Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1710 (2005) 57 – 62

BIOCHIMICA ET BIOPHYSICA ACTA BBA

http://www.elsevier.com/locate/bba

Localization of coenzyme Q_{10} in the center of a deuterated lipid membrane by neutron diffraction

Thomas Hauß [a,b,*], Silvia Dante [a,b], Thomas H. Haines [c], Norbert A. Dencher [b]

[a] *Hahn-Meitner Institute, Berlin Neutron Scattering Centre, Glienicker Strasse 100, D-14109 Berlin, Germany*

[b] *Darmstadt University of Technology, Dept. of Chemistry, Physical Biochemistry, Petersenstrasse 22, D-64287 Darmstadt, Germany*

[c] *Department of Chemistry, City College of CUNY, New York, NY 10031, USA*

Received 27 June 2005; received in revised form 26 August 2005; accepted 29 August 2005

Available online 19 September 2005

Abstract

Quinones (e.g., coenzyme Q, CoQ_{10}) are best known as carriers of electrons and protons during oxidative phosphorylation and photosynthesis. A myriad of mostly more indirect physical methods, including fluorescence spectroscopy, electron-spin resonance, and nuclear magnetic resonance, has been used to localize CoQ_{10} within lipid membranes. They have yielded equivocal and sometimes contradictory results. Seeking unambiguous evidence for the localization of ubiquinone within lipid bilayers, we have employed neutron diffraction. CoQ_{10} was incorporated into stacked bilayers of perdeuterated dimyristoyl phosphatidyl choline doped with dimyristoyl phosphatidyl serine containing perdeuterated chains in the natural fluid-crystalline state. Our data show CoQ_{10} at the center of the hydrophobic core parallel to the membrane plane and not, as might be expected, parallel to the lipid chains. This localization is of importance for its function as a redox shuttle between the respiratory complexes and, taken together with our recent result that squalane is in the bilayer center, may be interpreted to show that all natural polyisoprene chains lie in the bilayer center. Thus ubiquinone, in addition to its free radical scavenging and its well-known role in oxidative phosphorylation as a carrier of electrons and protons, might also act as an inhibitor of transmembrane proton leaks.

Keywords: Coenzyme Q_{10}; Ubiquinone; Neutron diffraction; Lipid membrane; Electron transfer; Proton permeability

1. Introduction

Redox-active quinones with long polyisoprenoid chains like coenzyme Q are ubiquitous in eukaryotes. They fulfill two established functions as electron/proton carriers in bioenergetic membranes and as antioxidants [1]. Since all of the organelle membranes that contain them are exposed to H^+ gradients, they may also serve as inhibitors of H^+ leakage [2].

Ubiquinone (CoQ_{10}) is a 50-carbon polyisoprene with a terminal quinone domain. It is synthesized in all of the mammalian organelle membranes investigated [3]. It is essential for ATP synthesis in mitochondria as a carrier of electrons and protons in oxidative phosphorylation and in other proton-pumping membranes, presumably by the Q-cycle [4]. The structurally and functionally very similar plastoquinone is employed in photosynthetic membrane systems. The function of the polyisoprenoid domain of CoQ_{10} is generally assumed to anchor the quinone to the hydrophobic domain of the bilayer. Nonetheless, a short aliphatic chain has been shown to suffice for this purpose in vitro [5].

The localization of CoQ_{10} within membranes has been the subject of many studies [6,7]. The studies, using NMR spectroscopy [5,8,9], fluorescence [10], differential infrared spectroscopy [11], linear dichroism [12], and differential scanning calorimetry [13] have concluded with differing and often conflicting interpretations.

It has recently been established that squalane, an analogue of squalene, which appears to inhibit proton leakage across alkaliphile membranes [14], lies in the bilayer center of phospholipid bilayers [15]. The "water cluster" model [2] for proton leakage across lipid bilayers suggests that hydrocarbons in the center of straight chain lipid bilayers may serve as an inhibitor of proton leakage in addition to any other functions it

* Corresponding author. Darmstadt University of Technology, Dept. of Chemistry, Physical Biochemistry, Petersenstrasse 22, D-64287 Darmstadt, Germany. Fax: +49 30 8062 2999.

E-mail address: hauss@hmi.de (T. Hauß).

