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Interactions between Ubiquinones and Phospholipid Bilayers

A Spin-Label Study

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The effect of two ubiquinones of different side chain length (Q-3; Q-9), on the fluidity of phospholipid vesicles has been investigated using stearic acid spin labels. While both oxidized quinones have a disordering effect on the lipid bilayers, the reduced forms behave in an opposite way, in that Q-3 enhances and Q-9 decreases the order of the bilayer. The ordering effect of reduced Q-3 and the attendant decreased motional freedom in the bilayer might be the result of the insertion and stacking of the quinone between the phospholipid molecules in the bilayer. Such insertion might be related to the incapability of short-chain quinones in restoring NADH oxidation in Q-depleted mitochondria.

The exact location and role of ubiquinone in the respiratory chain are still argument of debate. A pool function of ubiquinone as a mobile component between complex I and II and complex III has been suggested (1–3).

Studies, carried out in our laboratory, have provided evidence that short-chain Q[2] homologues are unable to restore NADH oxidation in Q-depleted mitochondria and have shown an inhibitory effect on NADH oxidation in submitochondrial particles (4). The hypothesis has been put forward that short-chain Q's, in contrast with long-chain Q's, are unable to cross the lipid bilayer in the inner mitochondrial membrane (5, 6).

This suggestion has prompted us to investigate the effect of Q's having different side chain length on the order and fluidity of lipid bilayers. We have chosen as a model system, vesicles of EL, DOL, whose purity was checked by thin-layer chromatography, or of soybean phospholipids (Asolectin) and we have investigated the bilayer fluidity by means of esr technique using spin-labeled stearic acid as probes. Q-3 and Q-9 have been used as representatives of short and long-chain quinones since these have already been employed in our previous studies (4–6).

MATERIALS AND METHODS

Lipid vesicles were obtained by sonic irradiation of EL, DOL (from Serdary Research Laboratory Inc., Ontario, Canada), or soybean phopholipids (Asolectin, from Associated Concentrates, Woodside, N.Y.).

EL and DOL, dissolved in chloroform, were dried under a stream of nitrogen. Tris–acetate, 20 mM (pH 8.0), EDTA, 1 mM, buffer was added to give a suspension of 6 mg PL/ml, homogenized by means of a Potter-Elvehjem homogenizer equipped with a Teflon pestle, and sonicated under nitrogen, at 4°C, using a MSE 150-watt ultrasonic disintegrator model MK2 until the suspension became translucent.

Asolectin vesicles were prepared as described previously (7).

The final concentration of the PL dispersion, determined as total phosphorus, was assayed according to Marinetti (8).

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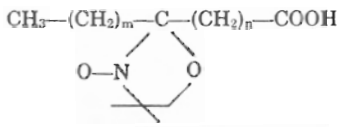
[2] Abbreviations used: Q, ubiquinone; Q-3, ubiqui-



where used as spin probes. In particular we used the I(1,14) and I(12,3) stearic acid spin labels. They were obtained from Synvar Co., Palo Alto, California, and were dissolved in pure ethanol at a concentration of 10 mM.

The incorporation of quinones into the vesicles was achieved by adding a proper amount of their ethanolic solutions to the PL-suspension and by vigorous shaking in a Vortex mixer for 2–3 min, followed by incubation of the samples at 37°C for 5 min. Spin labels were added also by vigorous shaking in a Vortex mixer for 2–3 min and the samples were allowed to equilibrate at room temperature for 5 min. The PL to spin label molar ratio was about 100:1. The quinones were reduced directly inside the vesicles by addition of $NaBH_3CN$ (from EGA Chemie, D 7924 Steinheim/Albuch, West Germany), in crystals: the addition of the reducing agent did not affect appreciably the physical state of the PL bilayers, as indicated by the data shown in Table I. In other experiments, quinones previously reduced and purified (9) were added directly to PL vesicles. Ethanol had very little effect on the mobility of the spin labels, at least at low concentrations. Only at the highest concentrations used, ethanol showed some interference with the effects of quinone addition (cf. Table II).

