during heating. Compared to the first heating scan, the H_{II} onset temperatures of the following scans were clearly reduced (Figure 3). From the first to the second heating scan, the reduction of the H_{II} onset temperature was 14°C, whereas the reduction from the second to the third and from the third to the fourth heating scan was 1°C.

Figure 3: H_{II} onset temperature during the first four heating scans of the sample containing 30 mol% UQ

3.3 Fully Hydrated POPE Dispersions Containing UH

UH did not influence the lattice parameters of the lamellar phases of fully hydrated POPE dispersions for concentrations of 12 mol%, 19 mol% or 33 mol% UH (Figure 4, Table 1). T_m during heating appeared to be slightly decreased and the characteristics of the main phase transition showed the same behavior like the UQ samples.

The H_{II} lattice parameter was strikingly affected by UH. In contrast to the UQ samples however, the values were clearly *decreased* compared to the pure POPE sample (Figure 4, Table 1). The higher the UH concentration was, the more was the decrease. For the samples containing 12 mol%, 19 mol% and 33 mol% UH, the decrease at 85°C had a value of 0.8 Å, 1.3 Å and 1.6 Å, respectively.

The L_α - H_{II} phase transition appeared at temperatures, which were even lower than the transition temperatures of the UQ samples (Table 2). Similar to the UQ samples, the H_{II} onset temperature was concentration independent and the L_α completion temperature was concentration dependent. The higher the UH concentration was, the lower was T_h . For the samples containing 12 mol%, 19 mol% and 33 mol% UH, the lowering was 24.3°C, 26.8°C and 27.5°C, respectively, compared to the pure POPE sample.

Figure 4: Lattice parameters d and a of the L_β , L_α and H_{II} phase, respectively, during the heating and cooling scan of a pure fully hydrated POPE dispersion and of fully hydrated POPE dispersions containing 12 mol%, 19 mol% or 33 mol% UH

Interestingly, a predominant L_α phase formed during the cooling scan only for the sample containing 12 mol% UH. For the sample containing 19 mol% UH, the maximum intensity of the H_{II} phase was for every temperature bigger compared to the L_α phase. For the sample containing 33 mol% UH, no L_α phase formed at all and a direct conversion from the H_{II} phase to the L_β phase took place.

The sample containing 33 mol% UH was temperature cycled for several times. From the second heating scan on, no L_α phase was observed. Instead, a direct conversion between an L_β phase and an H_{II} phase took always place.

3.4 Cubic phases

When looking at the diffraction patterns of the pure fully hydrated POPE dispersion of the cooling scan at small angles, small signals were visible (Figure 5, left). They formed during the H_{II} - L_α phase transition and coexisted with the L_α phase. When they were present, no signal of an H_{II} phase was detected. After temperature cycling, these signals enhanced and could be assigned to the cubic phases Im3m and Pn3m (Figure 5, right) (Tenchov, et al., 1998), which is in agreement with an earlier publication on POPE dispersions (Hickel, et al., 2008). Their lattice parameters a_{cub} could be calculated using the Miller indices hkl and the position s_{hkl} of the peaks of the reflections of the cubic phases by a linear fit using $1/s_{\text{hkl}} = a_{\text{cub}} \cdot 1/\sqrt{h^2 + k^2 + l^2}$. For the fourth cooling scan at 50°C, the Im3m phase had a lattice parameter of 176.5 Å and the Pn3m phase of 138.3 Å. The ratio of both lattice parameters is 1.28, which is in accordance with the theoretical predicted ratio for the coexistence of a Pn3m phase with an Im3m phase (Tenchov, et al., 1998).

Figure 5: Left: Diffraction patterns of a pure fully hydrated POPE dispersion of the H_{II} - L_α phase transition during the cooling scan. The black rectangle shows the region, in which the signals of the cubic phases Pn3m and Im3m are present. Right: Diffraction pattern of the pure fully hydrated POPE dispersion during the fourth cooling scan at 50°C and the linear fits for the determination of the lattice parameters of the detected cubic phases

In contrast to the pure POPE sample, no formation of cubic phases was observed during the H_{II} - L_α phase transition in the samples containing UQ (Figure 6, left). Instead, a trace of an H_{II} phase coexisted with the L_α phase. In the samples containing UH, cubic phases were neither observed during the cooling scan.