0005-2728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2005.08.007

may have in the cell. Thus, quinone polyisoprenoids may inhibit proton leakage in addition to their other biological roles.

Neutron diffraction is ideal and the most direct tool for localizing molecular building blocks in lipid bilayers. Due to the large difference in the coherent scattering length b of a proton (b_H=−3.74 fm) and a deuteron (b_D=+6.67 fm) an ideally isomorphous replacement can be achieved. In this study, a method is applied in which the membrane constituent CoQ_{10} is protonated and the bilayer lipids are deuterated. Using this approach we report that CoQ_{10} is resident in the center of perdeuterated dimyristoyl phosphatidyl choline (DMPC) bilayers doped with 5 mol% dimyristoyl phosphatidyl serine (DMPS) containing perdeuterated chains.

2. Materials and methods

Perdeuterated dimyristoyl phosphatidyl choline (*d*DMPC, 67 deuterons, 98% purity) and the net negatively charged lipid dimyristoyl phosphatidyl serine containing perdeuterated chains (*d*DMPS, 54 deuterons, 98% purity) were purchased from Avanti Polar Lipids, Alabaster, AL. Non-deuterated ubiquinone (89% minimum) was obtained from Sigma Chemicals.

2.1. Preparation of stacked bilayers

Chloroform solutions containing *d*DMPC/*d*DMPS (9:1 mol/mol) with or without an additional 5 mol% perprotonated ubiquinone were prepared. The total lipid weight for each preparation was 10 mg. Oriented samples were prepared as described [15]. Dry samples were then rehydrated for 24 h at room temperature in an atmosphere of 98% relative humidity, maintained with a saturated K_2SO_4 solution.

2.2. Neutron diffraction

Neutron diffraction measurements were carried out on the membrane diffractometer V1 at the Berlin Neutron Scattering Center of the Hahn-Meitner-Institute, Berlin (Germany) with a neutron wavelength of λ=0.452 nm in thermostated aluminum cans at 98% relative humidity. The procedure is described in detail elsewhere [15]. Briefly, the diffraction intensities where measured at four different D_2O/H_2O contrasts (100:0, 80:20, 50:50, and 0:100) at T=(25±0.1) °C with rocking scans around the expected Bragg position θ by θ±2°. Up to 6 orders were detected for each sample. Diffraction patterns of *d*DMPC/*d*DMPS bilayers were examined with or without 5 mol% protonated ubiquinone. The measured intensities were scaled with the integrated scattering length of the water layer using the known differences in scattering length of subsequent D_2O/H_2O ratios. The D_2O/H_2O contrast variation also facilitates the phase assignment [16]. The square roots of the scaled intensities, corrected with absorption and Lorenz factors, produced the structure factor amplitudes. The scattering length density profile $\rho(z)$ is given by:

$$\rho(z) = \rho_0 + \frac{2}{d}\sum_{h=1}^{n} \pm F(h)\cos\left(\frac{2\pi hz}{d}\right)$$

where ρ_o is the total scattering length density of the bilayer, $F(h)$ are the scaled structure factors and d the unit cell length. For more details to evaluate the localization of labels in biological membranes, see, e.g., [17,18].

3. Results

The rocking scans around the first order peaks display a mosaicity inferior to 0.5 degrees demonstrating the excellent quality of the samples. As already reported [19], few small yellow droplets of ubiquinone are expelled from the lipid bilayers, but do not disturb the alignment of the membranes.

