Fluidizing effect of endogenous ubiquinone in bovine heart mitochondrial membranes

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Extraction of endogenous ubiquinone from lyophilized beef heart mitochondria results in increases of both the order parameter of the spin label 5-NS and of the rotational correlation time of 16-NS; reconstitution with the pentane extract results in restoration of the original spectral parameters. On the other hand, addition of purified ubiquinone homologs restores the original spectra only in the case of 16-NS, whereas the order parameter of 5-NS is restored by addition of mixed phospholipids. The same amounts of ubiquinone homologs incorporated in mixed phospholipid vesicles induce much lower effects. It is suggested that ubiquinone in mitochondria is intercalated with the lipid chains of the membrane in such a way to perturb the fluidity of the hydrophobic core.

Ubiquinone *Spin label* *Intercalation* *Fluidity*

1. INTRODUCTION

The localization of quinones in energy conserving membranes is receiving considerable attention [1] for a better evaluation of their role in electron transfer and energy conservation. In mammalian mitochondria ubiquinones having long isoprenoid chains (largely Q_{10}) are the physiological forms, although short-chain homologs (Q_1–Q_3) are widely used as substrates for partial reactions of the respiratory chain [2]. Several approaches have been attempted to investigate the physico-chemical properties of ubiquinone in model systems and natural membranes, including UV [3] and fluorescence spectroscopy [4], NMR [5], differential scanning calorimetry [6], and EPR of the semiquinone form [7].

An approach, which might give further information on the state and localization of the quinone is represented by the perturbation, if any, induced by this molecule on the lipid bilayer. It is well known that several liphopilic compounds affect membrane fluidity; the effects of cholesterol and fat-soluble vitamins [8–10] have been useful to assess their localization in the membranes.

It was previously found by use of EPR spin labels [11] and fluorescence polarization of perylene [12] that ubiquinones induce fluidity changes in model lipid bilayers. In particular, whereas the short ubiquinone homologs, particularly in their reduced forms, were found to increase the rigidity and order of the bilayers, the long physiological ubiquinones were reported to enhance the bilayer fluidity.

We report here the effect of ubiquinone extraction and reconstitution on the fluidity of mitochondrial membranes, studied by the use of two lipid spin labels, viz 5- and 16-doxyl stearate derivatives, that probe the surface and the core of the membrane, respectively.

2. MATERIALS AND METHODS

Beef heart mitochondria [13] were depleted of endogenous ubiquinone by lyophilization and pentane extraction [14]. Occasionally a further extraction with 10% acetone in pentane was used to re-

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move additional ubiquinone [15]. Reconstitution was performed as in [16]. NADH oxidase was assayed as in [15]; also for analytical determinations cf. [15].

Phospholipid vesicles were prepared by sonic irradiation of mixed soybean phospholipids [17] (asolectin, Associated Concentrates, NY) purified according to [18], in the presence or absence of different proportions; of ubiquinone homologs.

The lipid–soluble spin labels used were 5- or 16-(*N*-oxyl-4′,4′-dimethyl-oxazolidine) derivatives of stearic acid (5-NS and 16-NS, respectively), obtained from Syva, Palo Alto, CA, and employed as in [19]. ESR spectra were recorded with a Varian E-4 ESR spectrometer as in [20]. In general the ordering of the molecules is related to the position and the separation (hyperfine splittings) of the resonance lines, while the dynamical properties are reflected in their width [21]. The ESR spectra of 5-NS exhibit probe ordering, proportional to the separation of the two hyperfine extrema ($2T_{\parallel}$). From the hyperfine values, order parameters S_n, corrected for polarity, were calculated according to [22]. The ESR spectra of 16-NS consist of three lines only, indicating that the segmental motion can be considered effectively isotropic; we have therefore used as an empirical measure of lipid fluidity a pseudoisotropic rotational correlation time τ_c, calculated according to [23]. Although the parameters used to study 5-NS and 16-NS mobility are not directly comparable, their changes depend on modification of the physical state of the environment in which they are localized [20].

3. RESULTS AND DISCUSSION

Repeated pentane washings of lyophilized mitochondria [14] result in extraction of about 95% of endogenous ubiquinone [15]; a further extraction by 10% acetone in pentane removes additional quinone with residual levels less than 0.1 nmol/mg protein. Reconstitution results in reincorporation of the added quinone to levels close to the physiological ones. NADH oxidase activities in pentane-extracted mitochondria are within 10% of the original activities (table 1).