All the esr spectra were recorded, at room temperature, using a Varian E-4 9.5 GHz spectrometer.

RESULTS

The spectra of the C-5 spin label in EL vesicles with and without Q-3 in its oxidized and reduced state are shown in Fig. 1. The spectra clearly exhibit probe ordering, proportional in this type of spin labels to the separation of the two hyperfine extrema, 2 T_{II} (10, 11). As usual (10), we shall take 2 T_{II} as empirical measure of the motional freedom of the spin-labeled chain segment of the probe and of its microenvironment. The values of 2 T_{II} in vesicles of EL, Asolectin, DOL containing Q-3 and Q-9 are reported in Table I. It has been pointed out (12) that measurements of ordering from the outer hyperfine separation rather than from a complete simulation of the spectrum can be subject to some error. In this study, however, we are not too interested in an absolute measure of order parameters but rather in the relative effect of Q-3 and Q-9 upon them. The values of 2 T_{II} for the C-5 probe have been determined as a function of Q concentration and are reported in Fig. 2. It can be seen that the addition of either

TABLE I

VALUES OF THE 2 T_{II} PARAMETER OF THE SPIN LABEL C-5 IN DIFFERENT SYSTEMS[a]

Phospholipids	Addition (mM)	2 T_{II} (gauss)	
		$-NaBH_3CN$	$+NaBH_3CN$
EL	None	51.2	51.2
	Q-3, 0.6	50.4	51.8
	Q-3, 1.0	49.8	52.0
	Q-9, 0.7	49.2	50.0
	Q-9, 0.4	50.0	50.4
Asolectin	None	51.2	51.2
	Q-3, 0.2	50.4	51.6
	Q-3 1.0	50.4	51.8
	Q-9 0.2	51.0	51.2
	Q-9 0.9	48.6	50.0
DOL	None	50.3	50.3
	Q-3, 0.2	50.1	51.3
	Q-9, 0.2	49.5	50.3

[a] All the results are the average of 4 reproducible experiments.

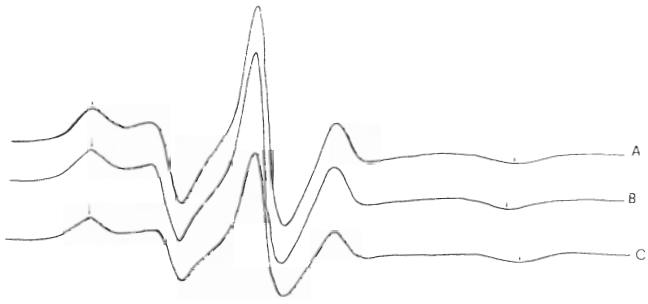


FIG. 1. Resonance spectrum of spin label stearic acid C-5 in: (A) egg-lecithin vesicles; (B) egg-lecithin vesicles + Q-3 oxidized 0.1 mM; (C) egg-lecithin vesicles + Q-3 reduced 0.1 mM. The arrows indicate the 2 T_{II} parameter.