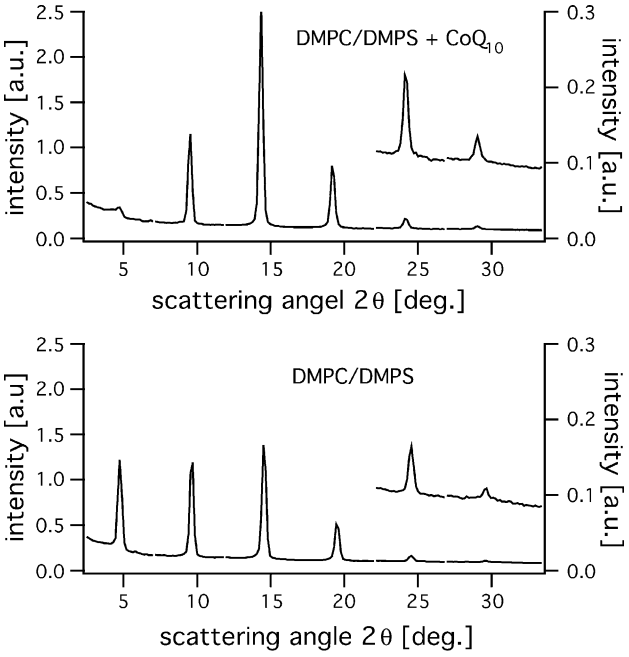

Fig. 1. Diffractograms of the stacked bilayers of *d*DMPC/*d*DMPS (9:1 mol/mol) sample at 25 °C with and without protonated ubiquinone at D_2O/H_2O=100:0 contrast. Up to six orders are visible. In particular, at 100% D_2O content, the diffractogram of the sample containing deuterated lipids and protonated ubiquinone is dominated by the third order peak.

Fig. 1 shows the diffraction patterns obtained from the two samples containing, or not containing, protonated ubiquinone at 100:0 D_2O/H_2O contrast. Up to six diffraction orders were recorded for each sample with pronounced relative intensity changes between the samples. The measured *d*-spacing for the samples were equal to (5.28±0.04) nm for the pure lipid sample and (5.38±0.04) nm for the lipid sample with the ubiquinone, respectively, suggesting no change in the membrane thickness. Profiles of the scattering length densities (i.e., the membrane profiles) are calculated as the Fourier sum of the structure factors. The scattering length density profiles for the deuterated lipid sample with protonated ubiquinone are illustrated for the various D_2O/H_2O atmospheres in Fig. 2, demonstrating the effect of the contrast variation. The unit cell is chosen so that the midplane of the lipid membrane is in the center and the water layers are at its outer edges. At 0% D_2O, the contrast between the water layer and the lipid membrane is largest, reflecting the negative scattering length density (sld) of H_2O (-0.56×10^{10} cm^{-2}) and the positive average scattering length density of the deuterated lipids at about $+5.9\times10^{10}$ cm^{-2}. At 100% D_2O contrast the scattering length densities of lipids and water nearly match (sld(D_2O)=6.33×10^{10} cm^{-2}) and the scattering density profile reflects smaller local changes in this entity. The scattering length density profiles for the two samples with and without ubiquinone in an atmosphere of 100% D_2O are shown in Fig. 3 together with the corresponding difference density profile. The protonated ubiquinone shows up as a negative density difference due to the negative scattering length of the protons. The difference densities between the samples with and without the ubiquinone for all four measured contrast ratios are displayed in Fig. 4 and reflect its location in

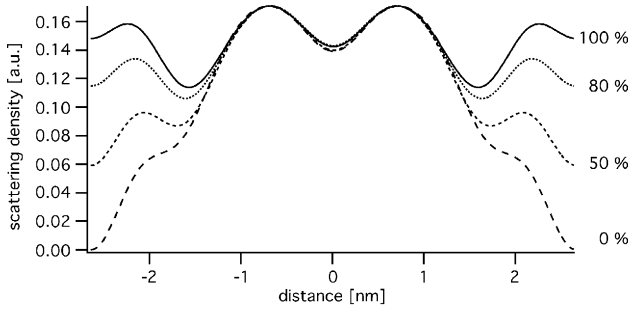

Fig. 2. The effect of the contrast variation on the calculated density profiles are shown in the case of the deuterated lipid membrane containing the protonated ubiquinone. The decreasing D_2O content from 100% to 0% in the water composition gives rise to a decrease in the scattering length density in the aqueous layers of the membrane stack. The scattering length density in the center of the bilayer is not effected in the experimental error limit.

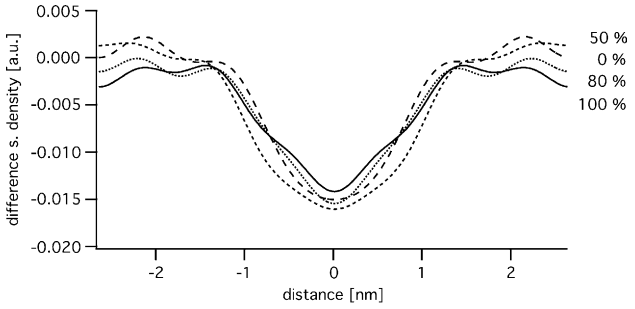

Fig. 4. Density profiles for all measured D_2O/H_2O contrasts. The central minimum in the scattering length density is caused by the protonated ubiquinone. This position corresponds to the lipid bilayer center. The wavy features at the outer edges of the density profiles are due to counting statistics and Fourier truncation errors and reflect the overall error in the membrane profiles calculated.