Ubiquinone extraction from lyophilized mitochondrial membranes induces a significant increase of the order parameter S_n of 5-NS, indicating ordering of the probe (fig.1). Readdition of the pentane extract restores the original value of S_n, whereas addition of either Q_3 or Q_{10}, although restoring completely succinoxidase activity [15], fails to change significantly the membrane order (fig.2). It is suggested that the effect is largely the result of extraction by pentane of lipids, since addition of purified mixed soybean phospholipids in pentane to the extracted mitochondria also induces a decrease of S_n: for example, in one experiment S_n of 0.74 in extracted mitochondria decreased to 0.70 by addition of the pentane extract and to

Table 1

Effect of pentane extraction on the ubiquinone content of lyopilized mitochondria

Mitochondria	Range of Q content (nmol/mg protein)	NADH oxidase (%)
Lyophilized	3.50–4.80	100
Extracted (pentane)	0.20–0.37	0–7
Extracted (pentane + acetone)	0.08–0.10	0
Reintegrated (pentane extract)	2.60–4.00	67–89

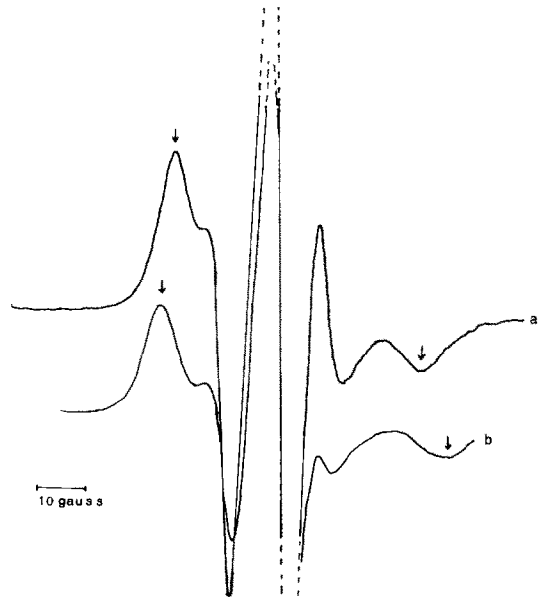

Fig.1. Spectra of 5-NS in lyophilized mitochondria (a) and pentane-extracted mitochondria (b). The arrows point to the hyperfine splitting extrema.

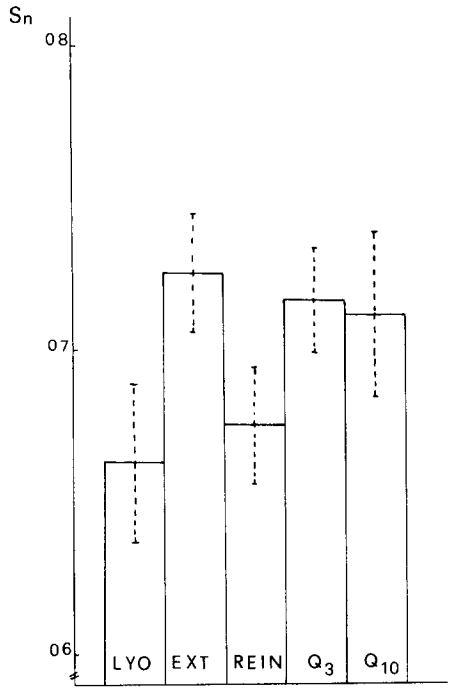

Fig.2. Effect of ubiquinone extraction and reconstitution on the order parameter S_n of 5-NS. The bars refer to standard deviations. The data are averages of 4 different preparations. Each spectrum was repeated 3 times without any change in the different determinations. Lyo, lyophilized; ext, extracted; rein, reintegrated with pentane extract. Statistical significance was established by Student's t-test and referred to the extracted samples.

0.69 by addition of phospholipids only. Clearly the membrane order is mostly affected by the phospholipid content. Analysis of several preparations has given an average of 5 μg lipid phosphorus extracted (about 26% of the total membrane content). This result is unexpected, since it was originally assumed that the solvent extracts only neutral lipids from lyophilized mitochondria [14]. It has been reported, however [24,25], that several conditions may induce extraction of phospholipids by nonpolar solvents.

The results with the deep spin label 16-NS are rather different (fig.3,4); pentane extraction induces a significant increase of τ_c, and significant restoration is achieved by addition of either the pentane extract or Q_{10} ($p < 0.01$). Phospholipids alone fail to enhance the mobility of 16-NS to any significant extent.

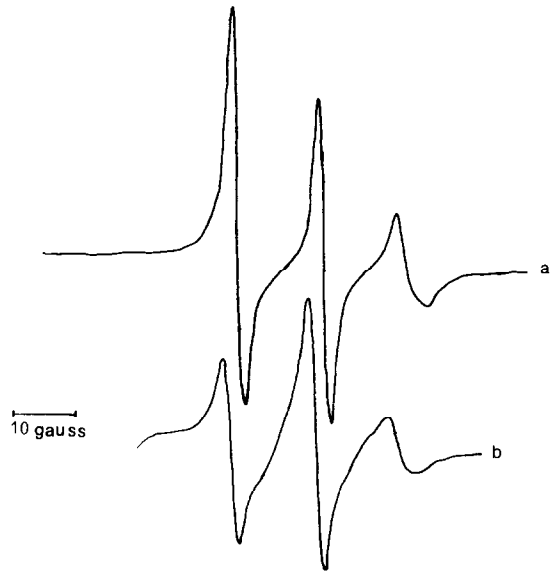

Fig.3. Spectra of 16-NS in lyophilized mitochondria (a) and pentane-extracted mitochondria (b).

