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The mobility of a fluorescent ubiquinone in model lipid membranes. Relevance to mitochondrial electron transport

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The diffusion and location of a functional, fluorescent ubiquinone molecule, NBDHA-Q, were determined as a function of temperature using microscopic observation, fluorescence recovery after photobleaching and fluorescence spectroscopy in protein-free, pure-lipid dimyristoylphosphatidylcholine and dimyristoylphosphatidylcholine/cholesterol multibilayers. The data reveal that in a liquid-crystalline membrane (1) ubiquinone is highly mobile, (2) ubiquinone uniformly diffuses laterally with the same diffusion coefficient ($3 \cdot 10^{-8}$ cm^2/s at 25°C) as the phospholipids in which it resides, (3) the diffusion coefficients of ubiquinone and phospholipid both decrease at the exothermic phase transition of the phospholipid, (4) cholesterol affects the diffusion coefficients of ubiquinone and phospholipids to the same degree, (5) cholesterol induces a lateral phase separation progressively excluding ubiquinone from cholesterol-containing domains. These data suggest that ubiquinone does not reside at the membrane surface or in the mid-plane for any appreciable length of time. Rather, the data indicate that ubiquinone is highly mobile laterally and transversely, spending the majority of its time in the acyl chain region of the membrane, where its lateral and transverse diffusion is limited by the lateral diffusion and the transverse microviscosity gradient of the phospholipids and where its lateral location can be affected by the presence of cholesterol. In addition, based upon a comparison of the diffusion coefficients for ubiquinone, phospholipids and mitochondrial redox complexes, we hypothesize that no significant portion of the ubiquinone pool remains bound to redox complexes for any significant length of time relative to that for electron transport as resolvable by fluorescence recovery after photobleaching.

Introduction

The mobility and location of ubiquinone is thought to be of fundamental importance to understanding the bioenergetic function of this lipoidal molecule in mitochondrial electron transport **. The concept of ubiquinone as a pool of mobile carriers has been developed and refined over the years [1–6]. Experimental evidence for the physical mobility of ubiquinone was shown in the kinetic results of Schneider et al. [7–10]. However, a direct determination of a lateral

Abbreviations: FRAP, fluorescence recovery after photobleaching; FQ, fluorescence quenching; D, lateral diffusion coefficient; Q, ubiquinone, (coenzyme Q); Q_n, ubiquinone with 'n' isoprenoid units; NBDHA-Q (Q_0Q_{10}NBDHA), fluorescent ubiquinone analogue composed of NBD hexanoic acid conjugated to 2,3-dimethyl-5-methyl-6-(10-hydroxyldecyl)quinone; NBD-Q_{10}, a fluorescent ubiquinone$_{10}$ analogue composed of NBD conjugated to the benzoquinol headgroup of ubiquinone; DiI, 3,3'-dioctyldecylindocarbocyanine; DPH, diphenylhexatriene; P, poise; DMPC, dimyristoylphosphatidyl choline; asolectin, soybean phospholipids; PE, phosphatidylethanolamine; MBL, multibilayers; SUV, single unilamellar vesicles; T_m, main phase transition temperature.

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** Electron transfer refers to the actual transmission of reducing equivalents between redox components. Electron transport is inclusive of the overall process of electron transfer and lateral diffusion of redox components.

diffusion coefficient (D) for any ubiquinone remained unknown prior to reports from this laboratory on a fluorescent ubiquinone in mitochondrial inner membranes [11,12].

Chazotte and Hackenbrock [13,14] concluded that the bioenergetic function of ubiquinone in the mitochondrial inner membrane is mediated by a multicollisional, obstructed, long-range ***, diffusion process and that this process is rate-limiting for maximum (uncoupled) rates of ubiquinone-mediated electron transport based on studies with protein-diluted (phospholipid-enriched) mitochondrial inner membranes by fluorescence recovery after photobleaching (FRAP). In marked contrast, Lenaz and co-workers [15–18], using fluorescence quenching (FQ), a short-range *** approach, concentrated mostly on pure-lipid single unilamellar vesicles (SUV) and reported D values for various ubiquinone analogues in excess of three orders of magnitude greater than those reported by us and concluded that ubiquinone diffusion is not rate-limiting in electron transport. The important test in resolving these differences would be FRAP measurements in protein-free membranes where long-range and short-ranges D values should be equivalent.

It has been conjectured that the mobility of ubiquinone is affected by its location in the bilayer's transverse plane. Since fluidity gradients have been reported to occur along the phospholipid acyl chains (e.g., Ref. 19) it has been argued that very rapid diffusion might be possible if ubiquinone were specifically located at the bilayer midplane and not intercalated with the phospholipid acyl chains [15,16]. This specific location is thought to have the lowest viscosity. Based on various conflicting physical measurements of ubiquinone by fluorescence, NMR and calorimetry, primarily in model membranes, arguments have been advanced for the absence and the presence of a separate (not interacting with phospholipid acyl chains) or enriched ubiquinone-phase in the bilayer midplane as well as for multiple locations in the bilayer [20–31]. Thus, the transverse location of ubiquinone is still debated due to these conflicting results.

We present here studies on the two-dimensional mobility of a functional, fluorescent ubiquinone analogue as a function of temperature and cholesterol concentration in a protein-free membrane. We report that within experimental error the D values for phospholipid and the ubiquinone molecule were the same. Our findings lead us to argue against the membrane surface or midplane as specific sites for the location of ubiquinone. Rather, for the majority of the time ubiquinone appears to be located in the region formed by the acyl chains of the membrane where its lateral and transverse diffusion is limited by the lateral diffusion and transverse microviscosity gradient of the phospholipids and where its lateral location can be affected by cholesterol. Comparison of the D values of phospholipid and ubiquinone in this study with our previous studies on phospholipids, ubiquinone and redox complexes in inner membranes leads us to hypothesize that no appreciable portion of the inner membrane ubiquinone population remains bound to redox complexes for any significant length of time relative to electron transport.

*** Short-range diffusion is defined to be ≤ 10 nm by Eisinger et al. [60] and long-range diffusion is defined to be $\sim \mu$m.