the bilayer center. In Fig. 4, this difference is clearly seen in the middle of the lipid bilayer. The experimental noise level due to counting statistics and Fourier truncation errors are reflected in the wavy features at the edges of the profiles. An artist view reflecting our neutron diffraction result is shown in Fig. 5, ubiquinone (dark gray) with its quinone ring is located in the center of the lipid bilayer near the terminal methyl groups, with minimal penetration into the acyl chain region.

4. Discussion

The aim of the present study is the unequivocal determination of the location of coenzyme Q_{10} in lipid bilayers, or more precisely, the orientation and the depth in the membrane profile. For this, we used the most direct method: neutron diffraction in combination with H/D contrast, here deuterated lipids and protonated CoQ_{10}. CoQ_{10} contains 10 isoprene units (50 carbons and 80 hydrogens) with a series of ten hinged planes (double bonds) and a quinone (9 carbons and 9 hydrogens). It needs not remain in an extended conformation, but may be curled in the membrane structure. Our approach is different from the "typical" neutron diffraction analysis on stacked lipid membranes wherein the localization of a small deuterated molecule (or domain) is determined in per-protonated bilayer lipids. In this study, we localized the per-protonated ubiquinone in a perdeuterated bilayer; an approach now possible due to the availability of per-deuterated lipids. Our neutron diffraction experiments yielded a set of scattering length density profiles from samples with deuterated lipids and deuterated lipids plus protonated ubiquinone at a series of D_2O/H_2O ratios of the solvent. From the respective difference density profiles (Fig. 4), the location of ubiquinone is demonstrated unambiguously in the bilayer center of lipid membranes in the physiologically relevant fluid-crystalline state.

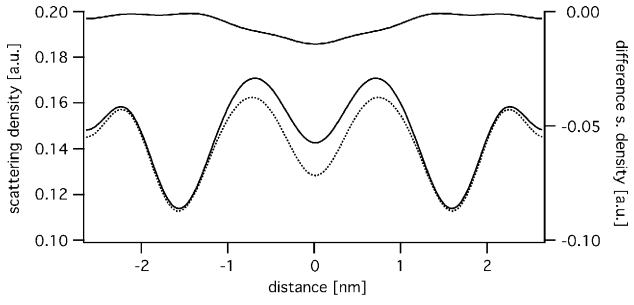

Fig. 3. Scattering density profile of the *d*DMPC/*d*DMPS (90:10 mol%) stacked bilayers without (lower solid line) or with (dotted line) protonated ubiquinone. The two minima represent the headgroups in the bilayers cross section. The trough in the middle is the bilayers center. The results are the Fourier transform of the structure factors calculated from the diffractogram in Fig. 1. Upper solid line: ubiquinone distribution calculated as the difference between the two scattering density profiles shown in the lower part of the figure. The central minimum reflects the position of the protonated ubiquinone, in the middle of the unit cell, i.e., in the center of the membrane.

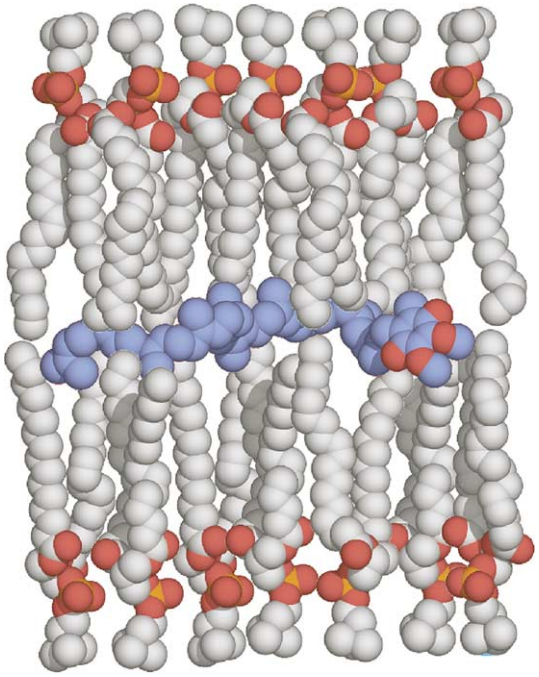

Fig. 5. An artist view of the lipid membrane with embedded ubiquinone derived from the neutron scattering density profiles (Fig. 4). The ubiquinone molecule (dark gray) is sandwiched between the two lipid layers of the membrane. Its conformation is not necessarily straight but its location is confined parallel to the membrane plane with only little intrusions into the lipid acyl chains.