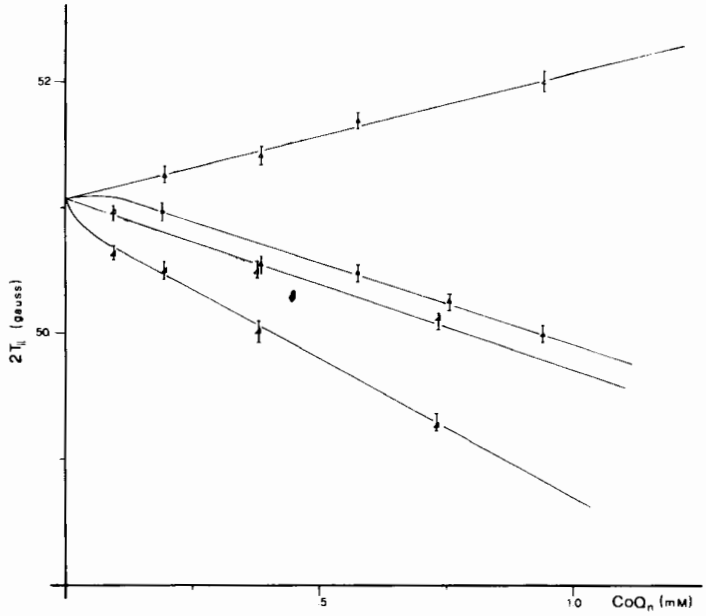


FIG. 2. Plots of the 2 T_{II} parameter of stearic spin label C-5 vs. Q-n concentration (mM). ▲——▲, egg-lecithin vesicles + Q-3 oxidized; △——△, egg-lecithin vesicles + Q-3 reduced; ▼——▼, egg-lecithin vesicles + Q-9 oxidized; ▽——▽, egg-lecithin vesicles + Q-9 reduced.

Q-3 or Q-9 in the oxidized state lowers 2 T_{II} indicating that they both decrease the order. However Q's in the reduced form behave rather differently: while reduced Q-9 still lowers 2 T_{II}, reduced Q-3, on the contrary, shows a noticeable ordering effect. The ordering effect of reduced Q-3 is found also when the quinone is added to lipid vesicles directly in its reduced form (Table II). This finding argues against the possibility that the ordering effect is the result of phospholipids being reduced by $NaBH_3CN$ with the quinone acting as a catalyst.

In order to investigate also changes in fluidity of the inner region of the bilayer, other experiments have been performed using the C-16 probe. The spectra consisted in every case of three lines only, thus hinting that, as in other similar experiments (13, 14) the C-16 probe segmental motion can be considered as effectively isotropic. We have therefore used as a simple empirical measure of the membrane fluidity a pseudo-isotropic correlation time calculated with the usual Kivelson relation (15).

Table III reports the correlation times of C-16 in lipid vesicles containing reduced and oxidized quinones; it appears that Q-3 in its reduced state produces an increase of the correlation time, which indicates a slower motion of the label. In order to compare the effects of Q's in the oxidized and

TABLE II

CONTROL EXPERIMENT SHOWING THE EFFECTS OF ETHANOL AND OF REDUCED Q-3 ON THE 2 T_{II} PARAMETER IN PHOSPHOLIPID VESICLES

Phospholipid	Addition (mM)	Final ethanol concentration (%, v/v)	2 T_{II} (gauss)	Change of 2 T_{II} (gauss)
DOL	—	0	50.6	—
	—	2	50.7	+0.1
	—	4	50.5	−0.1
	—	8	49.8	−0.8
	Q-3, 0.4	4	49.7	−0.9
	Q-9, 0.2	2	49.5	−1.1
	Q-9, 0.8	8	48.6	−2.0
Asolectin	—	0	45.6	—
	—	2	45.6	0
	Q-3, 0.2	2	45.1	−0.5
	Reduced Q-3, 0.2[a]	2	46.5	+0.9

[a] Q-3 was reduced with Na dithionite with the method of Rieske (9) and resuspended in absolute ethanol after washing of excess dithionite in a two-phase system and evaporation to dryness.

in the reduced states on the correlation time of C-16, we have considered the ratios between these two values. A decrease (increase) of this ratio is an indication of a decreased (increased) mobility of the spin label in presence of reduced Q in comparison with the mobility when the oxidized form is present. Figure 3 shows a plot of this ratio against the concentration of Q: it can be observed that only Q-3 is able to induce a decrease of this ratio.