Material and Methods

Multibilayer (MBL) membranes for FRAP measurements were prepared as described previously [32] using premixed lipid/fluorophore solutions (NBDHA-Q or 3,3'-dioctyldecylindocarbocyanine (DiI) as fluorophore). The lipid/fluorophore ratio of 4000/1 was chosen to be sufficient for fluorescence studies, low enough to eliminate membrane perturbation and no greater than the actual mole fraction (0.01–0.02) of ubiquinone in the inner mitochondrial membrane (cf. Ref. 24). The sealed, hydrated MBL membranes were attached to glass microscope slides and cycled several times between 4 and 37 °C prior to making FRAP measurements [32]. SUVs in suspension for fluorescence measurements were prepared from premixed lipid/NBDHA-Q (4000/1) solutions by hydration for 1–2 h at room temperature followed by sonication for 15 min at 24 W using a Branson Model 185 sonicator.

The fluorescent ubiquinone analogue, NBDHA-Q (Fig. 1), was synthesized by Drs. C.-A. Yu and L.Q. Gu (University Oklahoma, Stillwater, OK) as described elsewhere [33]. This functional analogue when reconstituted in ubiquinone-depleted mitochondrial inner membranes gives a ubiquinol-cytochrome c oxidoreductase activity of 6 μmol oxidized/min per mg protein [12]. DiI was obtained from Molecular Probes (Eugene, OR). Dimyristoylphosphatidylcholine (DMPC) and cholesterol were purchased from Sigma (St. Louis) or Applied Science (Deerfield, IL) and stored in chloroform/methanol (1:1, v/v) and were used without further purification. All chemicals were reagent grade and water was double distilled and deionized.

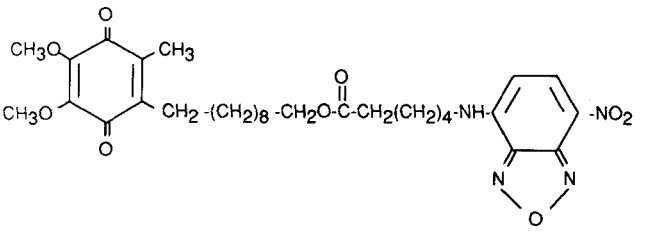

Fig. 1. Structural formula of the fluorescent ubiquinone, NBDHA-Q (Q_0C_{10} NBDHA). See Yu et al., [33].

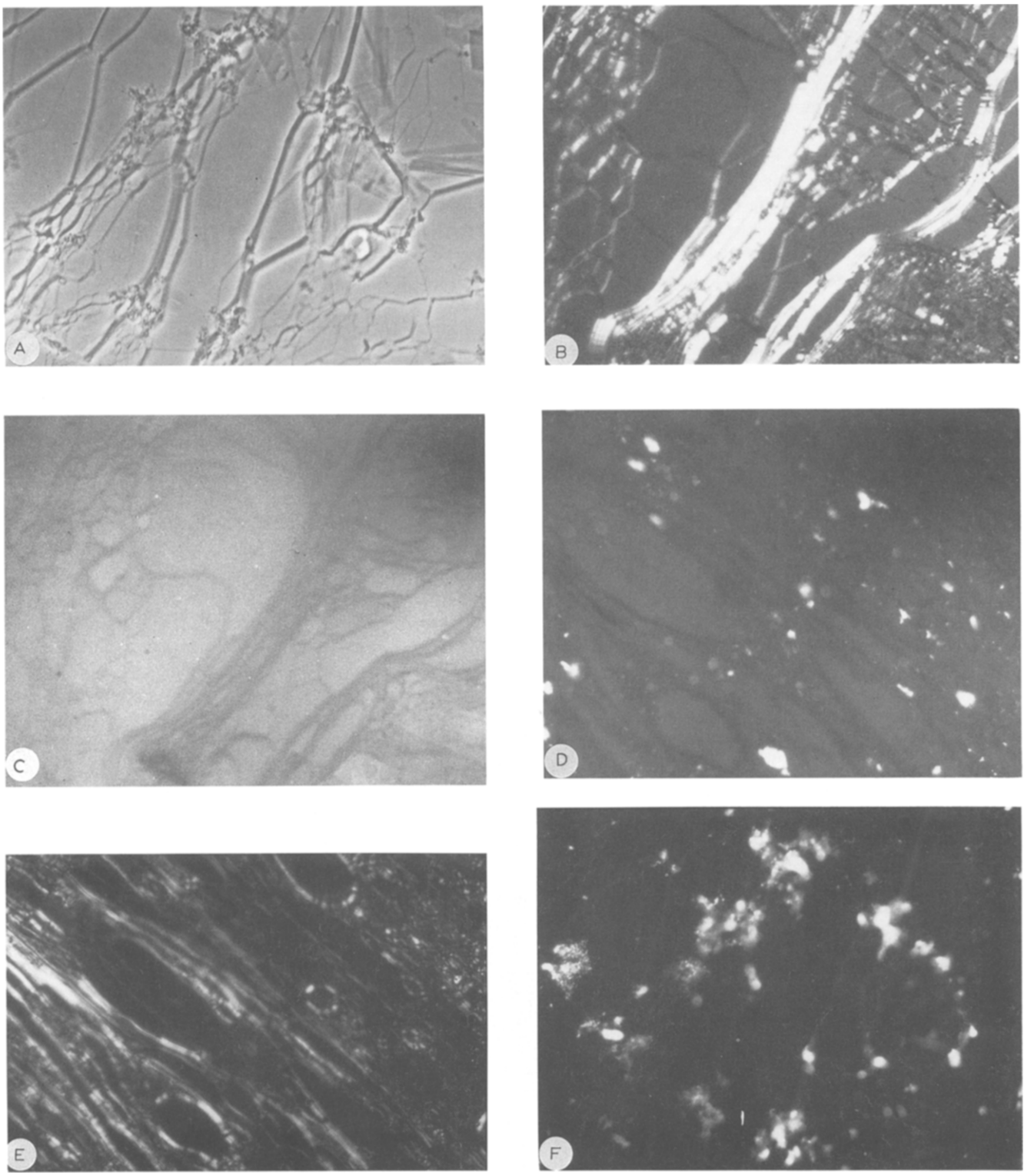

Fig. 2. Photomicrographs of DMPC MBLs all containing a NBDHA-Q:phospholipid ratio of 1:4000 (A) phase image; (B) polarized image; (C) the fluorescent image of pure (cholesterol-free) DMPC MBLs. (D) DMPC plus 20 mol% cholesterol, fluorescent image; (E) DMPC plus 20 mol% cholesterol, polarized image; (F) DMPC plus 50 mole% cholesterol, fluorescent image. Temperature, 30°C. ×400 magnification. Note the progressive displacement of NBDHA-Q fluorescence from the lamellar to the defect regions in Figs. 2C, D and F.