Our previous data on the localization of squalane in phospholipid bilayers [15], together with the present data for CoQ_{10} show that all polyisoprenes and polyisoprene domains lie flat in the bilayer center—between the bilayer leaflets. A careful review of the localization of polyisoprenes, including especially ubiquinone, lays out the contradictory data that leaves the question open to theoretical statements at best [7]. It is important therefore to have our definitive data on the subject.

The incorporation of CoQ_{10} into the lipid bilayer does not change the repeat distance of the membrane stack in the experimental error limit (the increase is only 0.1 nm). This finding is in accordance with previous X-ray experiments on DMPC and DPPC membranes in the presence of ubiquinone [19]. Inspection of the membrane profiles in Fig. 3 suggests that the membrane thickness does not change, the density minimum at ±1.6 nm does not change its position on the membrane profile.

Taking advantage of our data we have considered the following molecular description of ubiquinone in phospholipid bilayers. Using the volumetric lipid data of Nagle and Tristan-Nagle [20] and the neutron scattering length of elements [21], the neutron scattering length of deuterated myristoyl $(CD_2)_{12}$–CD_3 – the acyl chain of the lipid used in these experiments – is calculated to b_{myr}=266.5 fm, with a volume of 390×10^{-24} cm^3 we calculate the neutron scattering length density to 6.8×10^{10} cm^{-2}. This is to be compared to the scattering length density of the total protonated CoQ_{10}, which is estimated to 0.4×10^{10} cm^{-2}, using the scattering length of 78.81 fm and an estimated volume of 2000×10^{-24} cm^3. Incorporation of the protonated ubiquinone in the perdeuterated lipid matrix would therefore reduce the scattering length density where it resides. Our data (Figs. 3 and 4) prove that the polyisoprene domain of ubiquinone is at the center of the bilayer, i.e., sandwiched between the two lipid monolayers. From the width of the difference density feature, one can conclude that the polyisoprene chain runs predominantly parallel to the membrane plane. To illustrate the molecular distribution along the bilayer profile, we construct a model where molecular groups are represented by Gaussian distributions (Fig. 6). A comparison of these data with that of squalane [15] suggests that both polyisoprenes and polyisoprene domains in general have the same conformation in straight-chain lipid bilayers: they lie parallel to the membrane plane in the bilayer center. Thus, this statement applies to the myriad of polyisoprenes found in natural membranes. Only the membranes of the archaea lack straight-chain lipids, which are replaced by diphytanyl chains and which also contain polyisoprenes such as squalene [22]. The localization of polyisoprenes in these bilayers is yet to be established.

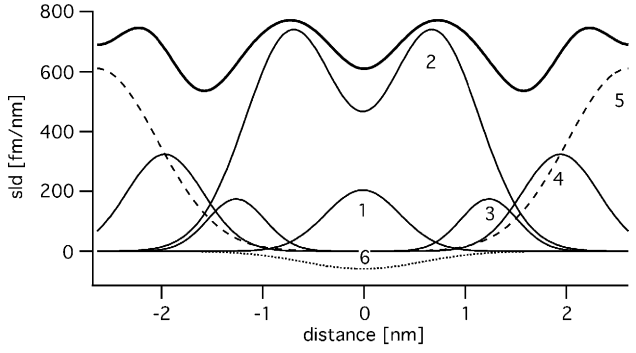

Fig. 6. Model of the deuterated DMPC bilayer containing the protonated ubiquinone with a 100% D_2O water layer. The different molecular groups of the lipids are represented by the Gaussian distributions 1,2,3,4, corresponding to the terminal methyl groups (1), the acyl chains (2), the glycerol backbone (3), and the phosphatidyl head group (4). The water distribution (5) is calculated by the difference of the density profiles at 100% and 0% D_2O, respectively. The model Gaussians 1 to 5 with the appropriate calculated scattering length densities were first fitted to the density profile of the membrane sample without ubiquinone, by varying the position, width, and amplitude, but keeping the integral to the calculated scattering length density. The parameters of the model Gaussian 6 (dotted curve), which describes the ubiquinone distribution, where then determined by fitting the parameters to the density profile of the membrane containing protonated ubiquinone, while keeping the parameters of Gaussians 1 to 5 constant.

