Impact of Coenzyme Q_{10} on the Physical Properties of Model Lipid Membranes

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Abstract: We investigated the influence of coenzyme Q_{10} on structural changes of model lipid membranes formed by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and by a mixture of phosphatidylcholine and sphingomyelin (2.4:1). Structural changes in the membranes were measured using electron paramagnetic resonance and differential scanning calorimetry. Two spin probes were used to monitor membrane characteristics: MeFASL (10,3) to monitor the changes close to the water-lipid interface, and MeFASL (2,11) to monitor the changes in the middle of the bilayer of the model lipid membranes. These data show that perturbation of CoQ_{10} in the lipid membranes, promotes a decrease in the dynamics of the lipid acyl chains, *i.e.*, it increases the ordering of the membrane interior. The results from DSC measurements suggested that the CoQ_{10} in the bilayer does not significantly perturb the thermal and enthalpic stability of DPPC (the gel-to-liquid transition does not change) but additionally stabilizes the lipid bilayer due to the aggregation of CoQ_{10} within the lipid bilayer. The CoQ_{10} fraction in an aggregated state increased in proportion to its concentration in the DPPC multilamellar liposomes.

Keywords: Coenzyme Q_{10}, Model lipid membranes, Electron paramagnetic resonance, Differential scanning calorimetry.

INTRODUCTION

Coenzyme Q_{10} (2,3-dimethoxy-5-methyl-6-multiprenyl-1, 4-benzoquinone) is also known as ubiquinone or ubidecarenone, and it is a lipid-soluble compound found in the cells of many organisms. CoQ_{10} is involved in a variety of essential cellular processes, such as acting as a redox component of transmembrane electron transport systems in the respiratory chain of mitochondria, and as a stabilising agent in cell membranes [1]. CoQ_{10} is also involved in the activation of signalling protein kinases related to the gene activation of cellular proliferation. In its reduced form CoQ_{10} functions as an antioxidant, protecting membrane phospholipids and serum low-density lipoproteins from the lipid peroxidation, and preventing oxidative damage of mitochondrial membrane proteins and DNA [2-4].

CoQ_{10} synthesis starts in the mevalonate pathway that produces farnesyl- pyrophosphate, precursor for cholesterol, CoQ10, dolihol and isoprenylated proteins [5]. In humans, CoQ_{10} is found in relatively higher concentrations in cells with high energy requirements such as heart, liver, muscle, and pancreas cells [6]. Exogenous CoQ_{10} is used as a nutritional supplement and is highly recommended in treatment of various cardiovascular disorders, degenerative muscle diseases, and during carcinogenesis [7].

Given the importance and a variety of the functions of CoQ_{10} in cell membranes, it is important to understand its interaction with lipid membranes. Model lipid membranes are particularly suitable for investigations into the permeability of drugs and drug delivery systems, and they allow the use of various physiochemical methods to study the biophysical interactions.

Several physicochemical methods can be used to study the structure, dynamics and intermolecular interactions of biomembranes. Numerous biophysical techniques, resulting in different hypotheses, were used to determine the interaction of CoQ_{10} with lipid membranes. Several studies have suggested that the quinone ring is buried in the hydrophobic core of the membrane [8, 9], some supported the idea that the quinone ring emerges near the lipid/water interface [10, 11], while the others hypotesized that ubiquinone might segregate in the membrane and form aggregates [12, 13].

The effects of CoQ_{10} on the structural properties of model lipid membranes was investigated using electron paramagnetic resonance (EPR) spectroscopy and differential scanning calorimetry (DSC). Two spin labels were used to monitor the membrane characteristics, one targeting the water–lipid interface, and the other targeting the middle of the bilayer.

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MATERIALS AND METHODS

1. Materials

The phospholipids phosphatidylcholine (PC; from egg), sphingomyelin (SM; from egg) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were from Avanti Polar Lipids (USA). HEPES was from Sigma Aldrich Chemical Company (USA), and organic solvents were from Merck (Germany). The spin label methyl esters of doxyl palmitic acid with the doxyl group on carbon 5 [MeFASL (10,3)] and on carbon 13 [MeFASL(2,11)] of the alkyl chain (counting from the methyl group) were synthesised by Prof. Slavko Pečar at the Faculty of Pharmacy, University of Ljubljana, Slovenia.

