Location and Activity of Ubiquinone 10 and Ubiquinone Analogues in Model and Biological Membranes†

B. A. Cornell,* M. A. Keniry,‡ A. Post,§ R. N. Robertson,* L. E. Weir,* and P. W. Westerman‖

Division of Food Research, Commonwealth Scientific and Industrial Research Organisation, North Ryde, Sydney, New South Wales 2113, Australia, School of Biological Sciences, Macquarie University, North Ryde, Sydney, New South Wales 2113, Australia, Department of Biochemistry and Molecular Pathology, Northeastern Ohio Universities' College of Medicine, Rootstown, Ohio 44272, and Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143

Received September 4, 1986; Revised Manuscript Received July 6, 1987

ABSTRACT: Deuteriated analogues of ubiquinone 10 (Q_{10}) have been dispersed with plasma membranes of *Escherichia coli* and with the inner membranes of beetroot mitochondria. Orientational order at various deuteriated sites was measured by solid-state deuterium nuclear magnetic resonance (^{2}H NMR). Similar measurements were made, using the compounds dispersed in dimyristoylphosphatidylcholine (DMPC) and egg yolk lecithin and dispersions prepared from the lipid extracts of beetroot mitochondria. In all cases only a single unresolved ^{2}H NMR spectrum (typically 1000-Hz full width at half-height) was observed at concentrations down to 0.02 mol % Q_{10} per membrane lipid. This result shows that most Q_{10} is in a mobile environment which is physically separate from the orientational constraints of the bilayer lipid chains. In contrast, a short-chain analogue of Q_{10}, in which the 10 isoprene groups have been replaced by a perdeuteriated tridecyl chain, showed ^{2}H NMR spectra with quadrupolar splittings typical of an ordered lipid that is intercalated into the bilayer. The NADH oxidase activity and O_2 uptake in *Escherichia coli* and in mitochondria were indepedent of which analogue was incorporated into the membrane. Thus, despite the major difference in their physical association with membranes, or their lipid extracts, the electron transport function of the long- and short-chain ubiquinones is similar, suggesting that the bulk of the long-chain ubiquinone does not have a direct function in electron transporting activity. The physiologically active Q_{10} may only be a small fraction of the total ubiquinone, a fraction that is below the level of detection of the present NMR equipment. However, our results do not support any model of Q_{10} electron transport action that includes intercalation of the long isoprenoid chain in lipid.

Ubiquinones (Q)[1] are lipoidal components of the respiratory and photosynthetic electron transport chains of several cellular and subcellular membranes (Trumpower, 1982). Mechanisms by which Q carries electrons through electron transport chains and protons across membranes, as postulated by the "protonmotive Q cycle" (Mitchell, 1976), have been discussed extensively [e.g., Crane (1977), Trumpower (1981), and Hauska and Hurt (1982)]. Central to an understanding of these mechanisms is a knowledge of the disposition of Q in artificial and native membranes. A widely held view has been that transmembrane "flip-flop" of long-chain quinones across membrane bilayers provides the mechanism for quinone-catalyzed proton transport (Hauska & Hurt, 1982). Since the oligoprenyl chain length in the major physiological quinones (Q_6–Q_{10}), in the extended all-trans configuration, is approximately the thickness of a membrane (see Figure 1), it has been proposed that the quinone could form a layer in the center of the bilayer (Crane, 1977). Other models that place a substantial fraction of the Q in the center of the bilayer have been proposed on the basis of calorimetry (Katsikas & Quinn, 1981), fluorescence quenching (Chatelier & Sawyer, 1985), and ^{1}H NMR (Kingsley & Feigensen, 1981; Ulrich et al., 1985; Ondarroa & Quinn, 1986).

In the present paper we have studied a series of deuteriated analogues of Q_{10} incorporated into dispersions of synthetic DMPC, *E. coli* plasma membranes, beet mitochondrial membranes, and hydrated lamellar dispersions of their lipid extracts. In addition, the Q_{10} analogues were incorporated into membranes of an *E. coli* mutant (AN750) that possessed no native ubiquinone and yet increased its electron transport activity when supplemented with synthetic Q_{10}. These dispersions have been characterized by solid-state deuterium NMR to determine the extent of orientational order at selected sites. One analogue possessed deuteriated methoxy groups on the benzoquinone ring (Q_{10}-OMe-d_6) and the other deuterons at 20 sites along the isoprenoid chain (Q_{10}-d_{20}). In addition, we studied the properties of a shorter chain analogue in which

† This work was supported by CSIRO visiting fellowships (to P.W.W. and M.A.K.) and by Grant GM 27127 from the National Institutes of Health (to P.W.W.). A.P. is the recipient of a Commonwealth Postgraduate Research Award. Preliminary accounts of this research have been presented (Westerman et al., 1986).

* Address correspondence to these authors at CSIRO.
‡ University of California.
§ Macquarie University.
‖ Northeastern Ohio Universities' College of Medicine.