FRAP measurements were carried out by standard methods [34] using the instrument described in a previous publication [35]. The 488 nm line of the argon-ion laser was used for excitation of the fluorescent analogues. Sample temperature was measured using a Digitec thermometer and was controlled using a micro-

scope temperature stage coupled to a thermostated water bath. Fluorescence recovery curves were analyzed by the method of Axelrod et al. [36].

Fluorescence spectra were obtained using a Perkin-Elmer 650-40 spectrofluorometer. The appropriate excitation wavelength for each sample was initially determined by the automatic scan mode of the instrument. Slit width for the excitation and emission monochromators were set at 2 nm. The sample temperature was measured with a Sensortek thermometer in a thermostated cuvette and was controlled by a Forma 2067 water bath. Microscopic observations were made using a Zeiss WL microscope equipped with phase and fluorescence optics and appropriate polarization filters. An Olympus OM-2N camera operated in the automatic exposure mode and Ektachrome ASA 400 film were used for microphotography.

Comparisons were made by a first order approximation between the FRAP determined D of our fluorescent ubiquinone probe and the D predicted for different sizes and shapes of the molecule based upon diphenylhexatriene (DPH)-derived membrane viscosities. In this approach the membrane is approximated as a continuous fluid and the diffusion of ubiquinone is quasi-three-dimensional within the thin viscous sheet of the membrane. There is no exact theory to currently describe the hydrodynamic behavior of such a molecule within the bilayer (e.g., Refs. 37, 38). The Saffman-Delbrück treatment [39–41] typically used for membranes, is only appropriate for the two-dimensional diffusion of a cylinder that projects onto the bilayer surface in contact with the aqueous phase. The Stokes-Einstein approach has been used as an approximation for the diffusion of small molecules within membranes [16,42]. On these bases we used the Stokes-Einstein equations [43,44], probable molecular dimensions for Q_{10}, Q_3 and NBDHA-Q adapted from Trumpower [45] and viscosities reported by steady-state fluorescence polarization measurements of DPH in DMPC bilayers as a function of temperature [46] for these calculations. We note that such derived viscosity measurements cannot be considered exact for a number of reasons, e.g., greater anisotropic environment in the membrane compared to solution.

Results

Two-dimensional distribution of NBDHA-Q in DMPC MBL

Phase, polarized and fluorescence microscopy were used to observe the effects of both NBDHA-Q and cholesterol incorporation on MBL structure and to ascertain the two-dimensional distribution of NBDHA-Q in MBL. The incorporation of NBDHA-Q or cholesterol did not alter the general appearance of the MBL which exhibited typical homogeneous, multilamellar domains bordered by crystal defect (disordered-non-lamellar) regions [47,48] (Fig. 2A,B). Below (or above) the T_m no evidence was seen for the type of large scale defects in the multilamellar domains like those reported by Schneider et al. [49] in the $P_{\beta'}$ membrane phase. In the absence of cholesterol, NBDHA-Q exhibited a strong and uniform fluorescence in the lamellar domains and was virtually nonexistent in the defect regions (Fig. 2C). At 20 mol% cholesterol the general structure of the MBL was unaffected (Fig. 2E), but the fluorescent NBDHA-Q decreased in the lamellar domains and accumulated in the defect regions (Fig. 2D). The amount of fluorescent NBDHA-Q in the defect regions progressively increased commensurate with a decrease in the lamellar domain fluorescence as the cholesterol content was progressively increased until most of the NBDHA-Q was present in the multibilayer defect regions at 50 mol% cholesterol (Fig. 2F). These results reveal that incorporation of cholesterol into the bilayer causes the lateral displacement of NBDHA-Q from the lamellar to the defect domains of MBL indicating that the ubiquinone analogue is located in the transverse domain of the bilayer occupied or affected by cholesterol.

Transbilayer location of NBDHA-Q

The relative transverse location of the fluorophore moiety of NBDHA-Q in the membrane was inferred from the comparison of the wavelength of the emission maximum for the fluorescence spectra (cf. Refs. 50, 51) of NBDHA-Q in solvents having different dielectric constants (polarities). NBDHA-Q in DMPC vesicles gave rise to a single, narrow peak with an emission maximum between 531–532 nm. This wavelength is close to that found for the reference solvents ethanol and methanol (Table I), but clearly not equivalent to that seen in aqueous buffer (see also Table II [52]). While it is not possible to precisely establish a location of NBDHA-Q in the transverse plane of the membrane bilayer from this data it is likely that its fluorophore moiety was closer to the polar headgroups of the phospholipids and its electron-transferring end, which is functional in electron transport and must therefore reach redox complex active sites, was closer to the bilayer midplane *. Since water is known to penetrate to at least C-2 of the fatty acyl chains [53], it is likely that the fluorophore was positioned proximate to C-2.

* The thickness of a bilayer is generally considered to be on the order of 50 Å and of that approx. 35 Å constitutes the acyl chain (hydrocarbon) region [73,74]. Given such dimensions, a nearly linear molecule [45] ~ 30 Å long oriented roughly parallel to the acyl chains [70] with one (fluorescent) end nearer the polar headgroups would have to have its opposite end closer to the bilayer midplane, or else it must project out of the bilayer and would be nonfunctional in electron transport.

Likewise, the diffusion studies discussed below indicate a ubiquinone location internal in the bilayer. We also determined that the fluorophore moiety of NBDHA-Q retained the same relative transverse location in the membrane irrespective of temperature. This follows from the observations that the emission maxima (Table I), relative intensity and peak width at half-height for the fluorescence spectra of NBDHA-Q (data not shown) were essentially unchanged at a range of temperatures both above and below the phase transition of the DMPC membrane. Thus, based on fluorescence emission criteria, we conclude that NBDHA-Q is internal in the bilayer with the fluorophore moiety of the molecule closer to the phospholipid polar headgroups such as the C-2 region of the acyl chains and the electron transferring end closer to the bilayer midplane.