2. Methods

2.1. Preparation of Liposomes

Multilamellar liposomes from DPPC or from PC and SM at a molar ratio of 2.4:1 (PC/SM) were prepared using the thin layer method [14]. These multilamellar liposomes (final lipid concentration, 5 mg/mL) were prepared by transferring 1 mL DPPC stock solution (10 mg/mL in chloroform) or 725 μL PC plus 275.5 μL SM stock solutions (10 mg/mL in chloroform; respectively) into rotary flasks. The solvents were completely evaporated under reduced pressure (17 mbar), to provide a thin phospholipid film in the flasks. These lipid films were hydrated by adding 2 mL 10 mM HEPES (buffered to pH 7.0 with NaOH), and the mixtures were heated to above the phase transition temperature of the phospholipids in the liposome preparations and shaken for 2 h; *i.e*., 45 °C for the formation of DPPC multilamellar liposomes, and 40 °C for the formation of PC/SM multilamellar liposomes [15].

Small unilamellar liposomes/ vesicles (SUVs) were prepared from these multilamellar liposomes before each measurement. The multilamellar liposomes were sonicated using a high intensity ultrasonic processor (Sonics Vibra Cell VCX 750) in an ice-cold bath for 15 min, as continuous repeating 10-s on-off intervals. The SUVs formed were used for EPR spectroscopy, while the multilamellar liposomes were used for DSC measurements. An *in-vitro* assay for quantitative determination of phospholipids was used for verification of the phospholipid concentrations (Phospholipid C; Wako Pure Chemical Industries, Ltd., Japan).

2.2. Electron Paramagnetic Resonance

The SUVs were first spin-labelled with either MeFASL (10,3), which was used to monitor the membrane properties close to the water–lipid interface, or MeFASL (2,11), which was used to monitor the properties in the middle of the membranes. For this, 35 μL aliquots of 10^{-4} M solutions of the spin probes in ethanol were dried in test-tubes, to obtain a uniform thin film of the probe on the walls of the test-tubes. Then 50 μL aliquots of the liposome suspensions (5 mg lipid/mL) were added. The suspensions were mixed for 10 min, and then 28.7 μL of the water-soluble CoQ_{10} formulation dissolved in ultrapure water (5 mg/mL CoQ_{10}) was added. The same volume of ultrapure water was used as the control. In the case of the pure CoQ_{10} solution in 99% ethanol at a concentration of 20 mg/mL, 7.2 μL were added, and the same amount of absolute ethanol was used as the control. The mixtures were vortexed for 5 min, transferred to 1-mm capillary tubes (Euroglas, Slovenia), and measured in an X-band EPR spectrometer (Elexsyse 500; Bruker, Germany) across the temperature range of 15 °C to 45 °C, at 10 °C intervals. The molar ratio of lipid to CoQ_{10} was 2:1.

The empirical correlation time (τ_{emp}) was calculated from the spectra using Equation (1) [16]:

$$\tau_{emp} = k\Delta H\left[\left(h_0 / h_{-1}\right)^{\frac{1}{2}} - 1\right] \quad (1)$$

where k is a constant typical for the spin probe, as 5.9387×10^{-11} mT^{-1} for MeFASL(10,3) and MeFASL(2,11) [17], and the line width (ΔH, in mT) and the height of the mid-field (h_0) and high-field (h_{-1}) lines were obtained from the EPR spectra. This provided rough estimates of the ordering and dynamics of the spin-probe motion and the changes caused by the incorporation of the CoQ_{10} or carriers into the membrane in each sample. A short empirical correlation time indicated low ordering of the phospholipid acyl chains, a fast motion of the nitroxide group of the spin probe, and consequently a high average membrane fluidity.