Even after temperature cycling, no signals of cubic phases were found during the cooling scan in the sample containing 30 mol% UQ (Figure 6, right). Instead, a small signal of an H_{II} phase reappeared. Temperature cycling of the sample containing 33 mol% UH did not reveal signals of cubic phases either.

Figure 6: Left: Diffraction patterns of the fully hydrated POPE dispersion containing 10 mol% UQ during the cooling scan at the H_{II} - L_α phase transition. The black rectangle shows the signals of the H_{II} phase. Right: Diffraction pattern of the pure fully hydrated POPE dispersion and of the fully hydrated POPE dispersion containing 30 mol% UQ at 30°C of the fourth cooling scan

3.5 Fully hydrated POPE dispersions containing mixtures of UQ and UH

We investigated fully hydrated POPE dispersions containing mixtures of UQ and UH but with a similar Q10 concentration. The phase behavior was similar to the phase behavior of POPE dispersions containing either UQ or UH (Figure 7, left). The higher the UQ concentration was the bigger was the H_{II} lattice parameter. Furthermore, the H_{II} onset temperature was always equal to the H_{II} onset temperature of the samples containing only UH.

A significant peculiarity of the samples containing mixtures of UQ and UH was the dependence of the H_{II} lattice parameter on the ratio of the mixtures, which showed a continuous relationship (Figure 7, right). Thus, a transformation of UH, which is prone to oxidation, to UQ, could be observed using the H_{II} lattice parameter. Indeed, the color of the UH samples, which was white before the measurements, changed to a pale yellow color after the measurements, whereas the extent was varying among the samples. The UQ samples

had an orange color. Thus, a small amount of UH transformed to UQ during the measurements, probably because of radiation effects. However, the H_{II} lattice parameter of the samples containing only UH was always smaller than the H_{II} lattice parameter of the pure POPE samples, which indicates, that the biggest amount of UH was not oxidized.

Figure 7: Left: Lattice parameters d and a of the L_β , L_α and H_{II} phase, respectively, during the heating scan of a fully hydrated POPE dispersion and fully hydrated POPE dispersions containing mixtures of UQ and UH. Right: H_{II} lattice parameter versus the UQ:Q10 ratio. The Q10 concentration of the samples presented in this plot varied between 14.7 mol% and 16.8 mol%. The samples containing only UQ or UH were prepared with 10mM TRIS buffer, which did not influence the phase behavior of the samples (data not shown here).

4 Discussion

Q10 did not influence the lattice parameters of the lamellar phases of fully hydrated POPE dispersions, which indicated that the transversal thermal expansivity (Uhríková, et al., 2004) remained unaffected. In agreement with a study on the effect of UQ on multibilayer stacks (Haus, et al., 2005), we conclude that the bilayer thickness was not changed by the incorporation of Q10. However, fully hydrated dispersions of DMPC (Ausili, et al., 2008) or DEPE (Gomez-Fernandez, et al., 1999) showed a change of the lattice parameter of the lamellar phases. We assume that the POPE bilayer center of the L_β phase was fluid (Cevc, 1991), such that it could fully accommodate Q10, whereas the DMPC and the DEPE bilayers in the L_β phase do not contain a similar fluid layer. Q10 clearly increased the L_β lattice parameter of these phases (Ausili, et al., 2008; Gomez-Fernandez, et al., 1999), which is an indication for the formation of a separate domain of Q10 (Quinn, 2012) inside of the center of these bilayers that increases the bilayer thickness. In contrast to the L_β phase, the fluid

phospholipid bilayers of the L_α phase can accommodate Q10 much better (Ausili, et al., 2008). Therefore, we did not observe a change of the L_α lattice parameter in our samples. Similarly, the fully hydrated dispersions of DMPC (Ausili, et al., 2008) or DEPE (Gomez-Fernandez, et al., 1999) showed no change of the L_α lattice parameter or a smaller change compared to the L_β lattice parameter, respectively. Thus, we propose that the bilayer center of the inner mitochondrial membrane can particularly well accommodate Q10, such that Q10 does not form a separate domain inside of it.