[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; Q, ubiquinone; Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; Q_n, ubiquinone with n isoprenoid groups; QC-13-d_{27}, 2,3-dimethoxy-5-methyl-6-tridecyl-d_{27}-1,4-benzoquinone; QC-13, 2,3-dimethoxy-5-methyl-6-tridecyl-1,4-benzoquinone; Q_{10}-d_{20}, 2,3-dimethoxy-5-methyl-6-[decakis(2,3-dideuterio-3-methylbutan-1,4-diyl)]-1,4-benzoquinone; Q_{10}-OMe-d_6, 2,3-dimethoxy-d_6-5-methyl-6-[decakis(3-methyl-2-buten-1,4-diyl)]-1,4-benzoquinone; TLC, thin-layer chromatography; Me_4Si, tetramethylsilane; Tris, tris(hydroxymethyl)aminomethane; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PVP, poly(vinylpyrrolidone); EFG, electric field gradient; NMR, nuclear magnetic resonance; *E. coli*, *Escherichia coli*; NADH, nicotinamide adenine dinucleotide, reduced.

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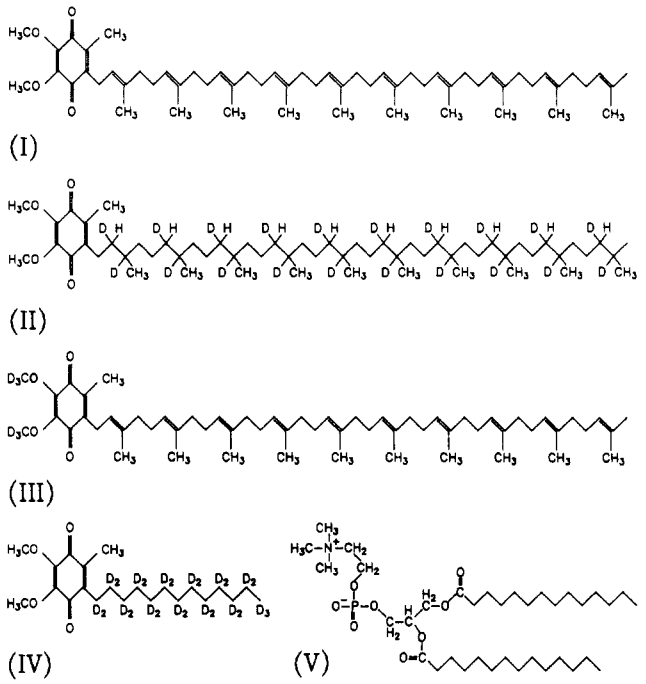

FIGURE 1: Structures of (I) ubiquinone 10 (Q_{10}), (II) the chain deuteriated analogue of ubiquinone 10 (Q_{10}-d_{20}), (III) the benzoquinone ring deuteriated analogue of ubiquinone 10 (Q_{10}-OMe-d_6), (IV) the short chain deuteriated analogue of ubiquinone 10 (QC-13-d_{27}), and (V) DMPC.

the chain had been replaced by a deuteriated tridecyl chain (QC-13-d_{27}).

Concurrent measurements were made of the ability of the analogue to restore electron transport activity to ubiquinone-depleted *E. coli* and beet mitochondrial membranes.

Experimental Procedures

Materials. DMPC was obtained from both Sigma Chemical Co. and Avanti Lipids. Q_0 was purchased from Fluka. Q_{10} was purchased from Calbiochem and its purity confirmed by TLC using a mixture of petroleum ether–ether–acetic acid (90:10:1) for development. Other chemicals were obtained commercially at the highest available purity, and organic solvents were distilled before use.

Organic Synthesis. Tetradecanoic acid was perdeuteriated with ^2H_2O in a Parr high-pressure reactor vessel (Nguyen-Dihn-Nguyen & Stenhagen, 1966). ^1H NMR indicated 93% replacement of protons after a single exchange reaction. The deuteriated acid was converted to the diacyl peroxide by the method of Silbert and Swern (1959).

QC-13-d_{27} was synthesized by a modification of the procedure of Wan and Folkers (1978). A stirred solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (0.91 g) in glacial acetic acid (25 mL), was heated to 90–95 °C under dry N_2. A suspension of perdeuteriated ditetradecanoyl peroxide (5 g) in glacial acetic acid (5 mL) and ethyl ether (20 mL) was added dropwise over 2 h and stirred for an additional 20 h at 90–95 °C. The products were evaporated to dryness and purified on a silica gel column. Byproducts were eluted with hexane, and the compound was removed with chloroform–hexane (1:10). Purity was checked by TLC using chloroform–hexane–ether (10:10:1). The yellow-orange crystalline product (0.64 g) gave a single spot (R_f = 0.48) on TLC and ^1H NMR spectrum consistent with related analogues (Yu et al., 1985). QC-13 was prepared by the same procedure.

Q_{10}-d_{20} was prepared from Q_{10} by low-pressure deuteriation in absolute ethanol over a 10% Pd/C catalyst at room temperature.