TABLE III

VALUES OF THE PSEUDO-CORRELATION TIME τ_c OF THE SPIN LABEL C-16 IN DIFFERENT SYSTEMS[a]

Phsospho-lipids	Addition (mM)	τ_c 10^{-9} s	
		$-NaBH_3CN$	$+NaBH_3CN$
EL	None	0.8	0.8
	Q-3, 0.4	0.8	1.0
	Q-3, 0.8	0.7	1.1
	Q-9, 0.2	0.8	0.8
	Q-9, 0.4	0.7	0.8
Asolectin	None	0.9	0.9
	Q-3, 0.2	0.9	1.1
	Q-3, 0.8	0.8	1.3
	Q-9, 0.2	0.8	0.9
DOL	None	0.8	0.8
	Q-3, 0.75	0.7	1.1
	Q-9, 0.75	0.6	0.7

[a] All the results are the average of 4 reproducible experiments.

CONCLUSIONS

The results of this investigation seem to support the suggestion that Q's with different side chain lengths, when in their reduced form, have different effects on the bilayer fluidity. This could be ascribed to the type of interaction with the lipid bilayer. The fluidization due to the presence of Q's in the oxidized state, as shown by the decrease of the 2 T_{II} parameter for C-5 probe and by the shorter correlation time for the C-16 probe, could be explained by the disordering effect of the side chain of Q intercalating in the hydrocarbon region of the lipid bilayer. The completely different effect of Q's in the reduced state is more difficult to explain.

One possibility is that in reduced Q's a very important role is played by their enhanced polarity. It is likely that reduced Q-3, which has the length of average phospholipid molecules, becomes inserted in its full

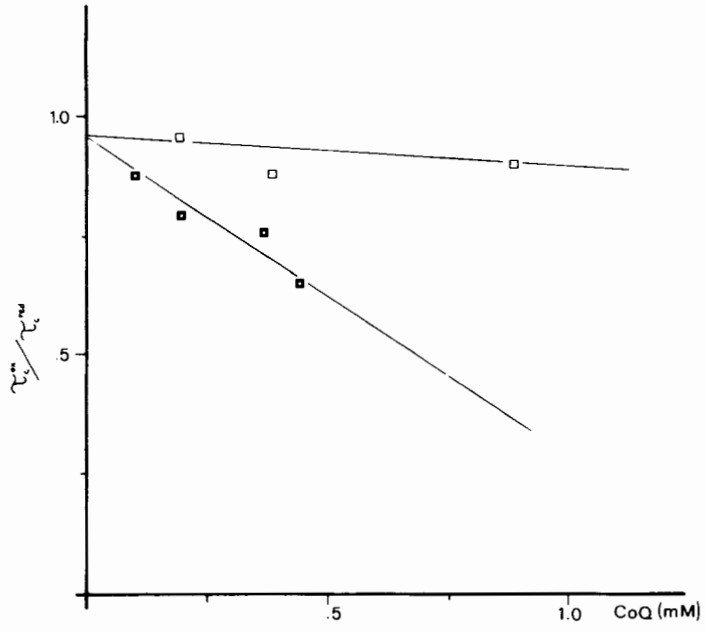


FIG. 3. Plots of the τ_c^{ox}/τ_c^{red} ratios of stearic and spin label C-16 vs. Q-n concentration (mM). ■——■, Egg-lecithin vesicles + Q-3; □——□, egg-lecithin vesicles + Q-9. A decrease of the ratios means a decrease in fluidity of the bilayer.

length between the fatty acyl chains, its interaction being possibly strengthened by hydrogen bonds linking the hydroquinone ring with the phospholipid polar heads (16). In the case of reduced Q-9 such an arrangement would be forbidden by the excessive length of the side chain.

In any case the fact that reduced Q-3 enhances the order and consequently decreases the fluidity of the lipid bilayer could be in good agreement with the inhibitory effect of Q-3 on mitochondrial respiration (4–6). In fact the quinone, once reduced, could become trapped in the lipid bilayer and prevented from crossing the membrane.

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