The presence of a fluid layer accommodating Q10 inside fully hydrated POPE dispersions in the L_β phase is in agreement with an unchanged value of T_m . It would only have changed, if Q10 had concentrated in the upper chain region above the 10th carbon atom (Ausili, et al., 2008).

Q10 strikingly lowered T_h , which is similar to the effect of alkanes on fully hydrated DEPE dispersions (Chen & Rand, 1998) or the effect of alkanols on fully hydrated dispersions of mixtures of DOPE and DOPC (Klacsová, et al., 2014). Similar to other hydrophobic substances, Q10 increases the chain volume term of the packing parameter due to its presence in the chain region of the bilayers (Gomez-Fernandez, et al., 1999). Thus, in addition to the high content of non-bilayer-forming phospholipids, Q10 creates tension inside the inner mitochondrial membrane.

Similar to fully hydrated DEPE dispersions, UH lowered T_h more than UQ (Gomez-Fernandez, et al., 1999). The interaction of UH with the chain region of the bilayers is thus stronger than the interaction of UQ.

The H_{II} onset temperature was concentration independent. The formation of line defects in the bilayers of the L_α phase drives the L_α - H_{II} phase transition (Rappolt, et al., 2003). We assume that Q10 influenced the kinetics: The higher the Q10 concentration was, the more Q10 induced line defects into the bilayers and the faster the conversion took place. Therefore, the L_α completion temperature was the lowest for the samples containing the highest amount of Q10.

Q10 strikingly affected the H_{II} lattice parameter of fully hydrated POPE dispersions. The higher the Q10 concentration was, the stronger was the effect. This result reflects the idea that only a proportion of Q10 present in the dispersions is incorporated into the phases of POPE. For DPPC liposomes, the degree of incorporation of Q10 is close to 100% and differs only slightly between UH and UQ (Jemiot-Rzeminska, et al., 2001). Since the partition coefficient of neutral compounds varies about a factor of 2 to 3 among the membrane lipids (Endo, et al., 2011), we conclude that the degree of incorporation is similar for POPE liposomes.

Similar to fully hydrated DEPE dispersions containing UQ (Gomez-Fernandez, et al., 1999), UQ increased the H_{II} lattice parameter of fully hydrated POPE dispersions. However, in

contrast to fully hydrated DEPE dispersions, UH *decreased* the $H_{||}$ lattice parameter of fully hydrated POPE dispersions.

We assume that Q10 fills the hydrophobic interstices of the $H_{||}$ lattice (Figure 8, left), which are energetically unfavorable (Seddon, 1990; Kirk & Gruner, 1985). Thus, the cylinders of the $H_{||}$ lattice can become larger in diameter and the $H_{||}$ lattice parameter becomes bigger than the $H_{||}$ lattice parameter of the unfilled $H_{||}$ lattice. Fully hydrated DEPE dispersions containing UH or UQ (Gomez-Fernandez, et al., 1999) and DOPE dispersions containing alkanes with a chain length between 12 and 18 carbon atoms (Chen & Rand, 1998) showed such an increase of the $H_{||}$ lattice parameter. The alkanes localized in the $H_{||}$ lattice at the end of the chain region. We propose that the situation is similar for the fully hydrated POPE dispersions containing UQ (Figure 8, right).

We propose that the *smaller* $H_{||}$ lattice parameter of the fully hydrated POPE dispersions containing UH compared to the pure POPE dispersion originates from an interaction of the headgroup of UH with the headgroup region of the monolayers of the $H_{||}$ lattice (Figure 8, right). This interaction lowers the headgroup repulsion such that the cylinders of the $H_{||}$ lattice become smaller in diameter and the $H_{||}$ lattice parameter becomes reduced. If this effect is bigger than the effect of filling the interstices, then a smaller $H_{||}$ lattice parameter is measured. Fully hydrated dispersions of mixtures of DOPE and DOPC containing alkanols, which contain a hydroxyl group like the headgroup of UH, showed such a decrease of the $H_{||}$ lattice parameter (Klacsóvá, et al., 2014). The authors similarly argue that the alkanols interact with the headgroup region of the monolayers of the $H_{||}$ lattice. Furthermore, the behavior of the $H_{||}$ lattice parameter of fully hydrated DEPE dispersions containing Q10 is in accordance with this idea. The $H_{||}$ lattice parameter of the samples containing UH is smaller than the $H_{||}$ lattice parameter of the samples containing UQ (Gomez-Fernandez, et al., 1999). In this case, however, the effect of filling the interstices might be bigger than the effect of UH on the headgroup region. A possible reason might be the size of the interstices of the fully hydrated $H_{||}$ lattice of DEPE, which is bigger than the size of the interstices of the fully hydrated $H_{||}$ lattice of POPE.