NBDHA-Q and phospholipid diffusion in DMPC MBL

The D values for NBDHA-Q and phospholipid (DiI) in pure DMPC MBL determined by FRAP were found to be virtually the same at temperatures at or above the T_m (23.7°C) of the membrane bilayer (Fig. 3). Diffusion was rapid above the T_m with both D values at $4.8 \cdot 10^{-8}$ cm^2/s at 32°C. An approximate 150-fold drop in the D values was observed from 32 to approx. 23°C. At temperatures below 23.7 °C the D values decreased further with the D values of phospholipid

TABLE I

Fluorescence characteristics of NBDHA-Q

Medium [a]	Emission (Max. nm.)	Temperature	Dielectric constant (20 ° C)
Hexane	507	23	1.89
Benzene	512	23	2.284
Ethanol	528	23	24.3
Methanol	532	23	33.63
H_{40} isolation medium [b]	554	23	–
Water	–		80.37
DMPC [d]	532	30	
	532	20	
	532	19	
	531	16.6	
	531	14.8	
	532	14.5 [c]	
Mitochondrial inner membrane [d]	531	23	

[a] 0.16 μM NBDHA-Q (Q_0C_{10}NBDHA).

[b] 40 mosM mitochondrial isolation medium made as 7.5-fold dilution of 220 mM mannitol, 70 mM sucrose and 2 mM Hepes without bovine serum albumin at pH 7.4.

[c] After 25 min at temperature.

[d] Main phase transition temperatures are 23.7°C for DMPC vesicles and −4°C for mitochondrial inner membranes. At each temperature the membranes were allowed to temperature equilibrate for 10 min prior to the initial reading. A 4000 DMPC:1 NBDHA-Q ratio was used in the vesicles.

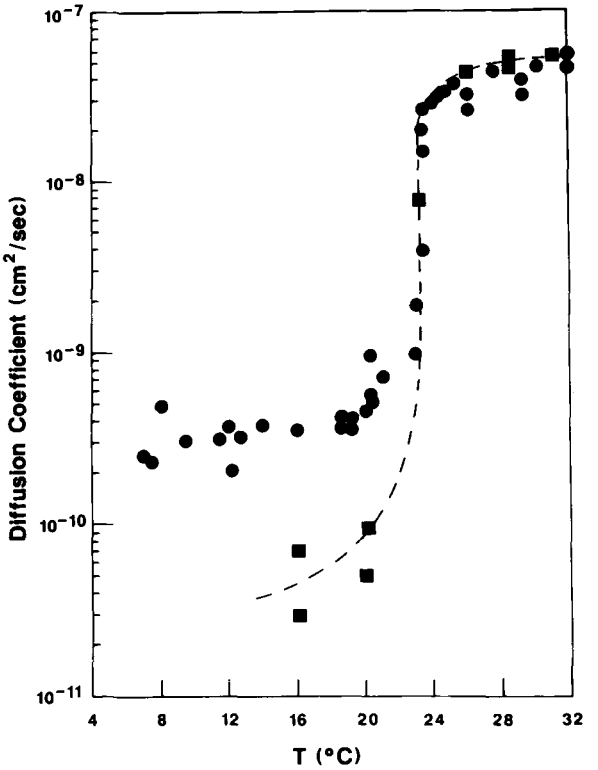

Fig. 3. Comparison of ubiquinone and phospholipid diffusion as a function of temperature in MBL. FRAP-determined D values of NBDHA-Q (●) and DiI (■) in pure DMPC MBLs.

decreasing on average, 7-fold lower than that for NBDHA-Q. We believe that the higher D values for NBDHA-Q compared to phospholipid below the T_m in pure DMPC reflects a location for NBDHA-Q closer to the bilayer midplane compared to the phospholipid headgroups. We found the fluorescence recovery of NBDHA-Q and phospholipid to be essentially complete and unicomponent at all temperatures studied indicating that the mobile fractions of both diffusants were approx. 100% of their respective populations. We also found no significant FRAP-detectable, temperature-induced change in the NBDHA-Q fluorescent intensity in MBL prior to photobleaching, indicating that NBDHA-Q remained in the lamellar domains at all temperatures studied. Thus, there was only one detectable uniformly mobile population for both phospholipid and the ubiquinone analogue indicating no significantly faster or slower diffusants in the respective populations or displacement of ubiquinone into the crystal defect regions of the MBL. We conclude that in the liquid crystalline membrane bilayer both ubiquinone and phospholipid are highly mobile, homogeneous diffusants with like D values.

NBDHA-Q diffusion in DMPC / cholesterol MBL

D values for NBDHA-Q in DMPC MBL progressively decreased above the T_m and increased below the T_m when cholesterol was progressively increased in the

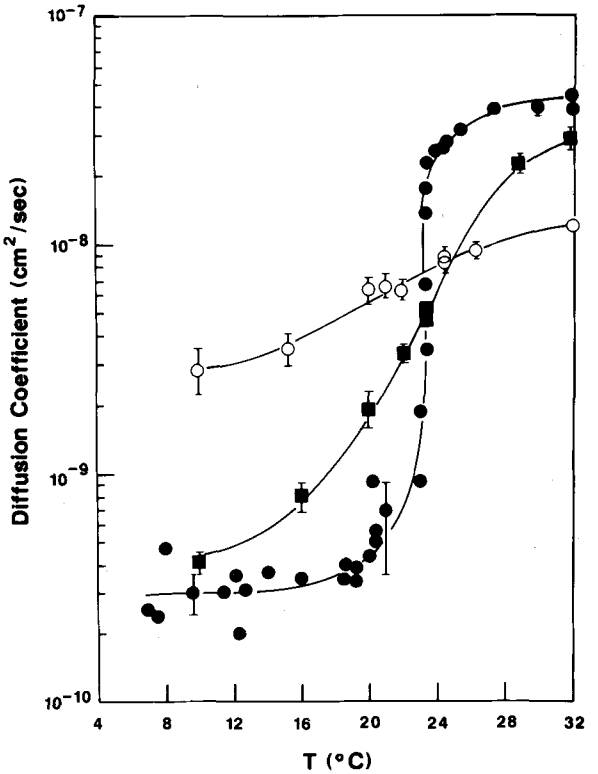

Fig. 4. Effect of cholesterol content and temperature on FRAP-determined D values of NBDHA-Q diffusion in MBL. 0 mol% cholesterol (●), 20 mol% cholesterol (■), 50 mol% cholesterol (○).