Differential Scanning Calorimetry

The excess heat capacity, $\langle c_p \rangle$, versus temperature profiles for the thermally induced phase transitions of the DPPC lipid (0.5 mg/mL) in the presence of CoQ_{10} (molar ratios, 1:1, 2:1, 4:1) were monitored using a Nano DSC series III DSC system (Calorimetry Science, Provo, USA). The samples were degassed under

vacuum and loaded into the calorimetric cell, in which they were heated/cooled repeatedly in the temperature range from 10 to 70 °C, 1 °C/min. The first DSC scan was used to obtain values of the transition temperature of the main transition, T_m, pre-transition, T'_m, the calorimetric enthalpy of the main gel-to-liquid transition, ΔH_{cal} and the enthalpy of petransition $\Delta H'_{cal}$. Subsequent scans were used to assess the reversibility of the phase transition. Transition enthalpies, ΔH_{cal}, were calculated from the area under the $<c_p>$ vs. T curves using the OriginPro 8.1 software (OriginLab Corporation, USA) (Equation. 2), where T_1 and T_2 are the temperatures where the transition begins and ends [18].

$$\Delta H = \int_{T_1}^{T_2} C_p dT \quad (2)$$

RESULTS

Electron Paramagnetic Resonance Spectroscopy

For the EPR spectroscopy, Figure **1** shows the temperature dependence of the empirical correlation time (τ_{emp}) for the spin probes MeFASL (10,3) and MeFASL (2,11) in the PS/SM SUVs in the presence of CoQ_{10}. With respect to the positions of the nitroxide groups, MeFASL (10,3) reports on the membrane fluidity characteristics close to the water–lipid interface,

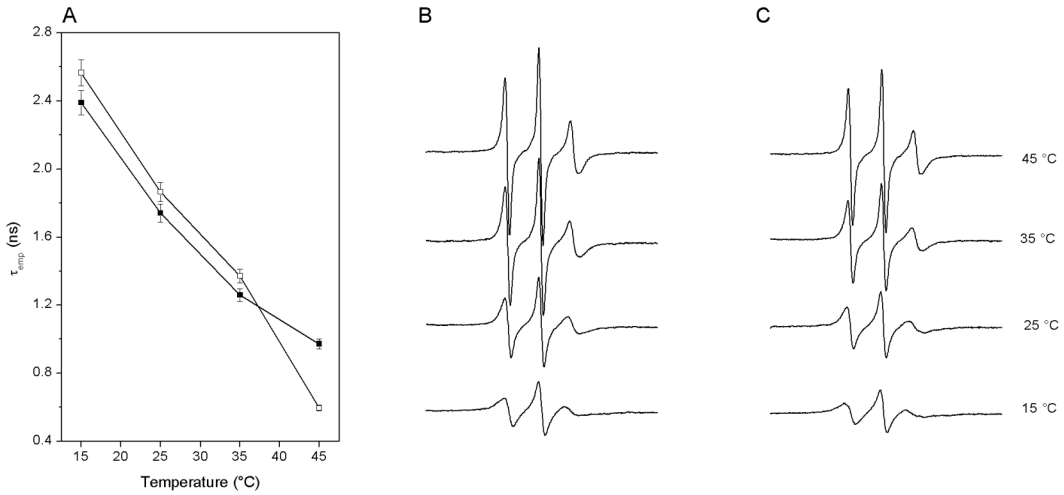

Figure 1. Temperature dependence of the empirical correlation time τ_{emp} of the spin probes MeFASL(10,3) in the PS/SM SUV (molar ratio, 2.4:1) without (control, ■), and with ethanol solution of CoQ_{10} (□). **(A)**. Experimental EPR spectra of MeFASL(10,3)-labelled PC/SM SUVs in the presence of ethanol **(B)** and ethanol solution of CoQ_{10} (lipid:CoQ_{10} ratio 2:1) **(C)**.