Figure 8: Left: The H_{II} lattice of POPE contains interstices (red color), which the chains fill by stretching. UQ (brown color) or UH fills the interstices, such that the chains do not stretch. Right: UH interacts with the headgroup region of the H_{II} lattice, whereas UQ does not. The side chain of Q10 remains at the end of the chain region.

The H_{II} onset temperature of the sample containing 30 mol% UQ was significantly larger during the first heating scan compared to the following heating scans. As for the sample containing 33 mol% UH, the H_{II} phase even formed directly out of the L_{β} phase from the second heating scan on. Furthermore, we observed a pronounced hysteresis behavior in the first temperature cycle for the samples containing Q10. We assume that a melting of UQ and UH took place during the heating scan, which happens for UQ at about 49°C (Castresana, et al., 1992). During the complete cooling scan, Q10 remained liquid. UQ and UH in the liquid phase interact stronger with the chain region than the crystalline phase such that T_h was lowered even more. This is in agreement with the assumption that liquid UQ and UH can exist in phospholipid bilayers even below the melting temperature of Q10 (Castresana, et al., 1992). A certain proportion of liquid Q10 might recrystallize during the cycling through the main phase transition (Katsikas & Quinn, 1982). Then, the residual proportion, which is also considered to be “molecularly dispersed” (Katsikas & Quinn, 1982) and not present in a separate phase, would induce the L_{α} - H_{II} transition at the lower T_h . Compared to the crystalline phase, the liquid phase speaks for a “conformational flexibility” (Cramer, et al., 2011), which is in favor of the conversion at the binding sites of respiratory complex III.

The interstices of the unfilled H_{II} lattice are a reason for the formation of cubic phases during the H_{II} - L_{α} phase transition (Seddon, 1990). Cubic phases represent an energetic compromise between the H_{II} phase, which has a curved structure, but also energetically unfavorable interstices, and the frustrated L_{α} phase. Since Q10 fills the interstices, no cubic phases formed in the fully hydrated POPE dispersions. Instead, traces of an H_{II} phase coexisting with the L_{α} phase were present. Dispersions of monoolein showed a similar behavior: Q10 promoted the phase transition from the Ia3d cubic phase to the H_{II} phase (Barauskas, et al., 1999).

The concentration dependent ratio of the H_{II} lattice parameter of the samples containing mixtures of UQ and UH indicates that UQ also has a specific effect on the chain region besides filling the interstices of the H_{II} lattice. We therefore assume that also the headgroup of UQ penetrates to a certain extent into the chain region of the H_{II} lattice, whereas the side chain of Q10 remains at the end of the chain region of the H_{II} lattice (Figure 8, right). Analogously, the side chain could remain in the center of a bilayer without penetrating deeply into the chain region.

5 Conclusion

UH and UQ interact differently with the H_{II} phase of fully hydrated dispersions of POPE. We propose that most of the side chain of Q10 fills the interstices of the H_{II} lattice whereas the headgroup of Q10 penetrates into the chain region of the H_{II} lattice. In contrast to the headgroup of UQ, the headgroup of UH penetrates up to the headgroup region of the H_{II} lattice (Figure 8, right). This result gives new evidence for the proposition that the headgroup of Q10 is located in the chain region of a phospholipid bilayer distant from the center, which might be beneficial for the conversion at the respiratory complexes and for the antioxidant function of Q10. Furthermore, our results are in agreement with the stable existence of liquid Q10 in a phospholipid bilayer below the melting temperature of Q10. Finally, our results suggest that the side chain of Q10 remains in the center of a phospholipid bilayer, which might represent a dense barrier against proton leakage in order to keep up the efficiency of the mitochondrial respiratory chain.

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