MBL from 0 to 50 mol% (Fig. 4). Thus cholesterol diminished the abrupt shift in D values of Q at the T_m (c.f. Figs. 3 and 4) which indicated again that NBDHA-Q is located proximate to cholesterol in the bilayer. Such temperature-dependent trends are consistent with similar cholesterol studies [32,54,55]. The fluorescent intensity of NBDHA-Q in the lamellar regions monitored by FRAP measurements decreased concomitant with the increase in MBL cholesterol content. This finding was in concert with our photographic results (Figs. 2C, D and F) and indicated that ubiquinone was progressively excluded from the lamellar bilayer regions with increasing amounts of cholesterol. Yet in all instances in the cholesterol/DMPC bilayers, fluorescence recovery of the NBDHA-Q that remained in the lamellar regions was essentially complete, indicative of an approx. 100% mobile fraction, and unicomponent, indicative of a single pool without distinctly fast and slow diffusant populations. These results indicated again that ubiquinone is located in the cholesterol region of the bilayer where direct or indirect physical interactions between NBDHA-Q and cholesterol can occur.

Diffusion of NBDHA-Q related to bilayer viscosity

Using Stokes-Einstein equations as a first approximation (see Materials and Methods) a reasonable agreement was noted for the D of NBDHA-Q determined by FRAP and the D predicted for a 30×6 Å long rod shape from DPH-derived viscosities in DMPC over a range of temperatures (Fig. 5). The long rod shape is considered the preferred shape for ubiquinones (e.g., Refs. 33, 45, 56) with the size based upon CPK molecular models [45]. A reverse approach of calculating approximate viscosities based upon FRAP-determined D values yielded similar agreement (data not shown). (The Stokes-Einstein equations are not valid below the membrane T_m, since in the gel-state lipids the basic hydrodynamic assumptions are physically incorrect. Hence, such fittings, although mathe-

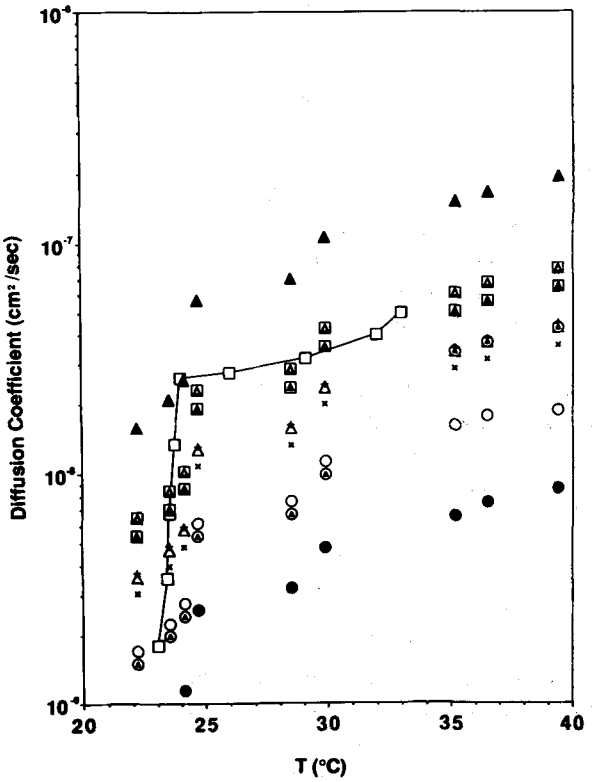

Fig. 5. Comparison of D values determined by FRAP or calculated for various shapes based on DPH-derived viscosities as a function of temperature at and above the T_m (~ 23°C) in DMPC bilayers. D for NBDHA-Q determined by FRAP. DPH viscosites from Lentz et al. [37]. Ubiquinone dimensions derived from Trumpower [45]; Q_{10} approx. 56×6 Å and Q_3 and NBDHA-Q approx. 30×6 Å in size. Calculations carried out per Methods substituting the appropriate expression for the frictional coefficient, f, into the equation $D = kT/f$, where k = Boltzman constant and T = temperature, η = viscosity, a = major axis or radius, e.g., $f_{sphere} = 6\pi\eta a$. FRAP-determined D for NBDHA-Q (□) determined by FRAP. D calculated based on DPH-derived DMPC bilayer viscosity for 50 Å sphere (●), 21 Å sphere (○), 21×6 Å prolate ellipsoid (△), 21×5 Å prolate ellipsoid (▲), 30×6 Å prolate ellipsoid (◓), 21×6 Å long rod (▨), 30×6 Å long rod (▩), 21 Å disk edge-on (×), 21×6 Å prolate ellipsoid length-wise (+). Note that calculations of D values for a longer rod-shaped ubiquinone, 56×6 Å, from DPH-derived viscosities yielded the same shaped plot as for the shorter rod-shaped ubiquinones shown here, but offset slightly to lower D values.

matically calculable, are not presented in Fig. 5.) Based upon these calculated agreements between D values and viscosity it can be concluded that our FRAP-determined, near identical D values for ubiquinone and phospholipid are accurate values and are a reflection of the common viscosity of the fluid DMPC lipid bilayer through which both Q and phospholipid must diffuse.

Discussion

Diffusion of ubiquinones, phospholipids and lipids in model membranes

Our finding that the D values for phospholipids and ubiquinones are virtually the same in liquid-crystalline ($T > T_m$) pure lipid bilayer membranes and that both are capable of long-range diffusion agrees with our earlier studies which revealed similar D values for NBDHA-Q and phospholipid in the 10^{-9} cm^2/s range and long-range diffusion in the protein-dense mitochondrial inner membrane [11–14]. This finding for the D values of lipoidal molecules is in agreement with other studies on diffusion in liquid-crystalline membranes [32,48,55,57]. This finding is also consistent with the self-diffusion-like nature of ubiquinone diffusion, i.e., since the phospholipids are in essence a solvent for proteins and lipoidal molecules, similarly sized molecules such as ubiquinones and phospholipids should diffuse at the same rate [48,57–60]. The fact that the D of NBDHA-Q decreased above T_m and increased below T_m with increasing cholesterol content in the bilayer clearly indicates that NBDHA-Q is integral to the bilayer, interacting with the phospholipid acyl chains in a transverse bilayer location affected by cholesterol and diffusing at a rate similar to the phospholipids above the T_m.