4.1. Previous attempts to localize ubiquinone in bilayers

There have been many attempts using indirect measurements to localize ubiquinone in bilayers. Our neutron diffraction experiments have unequivocally localized its polyisoprene domain at the bilayer center. Using differential scanning calorimetry (DSC) to measure the phase transition temperature T_m, Katsikakis and Quinn [10,23,24] concluded, that coenzyme Q_{10} at up to 20 mol% codispersions in dipalmitoyl phosphatidyl choline (DPPC) lies in the center of the bilayers. Fluorescence quenching measurements [25] are also consistent with the conclusion that ubiquinone resides in the bilayer center. In contrast, Lenaz et al. [26] have concluded from fluorescence studies, having the fluorophore at different heights in the bilayer, "that CoQ analogues oscillate between the two bilayer surfaces not extending beyond the glycerol region."

Katsikas and Quinn have shown [23], using X-ray diffraction, that tocopherol, another polyisoprene, thickens the bilayer, in contrast to the present study for CoQ_{10}. They suggested on this basis, that tocopherol is in or near the bilayer center. In contrast, Stidham et al. [19] reported in their ^{13}C NMR study on egg phosphatidylcholine liposomes containing ubiquinone, a marked effect on the ^{13}C spin lattice relaxation times of the hydrocarbon chain atoms near the lipid head groups, but nearly no effect on the atoms nearest to the bilayer center, suggesting ubiquinone's location in the head group region. They concluded that the ubiquinone is not located preferentially between the monolayers. A more extensive study was conducted by Cornell et al. [5]. Using deuterated ubiquinone and examining its ^2H NMR quadrupole coupling they found that the Q_{10} isoprenoid chain is in a mobile environment, physically separated from the cooperative motions and orientational constraints of the bilayer lipid chains. They thus suggested that the Q_{10} isoprenoid chain *might* lie in the center of the bilayer. These investigators also synthesized a ubiquinone analogue in which the ten isoprene groups have been replaced by a perdeuterated saturated tridecyl chain. The ^2H NMR quadrupole coupling showed

that this analogue is oriented *parallel to the chains* of the phospholipids and that its quinone domain is in the region of the phospholipid headgroups. Thus two features of straight-chain lipid bilayers seem to provoke the isoprenes to reside in the bilayer center. First, the cooperative motions of the chains in each monolayer are disrupted by intrusion of branched hydrocarbons within that monolayer. Second, the two monolayers are uncoupled so that the *lateral* cooperative motions [27] of the phospholipid acyl chains within one monolayer do not affect the motions of the other. In a recent study using NMR chemical shift-polarity correlation Afri et al. [9] concluded that the ubiquinone ring lies in the lipid head group region but distant from the lipid–water interface and place a considerable portion of the isoprenoid side chain parallel to the lipid acyl chains, an interpretation which is not in line with our neutron diffraction result.

4.2. Implications of CoQ in the bilayer center

CoQ is best known as a carrier of electrons and protons in mitochondria during oxidative phosphorylation as is the ubiquinone analog plastoquinone in chloroplasts during photophosphorylation [28]. As part of the mitochondrial electron transport chain CoQ transfers electrons from Complex I and II to Complex III, all embedded in the inner mitochondrial membrane. To facilitate electron transport, CoQ should be freely diffusing in its membrane environment to encounter the binding pockets in the protein complexes involved [6,29]; this is best if CoQ_{10} resides in the membrane midplane, as shown by our investigation.

Ubiquinone is also well known as an antioxidant in eukaryotes where it has wide distribution throughout many organelle membranes [30]. Interest has increased in the specific location of the quinone in the lipid bilayer cross-section with the emergence of high-resolution structures of the proteins that bind it [31].