It is tempting to suggest that a fluidizing effect of ubiquinone is present particularly in the membrane hydrophobic core (probed by 16-NS); it is however not possible to compare properly values

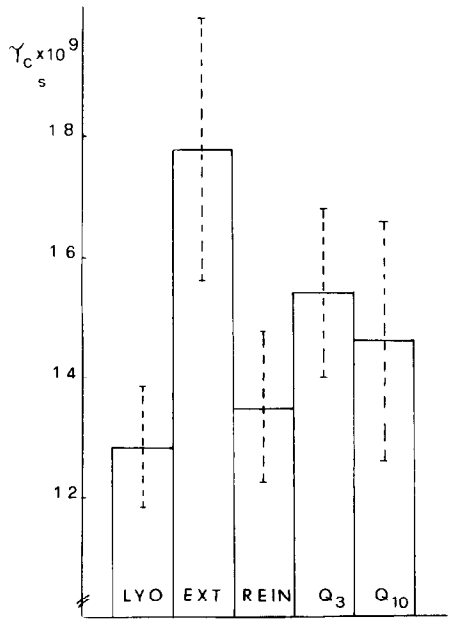

Fig.4. Effect of ubiquinone extraction and reconstitution on the rotational correlation times τ_c of 16-NS. For statistical analysis cf. legend to fig.2.

calculated from different spectral parameters of the two probes.

For comparison we have investigated the effect of different proportions of ubiquinone homologs in lipid vesicles from asolectin. In these experiments the quinones were incorporated by sonication with the lipids, and Q_9 was used preferentially to Q_{10} to avoid a non-homogeneous distribution in different phases [6]. The results, shown in table 2, indicate that the effects of both Q_3 and Q_9 on either the order parameters of 5-NS or the rotational correlation times of 16-NS are negligible, when the ubiquinones are incorporated at ratios of 8–24 nmol/mg phospholipid. A fluidizing effect was previously found [11] when ubiquinone homologs were added in higher amounts to lipid vesicles. A strong rigidizing effect of reduced Q_3 found in lipid bilayers could not be investigated in this study, because the reduced respiratory chain destroys the spin label signals by fast reduction of the nitroxides [26].

Although no direct model can be built from the results of the experiments about the disposition of ubiquinone in the mitochondrial membrane, it clearly appears that this molecule perturbs the hydrocarbon membrane core. A disposition of ubiquinone intercalated between the two lipid monolayers, parallel to the membrane surface [27], would not significantly perturb the bilayer; dispositions compatible with the present data would be either an irregular folding in the hydrophobic core or a micellar structure inserted within the membrane [28]. A stacking of the quinone molecules within the fatty acyl chains would induce an ordering effect, comparable to that obtained by cholesterol [8].

Table 2

Effect of Q homologs on the order parameter S_n of 5-NS and the rotational correlation times τ_c of 16-NS in asolectin vesicles

Addition (nmol/mg P1)		5-NS (S_n) (±SD)	16-NS τ_c(ns) (±SD)
	–	0.54 ± 0.01	1.15 ± 0.13
Q_3	(8)	0.56 ± 0.02	1.15 ± 0.20
	(16)	0.56 ± 0.02	1.24 ± 0.22
	(24)	0.56 ± 0.03	1.23 ± 0.13
Q_9	(8)	0.56 ± 0.02	1.11 ± 0.12
	(16)	0.56 ± 0.04	1.14 ± 0.08
	(24)	0.54 ± 0.04	1.20 ± 0.13

The differences found between the effects on the fluidity of mitochondrial membranes and of phospholipid vesicles may point out that mitochondrial proteins are somehow involved. It should be stressed that the fluidity of phospholipid vesicles is very high per se: the strong immobilizing effect of membrane proteins on phospholipids is well known [8]; alternatively the low radius of curvature of sonicated vesicles may affect the distribution of ubiquinones in the bilayer [5].

The present investigation throws light on the physical state of endogenous ubiquinone within the mitochondrial membrane; in addition, it also suggests a possible role of ubiquinone in establishing optimal fluidity of the lipid bilayer. The finding of high levels of Q_{10} in the other membranes as well as in mitochondria [29] suggests that ubiquinone, besides having a well known redox function, is also involved in regulation of the fluidity level of several biological membranes.

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