The similarity in D values of Q and phospholipid is supported by our experimentally based theoretical calculations on the relationship of viscosity and D in the DMPC bilayer. We found that the apparent viscosity derived from DPH measurements in the phospholipid bilayer, which reflect the local environment affecting DPH motions, could be used to reasonably predict the D values for NBDHA-Q. By using a range of temperatures to give a range of viscosities, we determined that the classically preferred long rod shapes for ubiquinones best fit our FRAP data. The agreement we noted between D values predicted by the apparent viscosity and reported by FRAP reflects the common viscosity through which both phospholipid and ubiquinone must diffuse. In contrast, the DPH and NBDHA-Q determinations do not agree with the D values at 25°C in the 10^{-6} cm^2/s range and the resultant approx. 0.05 P viscosity based on the criteria of Lenaz and Fato [17] and Fato et al. [16]. This low viscosity calculated by these investigators using the Stokes-Einstein equations was thought by them to reflect a location for ubiquinone exclusively in the bilayer midplane. However, others [4,20,28,29,33,45,61–67] and Lenaz [16,17] on structural, kinetic and physical grounds reported that ubiquinone must access regions near the phospholipid polar headgroups where effective viscosities are greater than at the bilayer midplane [19]. In addition, we note that the presence of functionally required integral proteins in mitochondrial inner membrane would increase the effective membrane viscosity, further decreasing the rate of long-range lateral diffusion in the inner membrane [14]. Therefore, our results that both NBDHA-Q and phospholipids experience the same environment, hence the similar D values ($\sim 3 \cdot 10^{-8}$ cm^2/s in lipid model membrane and $\sim 4 \cdot 10^{-9}$ in native inner membrane), are compatible with electron transport requirements.

Recently Ferguson-Miller and coworkers [52,68] used a different fluorescent ubiquinone having the NBD fluorophore attached to the quinol ring of ubiquinone-10 which was shown to partition in the bilayer interior of both phospholipid and mitochondrial inner membranes. in close agreement with our results, their NBD-Q_{10} had D values at 25°C of $3 \cdot 10^{-9}$ and $1.1 \cdot 10^{-8}$ cm^2/s in mitochondria and lipid (asolectin) vesicles, respectively, virtually the same as for the D values of their NBD-PE phospholipid probe. In agreement with our findings that the fluorophore moiety of our Q probe was located in the acyl chain region of the membrane, their fluorescence emission maxima data showed NBD-PE (fluorophore in headgroup region) at 537 nm and their Q probe was at 529 nm (fluorophore in acyl chain region). Since, our shorter chain, functional NBDHA-Q, their longer chain NBD-Q_{10} and the bilayer phospholipids all have the same D values for a particular membrane, it must be concluded that native ubiquinone most likely diffuses in the low 10^{-8} cm^2/s range in model membranes and in the mid 10^{-9} cm^2/s range in mitochondrial inner membranes.

Our FRAP-determined, unobstructed, long-range D values in pure phospholipid in theory should be the same as that determined by an accurate short-range method. However, in contrast to our findings, Lenaz and co-workers [15–17] using a FQ based approach, estimated short-range D values for ubiquinones in the 10^{-6} cm^2/s range. Consequently, their studies are not in agreement with our conclusion that the diffusion-based collisions of ubiquinone with its redox partners in the native inner membrane is rate-limiting for maximum rates of mitochondrial electron transport. We have discussed elsewhere [13,65] the problems with and the relevance of, the FQ approach in the estimation of D values which yields values orders of magnitude higher than that determined by FRAP and other techniques. We have also shown that functionally mitochondrial electron transport must be a long-range process [14].

Recently, an experimental and theoretical analysis of the FQ approach [69] has led Lenaz and co-workers [18] to revise their D values downward by an order of magnitude to the 10^{-7} cm^2/s range, closer to the values we report for D values in protein-free membranes, but in our opinion still an order of magnitude too high. Following Lenaz and coworkers' reasoning, shorter chain Q_ns which are located closer to the more viscous polar headgroup region and oriented parallel to the lipid acyl chains [20,27,70] would be expected to have lower D values than Q_{10} which they hypothesized is oriented parallel to and located in the highly fluid bilayer midplane. However experimentally they found only a approx. 3–4-fold range of D values with Q_1 being the highest and Q_{10} the lowest [16]. Thus, the relationship among the experimental D values reported by Lenaz tends not to support the exclusive bilayer midplane location, rather we believe it tends to support the FRAP-based findings in which the shorter chain NBDHA-Q probe and the long chain NBD-Q_{10} probe diffuse at the same rate consistent with the viscosity of the phospholipids in which they reside.

The relationship of ubiquinone location, diffusion and kinetics

Our results on ubiquinone's diffusion and location (i.e., the acyl chain region) in model membranes are consistent with our earlier reports on the fundamental significance of ubiquinone diffusion in mitochondrial electron transport which showed that (1) multi-redoxcomponent electron transport rates are affected by the density and distribution of the redox components in the inner membrane [7–10], (2) electron transport is a multicollisional, obstructed, long-range, diffusional process [14] and (3) the diffusion-based collisions of ubiquinone with its redox partners is a rate-limiting step [13]. Kinetic and ultrastructural studies showed that cholesterol incorporation into the mitochondrial inner membrane induces clustering of the integral proteins thereby increasing electron transport rates [9]. The similar effect of cholesterol on the diffusion and distribution of ubiquinone in the present study and of integral proteins in our earlier study [9], coupled with the functional requirement of ubiquinone for a low resistance to motion in the inner membrane [14], may well explain the observed absence of cholesterol from the native inner membrane. In addition, analogous to the treatment of Jacobson et al. [71] for a transiently bound diffusant, we hypothesize that our FRAP results showing that ubiquinones have the same D as phospholipids in two different environments, unobstructed diffusion in pure phospholipid membranes ($\sim 3 \cdot 10^{-8}$ cm^2/s at 25°C) and obstructed diffusion in mitochondrial inner membranes ($\sim 4 \cdot 10^{-9}$ cm^2/s) [14], but not the same D for proteins in inner membranes ($\sim 4 \cdot 10^{-10}$ cm^2/s) [12,14], indicate that no significant portion of the ubiquinone population remains bound to redox complexes for any significant length of time relative to that for electron transport.