Our data in Figs. 3 and 4 prove that the polyisoprene domain of ubiquinone, like that of squalane [15], lies in the bilayer center parallel to the membrane plane. Thus long polyisoprenes and polyisopranes can be assumed to have such a residence. Long polyisoprenes include tocopherol, dolichol, plastoquinone, carotenes, and lycopene.

The presence of these polyisoprenes in the bilayer center raises a question as to the area occupied by them since they appear to be restricted to the cleavage plane. Each isoprene unit based on models and assuming a conformation parallel to the bilayer plane, occupies about 0.4–0.5 nm^2. Since the isoprene units are sandwiched between the two monolayers we need only consider the area of one monolayer with an area of 0.64 nm^2 per lipid. Each CoQ_{10} contains 10 isoprenes and a quinone ring. Thus, it covers, if it resides as a monolayer, 4.4–5.5 nm^2, or an average of 5 nm^2. This occupies the area of about 8 lipid molecules. Thus a 6 mol% of CoQ_{10} is sufficient to provide a full surface coverage of each monolayer for the total lipids of the bilayer. The concentration of ubiquinone in biological membranes is widespread and largest in mitochondria [1]. Using published data with up to 7 nmol CoQ per mg protein [32], the surface coverage in mitochondria membranes lies between 10% and 30%. Such a calculation suggests that CoQ_{10} can represent a serious barrier for, e.g., proton diffusion across the bilayer of, say, the inner membrane of the mitochondrion. Taken this together with our localization of both squalane and ubiquinone in the bilayer center, suggests, in accordance with the "water cluster" mechanism for proton leakage [2], that these polyisoprenes may inhibit proton leakage. Such inhibition would be another biological function for them, in addition to their role in redox systems as electron/proton carriers and as antioxidants.

Acknowledgements

This work was supported by grants of the Bundesministerium für Bildung und Forschung (03-DE5DAR2-0), the Deutsche Forschungsgemeinschaft (Sfb 472) and the Fonds der Chemischen Industrie to N.A.D., as well as grant PSC/CUNY 62230-00 31 to T.H.H.

References

[1] M. Tutunen, J. Olsson, G. Dallner, Metabolism and function of coenzyme Q, Biochim. Biophys. Acta 1660 (2004) 171–199.
[2] T.H. Haines, Do sterols reduce proton and sodium leaks through lipid bilayers? Prog. Lipid Res. 40 (2001) 299–324.
[3] L. Ernster, G. Dallner, Biochemical, physiological and medical aspects of ubiquinone function, Biochim. Biophys. Acta 1271 (1995) 195–204.
[4] P. Mitchell, The protonmotive Q cycle: a general formulation, FEBS Lett. 59 (1975) 137–139.
[5] B.A. Cornell, M.A. Keniry, A. Post, R.N. Robertson, L.E. Weir, P.W. Westerman, Location and activity of ubiquinone 10 and ubiquinone analogues in model and biological membranes, Biochemistry 26 (1987) 7702–7707.
[6] G. Lenaz, A critical appraisal of the mitochondrial coenzyme Q pool, FEBS Lett. 509 (2001) 151–155.
[7] P.J. Quinn, in: V.E. Kagan, P.J. Quinn (Eds.), Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, New York, 2000, pp. 29–42.
[8] J. Salgado, J. Villalain, J.C. Gomez-Fernandez, Magic angle spinning 12C-NMR spin-lattice relaxion study of the location and effects of alpha-tocopherol, ubiquinone-10 and ubiquinol-10 in unsonicated model membranes, Eur. Biophys. J. 22 (1993) 151–155.
[9] M. Afri, B. Ehrenberg, Y. Talmon, J. Schmidt, Y. Cohen, A.A. Frimer, Active oxygen chemistry within the liposomal bilayer: Part III. Locating vitamin E, ubiquinol and ubiquinone and their derivatives in the lipid bilayer, Chem. Phys. Lipids 131 (2004) 107–121.
[10] H. Katsikas, P.J. Quinn, Fluorescence probe studies of the distribution of ubiquinone homologues in bilayers of dipalmitoylglycerophosphocholine, Eur. J. Biochem. 131 (1983) 607–612.
[11] M. Ondarroa, P.J. Quinn, A difference infrared-spectroscopic study of the interaction of ubiquinone-10 with phospholipid bilayers, Biochem. J. 240 (1986) 325–331.
[12] B. Samori, G. Lenaz, M. Battino, G. Marconi, I. Domini, On coenzyme Q orientation in membranes: a linear dichroism study of ubiquinones in a model bilayer, J. Membr. Biol. 128 (1992) 193–203.
[13] F.J. Aranda, J.C. Gomez-Fernandez, The interaction of ubiquinone-10 and ubiquinol-10 with phospholipid bilayers. A study using differential scanning calorimetry and turbidity measurements, Biochim. Biophys. Acta 820 (1985) 19–26.
[14] S. Clejan, T.A. Krulwich, K.R. Mondrus, D. Seto-Young, Membrane lipid composition of obligately and facultatively alkalophilic strains of Bacillus spp, J. Bacteriol. 168 (1986) 334–340.