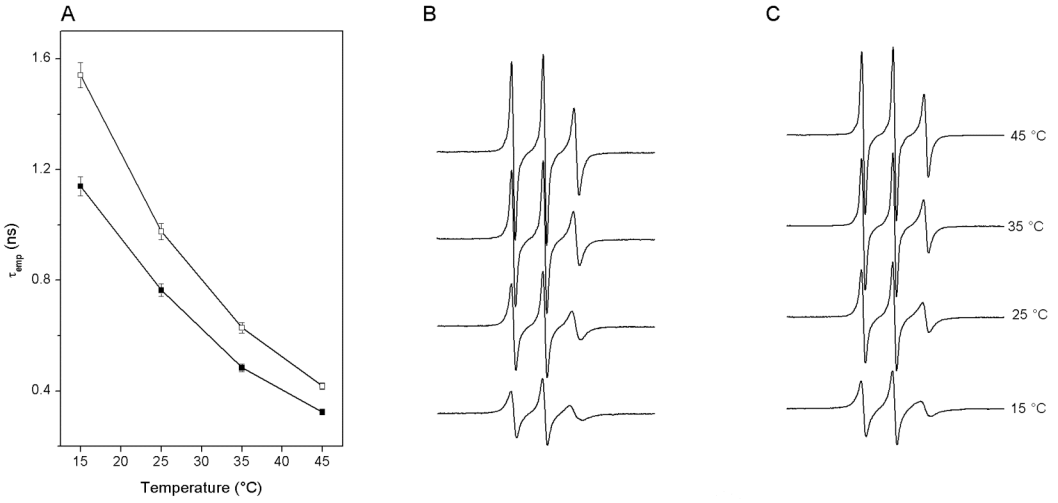

Figure 2. Temperature dependence of the empirical correlation time τ_{emp} of the spin probes MeFASL (2,11) in the PS/SM SUV (molar ratio, 2.4:1) without (control, ■), and with ethanol solution of CoQ_{10} (□). **(A)**. Experimental EPR spectra of MeFASL (10,3)-labelled PC/SM SUVs in the presence of ethanol **(B)** and ethanol solution of CoQ_{10} (lipid:CoQ_{10} ratio 2:1) **(C)**.

while MeFASL (2,11) reports on the changes in the middle of the bilayer [19].

In the presence of the CoQ_{10}, there was a slight increase (approximately 0.2 ns) in the empirical correlation time τ_{emp} of the spin probe MeFASL (10,3) compared to the ethanol only control over the temperature range from 15 °C to 35 °C. At 45°C, the presence of the CoQ_{10}, caused a decrease of the empirical correlation time compared to control sample (Figure **1**). This effect was more pronounced for the middle of the membranes, as revealed by the MeFASL(2,11) (Figure **2**), where a larger increase in the correlation time was observed througout the measured temperature range.

Differential Scanning Calorimetry

From the DSC measurements on the multilamellar liposomes, the changes in the enthalpy and the temperatures of the phase transitions of the lipids from the gel-to-liquid-crystalline state can be determined. The thermodynamic values of the phase transitions of the DPPC multilamellar liposomes for the full range of the samples tested are given in the Table **1**.

In SUV suspensions that are composed of mixtures of different lipids (*e.g.*, PC/SM in the molar ratio of 2.4:1), phase transitions cannot be determined in the temperature range from 0 °C to 100 °C using DSC. Since only pure lipid systems of saturated fatty acids have sharp phase transitions that can be easily determined across a narrow temperature interval [20], DPPC multilamellar liposomes were used for DSC experiments.

When the ethanol solution of CoQ_{10} (DPPC to CoQ_{10} ratio, 1:1) was added to the DPPC multilamellar liposomes after their formation, there were no changes in the thermal properties of this DPPC. When the CoQ_{10} was added (molar ratio, 1:1) prior to the DPPC multilamellar liposomes, there was a slight decrease in the enthalpy (23.3 ±0.5 kJ/mol) and an additional peak at 48.1 ±0.1 °C (ΔHcal = 38.7 ±0.5 kJ/mol) that corresponded to CoQ_{10} aggregates in the lipid bilayer. When lower molar ratios of CoQ_{10} were tested (DPPC to CoQ_{10} molar ratio 2:1 and 4:1) (Figure **3**), the CoQ_{10} fraction in an aggregated state decreased, (ΔHcal = 6.9 ±0.5 kJ/mol at molar ratio 2:1 and ΔHcal = 1.5 ±0.5 kJ/mol at molar ratio 4:1) in proportion to its concentration in the DPPC multilamellar liposomes.