A transbilayer electron transport capability for almost all ubiquinone homologues in model membranes has been shown by others [27,61,62,72] which suggests a capability for transbilayer diffusion and/or marked oscillation of the ubiquinone headgroup in addition to the lateral diffusion we report. Transbilayer excursions of ubiquinone are required for shuttling reducing equivalents to opposite sides of the bilayer as required in the Q-cycle [4] and possibly to interact with redox component active sites [67]. It would appear that ubiquinones must experience a significant portion of the total volume in the membrane bilayer in order to carry out bioenergetic functions. These functional studies are supported by our present findings but are in conflict with some physical studies in model membranes arguing for a separate or ubiquinone-rich phase at the bilayer midplane (e.g., Refs. 22, 23).

Based on our diffusion and fluorescence studies we ascribe a location to ubiquinone that is integral to the bilayer and interacting with the phospholipid acyl chains below C-2. This may be an equilibrium position that is isoprenoid chain-length dependent but allowing ubiquinone to oscillate across the membrane midplane to experience a large fraction of the total membrane volume, as suggested by our approximate diffusion-viscosity calculations. Such a concept is consistent with the fact that ubiquinones like Q_3 and longer are all capable of restoring high rates of electron transport to ubiquinone-depleted mitochondria [56]. We have not detected a separate or ubiquinone-rich phase in the cholesterol-free inner membrane or pure DMPC MBL. In these contexts our findings are in concert with the report of Stidham et al. [24], who found (1) no ubiquinone-induced ^{13}C NMR-detectable perturbation of the terminal acyl carbons, (2) no freeze fracture electron microscopy-detectable change in the $P_{\beta'}$ phase appearance of the DMPC bilayer midplane and (3) no modification of the membrane's X-ray diffraction angular reflections. The agreement of our findings with those of Stidham et al. for ubiquinone's membrane behavior versus a separate or ubiquinone-rich phase in other physical studies [20–23,25,26–31] is that the latter used very high ubiquinone concentrations, e.g., 0.05–0.50 mol fraction, in order to make their physical measurements, compared to the actual 0.01–0.02 mol fraction in the inner membrane. High concentrations alone may be the reason for a finding of a separate or ubiquinone-rich phase in these studies. Consistent with the Random Collision Model and the bioenergetic functions of ubiquinone, we believe the data collectively indicate that ubiquinone is highly mobile, transversely as well as laterally, in the fluid phospholipid milieu of the membrane bilayer.