[15] T. Hauss, S. Dante, N.A. Dencher, T.H. Haines, Squalane is in the midplane of the lipid bilayer: implications for its function as a proton permeability barrier, Biochim. Biophys. Acta 1556 (2002) 149–154.
[16] N.P. Franks, W.R. Lieb, The structure of lipid bilayers and the effects of general anaesthetics. An X-ray and neutron diffraction study, J. Mol. Biol. 133 (1979) 469–500.
[17] S. Dante, T. Hauss, N.A. Dencher, Insertion of externally administered amyloid beta peptide 25–35 and perturbation of lipid bilayers, Biochemistry 42 (2003) 13667–13672.
[18] T. Hauß, G. Büldt, M.P. Heyn, N.A. Dencher, Light-induced isomerization causes an increase in the chromophore tilt in the M intermediate of bacteriorhodopsin: a neutron diffraction study, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11854–11858.
[19] M.A. Stidham, T.J. McIntosh, J.N. Siedow, On the localization of ubiquinone in phosphatidylcholine bilayers, Biochim. Biophys. Acta 767 (1984) 423–431.
[20] J.F. Nagle, S. Tristram-Nagle, Structure of lipid bilayers, Biochim. Biophys. Acta 1469 (2000) 159–195.
[21] V.F. Sears, Neutron scattering length and cross sections, Neutron News 3 (1992) 26–37.
[22] A. Corcelli, M. Colella, G. Mascolo, F.P. Fanizzi, M. Kates, A novel glycolipid and phospholipid in the purple membrane, Biochemistry 39 (2000) 3318–3326.
[23] H. Katsikas, P.J. Quinn, The polyisoprenoid chain length influences the interaction of ubiquinones with phospholipid bilayers, Biochim. Biophys. Acta 689 (1982) 363–369.
[24] H. Katsikas, P.J. Quinn, The distribution of ubiquinone-10 in phospholipid bilayers. A study using differential scanning calorimetry, Eur. J. Biochem. 124 (1982) 165–169.
[25] D.A. Haydon, B.M. Hendry, S.R. Levinson, J. Requena, The molecular mechanisms of anaesthesia, Nature 268 (1977) 356–358.
[26] G. Lenaz, B. Samori, R. Fato, M. Battino, G. Parenti Castelli, I. Domini, Localization and preferred orientations of ubiquinone homologs in model bilayers, Biochem. Cell. Biol. 70 (1992) 504–514.
[27] T.H. Haines, Water transport across biological membranes, FEBS Lett. 346 (1994) 115–122.
[28] D. Voet, J.D. Voet, Biochemistry, John Wiley and Sons, Inc., New York, 1995.
[29] S. Gupte, E.S. Wu, L. Hoechli, M. Hoechli, K. Jacobson, A.E. Sowers, C.R. Hackenbrock, Relationship between lateral diffusion, collision frequency, and electron transfer of mitochondrial inner membrane oxidation–reduction components, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 2606–2610.
[30] K. Mukai, in: V.E. Kagan, P.J. Quinn (Eds.), Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, New York, 2000, pp. 43–61.
[31] H. Palsdottir, C.G. Lojero, B.L. Trumpower, C. Hunte, Structure of the yeast cytochrome bc1 complex with a hydroxyquinone anion Qo site inhibitor bound, J. Biol. Chem. 278 (2003) 31303–31311.
[32] A. Lass, R. Sohal, Comparison of coenzyme Q bound to mitochondrial membrane proteins among different mammalian species, Free Radical Biol. Med. 27 (1999) 220–226.