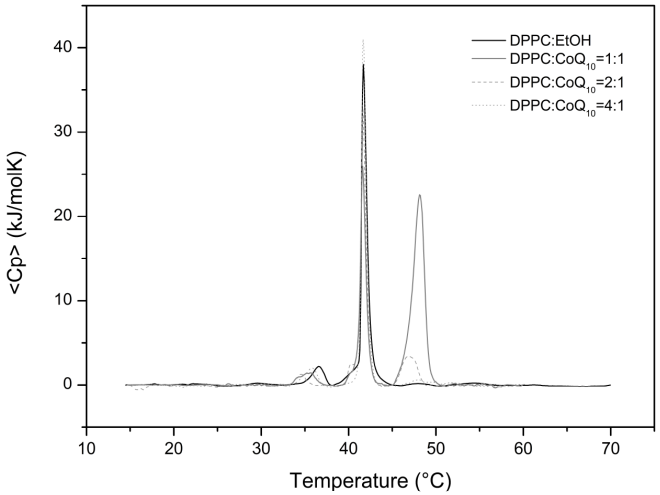

Figure 3. DSC thermograms of DPPC lipid vesicles (black line) prepared with pure CoQ_{10} with DPPC:CoQ_{10} molar ratio of 1:1 (grey solid line), 1:2 (grey dashed line) and 1:4 (grey dotted line) at pH 7.0.

Table 1. Thermodynamic Profile of the Phase Transitions of the DPPC Multilamellar Liposomes in the Presence of CoQ_{10} (DPPC: CoQ_{10} Molar Ratios of 1:1, 2:1 and 4:1)

DPPC Additions	T'_m [a] (°C)	$\Delta H'_{cal}$ [b] (kJ/molK)	T_m [c] (°C)	ΔH_{cal} [d] (kJ/molK)
None	36.7 ±0.1	3.5 ±0.5	41.8 ±0.1	35.5 ±0.5
CoQ_{10} 1:1	35.6 ±0.1	3.0 ±0.5	41.6 ±0.1	23.3 ±0.5
			[e]48.1 ±0.1	38.7 ±0.5
CoQ_{10} 2:1	35.1 ±0.1	2.8 ±0.5	41.6 ±0.1	27.0 ±0.5
			[e]46.9 ±0.1	6.9 ±0.5
CoQ_{10} 4:1	35.8 ±0.1	3.3 ±0.5	41.3 ±0.1	27.8 ±0.5
			[e]47.9 ±0.1	1.5 ±0.5

[a]T'_m, phase pretransition temperature
[b]$\Delta H'_{cal}$, pretransition enthalpy
[c]T_m, main phase transition temperature
[d]ΔH_{cal}, enthalpy of the gel-to-liquid crystalline transition
[e]temperature and enthalpy of the phase transition corresponding to CoQ_{10}

DISCUSSION AND CONCLUSIONS

The aim of this study was to better understand the interaction between CoQ_{10} and model lipid membranes. Based on the EPR results we can conclude that addition of CoQ_{10} resulted in an increase in the empirical correlation time which indicated that CoQ_{10} promotes a decrease in the dynamics of the lipid acyl chains; *i.e*., it increases the ordering of membrane interior. The effect on the water–lipid interface was far less pronounced.

Investigation of the energetic impact of CoQ_{10} on the DPPC gel-to-liquid phase transition revealed that addition of the CoQ_{10} prior to the DPPC multilamellar liposomes, causes a slight decrease in the enthalpy and an occurrence of an additional peak between 46.9 and 48.1 ±0.1 °C that corresponded to CoQ_{10} aggregates in the lipid bilayer. This suggested that the CoQ_{10} in the bilayer does not significantly perturb the enthalpic stability of DPPC (the gel-to liquid transition does not change) but additionally stabilizes the lipid bilayer due to the aggregation of CoQ_{10} within the lipid bilayer (the new peak at higher temperatures), as previously reported by Katsikas and Quinn [21]. According to Ulrich *et al*. CoQ_{10} is hydrophobic enough for a large fraction to be located in a mobile pool near the centre of the lipid bilayer [13]. When lower molar ratios of CoQ_{10} were tested, the CoQ_{10} fraction in an aggregated state decreased in proportion to its concentration in the DPPC multilamellar liposomes, suggesting the effect is dose dependent.

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