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References

1 Green, D.E. and Wharton, D.C. (1963) Biochem. Z. 338, 335–346.
2 Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 34, 358–368.
3 Kröger, A. and Klingenberg, M. (1973) Eur. J. Biochem. 39, 313–323.
4 Mitchell, P. (1976) J. Theor. Biol. 62, 327–367.
5 Ragan, C.I. and Cottingham, J.R. (1985) Biochim. Biophys. Acta 811, 13–31.
6 Ragan, C.J. and Reed, J.S. (1986) J. Bioenerg. Biomemb. 18, 403–418.
7 Schneider, H., Lemasters, J.J., Hoechli, M. and Hackenbrock C.R. (1980) Proc. Natl. Acad. Sci. USA 77, 442–446.
8 Schneider, H., Lemasters, J.J., Höchli, M. and Hackenbrock, C.R. (1980) J. Biol. Chem. 255, 3748–3756.
9 Schneider, H., Höchli, M. and Hackenbrock, C.R. (1982) J. Cell Biol. 94, 387–393.
10 Schneider, H., Lemasters, J.J. and Hackenbrock, C.R. (1982) J. Biol. Chem. 257, 10789–10793.
11 Wu, E.-S., Yu, C.-A. and Hackenbrock, C.R. (1983) Biophys. J. 41, 374a.
12 Gupte, S.S., Wu, E.-S., Hoechli, L., Hoechli, M., Sowers, A.E., Jacobson, K.A. and Hackenbrock, C.R. (1984) Proc. Natl. Acad. Sci. USA 81, 2606–2610.
13 Chazotte, B. and Hackenbrock, C.R. (1989) J. Biol. Chem. 264, 4978–4985.
14 Chazotte, B. and Hackenbrock, C.R. (1988) J. Biol. Chem. 263, 14359–14367.
15 Fato, R., Battino, M., Degli Eposti, M., Parenti Castelli, G. and Lenaz, G. (1985) FEBS Lett. 179, 238–242.
16 Fato, R., Battino, M., Degli Eposti, M., Parenti Castelli, G. and Lenaz, G. (1986) Biochemistry 25, 3378–3390.
17 Lenaz, G. and Fato, R. (1986) J. Bioenerg. Biomemb. 18, 369–401.
18 Lenaz, G., Fato, R., Castelluccio, C., Degli Esposti, M., Samworth, C.M., Battino, M. and Parenti Castelli, G. (1988) in Integration of Mitochondrial Function (Lemasters, J.J., Hackenbrock, C.R., Thurmond, R.G. and Westerhoff, H.V., eds.), pp. 33–52, Plenum Press, New York.
19 Moser, M., Marsh, D., Meier, P., Wassmer, K.-H. and Kothe, G. (1989) Biophys. J. 55, 111–123.
20 Kingsley, P.B. and Figenson, G.W. (1981) Biochim. Biophys. Acta 635, 602–618.
21 Katsikas, H. and Quinn, P.J. (1982) Biochim. Biophys. Acta 689, 363–369.
22 Katsikas, H. and Quinn, P.J. (1982) Eur. J. Biochem. 124, 165–169.
23 Katsikas, H. and Quinn, P.J. (1983) Eur. J. Biochem. 131, 607–612.
24 Stidham, M.A., McIntosh, T.J. and Siedow, J.N. (1984) Biochim. Biophys. Acta 767, 423–431.
25 Chatelier, R.C. and Sawyer, W.H. (1985) Eur. Biophys. J. 11, 179–185.
26 Michaelis, L. and Moore, M.J. (1985) Biochim Biophys. Acta 821, 121–129.
27 Ulrich, E.L., Girvin, M.E., Cramer, W.A. and Markley, J.L. (1985) Biochemistry 24, 2501–2508.
28 Aranda, F.J. and Gomez-Fernadez, J.C. (1985) Biochim. Biophys. Acta 820, 19–26.
29 Aranda, F.J. and Gomez-Fernadez, J.C. (1986) Biochem. Int. 12, 137–143.
30 Ondarroa, M. and Quinn, P.J. (1986) Eur. J. Biochem. 155, 353–361.
31 Cornell, B.A., Keniry, M.A., Post, A., Robertson, R.N., Weir, L.E. and Westerman, P.W. (1987) Biochemistry 26, 7702–7707.
32 Wu, E.-S., Jacobson, K. and Paphadjopoulos, D. (1977) Biochemistry 16, 3936–3941.
33 Yu, C.-A., Gu, L., Lim, Y. and Yu, L. (1985) Biochemistry 24, 3897–3902.
34 Kapitza, H.G. and Jacobson, K.A. (1985) in Techniques for the Analysis of Membrane Proteins (Ragan, C.I. and Cherry, R.J., eds.), pp. 345–375, Chapman and Hill, London.
35 Wu, E.-S. and Yang, C.S. (1984) Biochemistry 23, 28–33.
36 Axelrod, D. Koppel, D.L., Schlessinger, J., Elson, E. and Webb, W.W. (1976) Biophys. J. 16, 1055–1069.
37 Lentz, B.R. (1988) in Spectroscopic Membrane Probes (Loew, L., ed.), Vol. 1, pp. 14–42, CRC Press, Boca Raton.
38 Clegg, R.M. and Vaz, W.L.C. (1985) in Progress in Protein–Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), pp. 174–229, Elsevier/North Holland, Amsterdam.
39 Saffman, P.G. and Delbrück, M. (1975) Proc. Natl. Acad. Sci. USA 72, 3111–3113.
40 Saffman, P.G. (1976) J. Fluid Mech. 73, 593–602.
41 Hughes, B.D., Pailthorpe, B.A., White, L.R. and Sawyer, W.H. (1982) Biophys. J. 37 673–676.
42 Dix, J.A., Kivelson, D. and Diamond, J.M. (1978) J. Membr. Biol. 40, 315–342.
43 Marshall, A.G. (1978) Biophysical Chemistry, pp. 197–205, J. Wiley and Sons, New York.
44 Berg, H.C. (1983) Random Walks in Biology, Prentice Hall, Princeton, N.J.
45 Trumpower, B.L. (1981) J. Bioenerg. Biomemb. 13, 1–24.
46 Lentz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528.
47 Powers, L. and Pershan, P.S. (1977) Biophys. J. 20, 137–143.
48 Derzko, Z. and Jacobson, K. (1980) Biochemistry 19, 6050–6057.
49 Schneider, M.B., Chan, W. and Webb, W.W. (1983) Biophys. J. 43, 157–165.
50 Guibault, G.G. (1973) Practical Fluorescence, Marcel Dekker, New York.
51 Lakowicz, J.R. (1983) Principles of Fluorescence Spectroscopy, Plenum Press, New York.
52 Rajarathnam, K., Hochman, J., Schindler, M. and Ferguson-Miller, S. (1989) Biochemistry 28, 3168–3176.
53 Jain, M.K. and Wagner, R.C. (1980) Introduction to Biological Membranes, pp. 74–78, J. Wiley and Sons, New York.
54 Rubinstein, J.L., Smith, B.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. USA 76, 15–18.
55 Alecio, M.R., Golan, D.E., Veatch, W.R. and Rando, R.R. (1982) Proc. Natl. Acad. Sci. USA 79, 5171–5174.
56 Crane, F.L. (1977) Annu. Rev. Biochem. 46, 439–470.
57 Vaz, W.L.C., Derzko, Z.I. and Jacobson, K.A. (1982) Cell Surface Rev. 8, pp. 84–136.
58 Saxton, M.J. (1982) Biophys. J. 39, 165–173.
59 Vaz, W.L.C., Goodsaid-Zalduondo, F. and Jacobson, K. (1984) FEBS Lett. 174, 199–207.
60 Eisinger, J., Flores, J. and Peterson W.J. (1985) Biophys. J. 49, 987–1001.
61 Futami, A., Hurt, E. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 583–596.
62 Futami, A., Hurt, E. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 597–608.
63 Crane, F.L. (1986) in Biomedical and Clinical Aspects of Coenzyme Q (Folkers, G. and Yamamura, Y., eds.), Vol. 5, pp. 1–14, Plenum Press, New York.

64 Hackenbrock, C.R. Gupte, S.S. and Chazotte, B. (1985) in Achievements and Perspectives in Mitochondrial Research (Quaglierello, E., Slater, E.C., Palmieri, F., Saccone, C. and Kroon, A.M., eds.), Vol. 1, pp. 83–102, Elsevier/North Hollaand, Amsterdam.
65 Hackenbrock, C.R., Chazotte, B. and Gupte, S.S. (1986) J. Bioenerg. Biomemb. 18, 331–368.
66 Hackenbrock, C.R., Chazotte, B. and Gupte, S.S. (1986) in Biomedical and Clinical Aspects of Coenzyme Q (Folkers, G. and Yamamura, Y., eds.), Vol. 5, pp. 25–38, Plenum Press, New York.
67 Ohnishi, T., Schägger, H., Meinhardt, S.W., LoBrutto, R., Link, T.A. and Von Jagow, G. (1989) J. Biol. Chem. 264, 735–744.
68 Ferguson-Miller, S., Rajarathnam, K., Hochman, J. and Schindler, M. (1988) in Integration of Mitochondrial Function (Lemasters, J.J., Hackenbrock, C.R., Westerhoff, H. and Thurman, R.G., eds.), pp. 23–32, Plenum Press, New York.
69 Blackwell, M.F., Gounaris, K., Zara, S.J. and Barber, J. (1987) Biophys. J. 51, 735–744.
70 Cain, J., Santillan, G. and Blasie, J.K. (1972) in Membrane Research, (Fox, C.E., ed.), pp. 3–14, Academic Press, New York.
71 Jacobson, K., O'Dell, D. and August, J.T. (1984) J. Cell Biol. 99, 1624–1633.
72 Ilani, A. and Krakover, T. (1987) Biophys. J. 51, 161–167.
73 Levine, Y.K. and Wilkins, M.H.F. (1971) Nature 230, 69–72.
74 Zacci, G., Blasie, J.K. and Schoenborn, B.P. (1975) Proc. Natl. Acad. Sci. USA 72, 376–380.