Q_{10}-OMe-d_6 was prepared by a modification of the method of Shunk et al. (1960). A mixture of Q_{10} (400 mg) and pyrogallol (400 mg), in a 1 M solution of sodium methoxide-d_3 in methanol-d_4 (10 mL), was heated at 90 °C for 5 h in a sealed glass tube. On cooling, the contents were neutralized with a solution of ^2HCl in ^2H_2O and extracted with ether. The combined extracts were dried over anhydrous $MgSO_4$ and filtered, and the filtrate was stirred with Ag_2O (0.8 g) for 15 min. Solids were filtered off and the ether was removed under reduced pressure. The orange-red residue was purified on a Florisil column packed in hexane. A 2% ether–hexane solution eluted the product (150 mg), which TLC showed was Q_{10}. Integration of the ^1H NMR spectrum indicated approximately 20% isotopic incorporation. The procedure was repeated to give Q_{10} (43 mg) with 47% exchange at the methoxyl groups. The ^1H NMR spectral parameters were confirmed (Wilczynski et al., 1968).

Membrane Preparative Procedures. E. coli membranes prepared by the method of Cox et al. (1983) were a gift from Dr. Cox and Professor Gibson. Beet mitochondria were prepared by a method suggested by Bryce and Wiskich (personal communication). Fresh beets (400 g) were peeled, sliced, and ground in a juice extractor at 4 °C. The extract (approximately 200 mL) was filtered into 80 mL of a medium consisting of 1.0 M sucrose (32 mL), 0.5M TES (pH 7.4, 50 mL), 0.25 M EGTA (16 mL), 1.0 M Tris (7 mL), water (48 mL), and 1% w/v PVP. After filtering, cell debris was removed by centrifugation at 1500*g* for 10 min. The supernatant was spun at 10000*g* for 20 min, and the pellet was resuspended in 0.4 M sucrose and rinsed to give the mitochondrial membranes. Lipids were extracted by the method of Bligh and Dyer (1959). Egg yolk lecithin was extracted and purifed by the method of Singleton et al. (1965).

NMR Sample Preparation. Multilamellar dispersions of Q_{10} and its deuteriated analogues in model membrane systems were prepared by dissolving a known ratio of ubiquinone and lipid in benzene–methanol or cyclohexane–methanol (95:5 v/v). The mixture was lyophilized and then hydrated with an equal weight of deuterium-depleted water. The sample was lyophilized a second time and rehydrated to allow back exchange of labile protons.

Q_{10} and the deuteriated analogues were partitioned into the *E. coli* and mitochondrial membranes according to the procedures of Ernster et al. (1969). Concentrations of the ubiquinones in the reconstituted *E. coli* and mitochondrial membranes were in the range 0.4–0.05 mol % relative to the membrane lipid. Samples were hydrated with deuterium-depleted water (×1.5 weight of dry sample) and dispersed by repeated centrifuging through a constriction in a sealed glass tube.

Enzyme Assays. Electron-transfer activities of *E. coli* membranes with ubiquinone extracted and analogues reconstituted were assayed. NADH oxidase activity was measured spectrophotometrically (Mackler, 1967) in 50 mM phosphate buffer at pH 7.2. Cyanide inhibition experiments were also performed under these conditions. The oxygen uptake with NADH as substrate was measured with an oxygen electrode (Raison & Lyons, 1970). Proton translocation of membranes, in unbuffered solution, was measured by the change in pH with time by a recording pH meter. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

NMR Spectroscopy. High-resolution ^1H NMR spectra were recorded at ambient temperature on either a Bruker WP-80 or a Varian XL-200 spectrometer. Samples (ap-

proximately 20 mg) were dissolved in C^2HCl_3 and chemical shifts expressed relative to the internal standard Me_4Si. Typically, 100–200 pulses were accumulated. Solid-state ^2H NMR measurements were made with a Bruker CXP-300 spectrometer operating at 46 MHz and a 90° pulse duration of 5–6 μs. A solid echo (Gerstein & Dybowski, 1985) sequence with an echo delay of 40 μs was used to avoid pulse breakthrough and acoustic ringing. Owing to the use of a solid echo sequence, any changes that occur in the transverse relaxation time (T_2) will be reflected in a change in signal intensity.

Integrals of the spectral intensity were compared to the integrals of the ^2H-labeled quinones after being extracted from the sample with pentane, lyophilized, and dissolved in chloroform. This permitted a measure of the total ^2H NMR signal intensity and included any signal that was broadened below the level of detection in the dispersion. The precision of these measurements was typically 5–10%. In order to detect the ^2H NMR signals at the low concentrations of label employed, the probe was modified to accept an active sample volume of 5 mL (15-mm o.d.) tubes.

Interpretation of NMR Spectra (Davis, 1983; Strenk et al., 1985). The ^2H nucleus has a spin $I = 1$ and thus a nuclear quadrupole moment. In the presence of a strong magnetic field, the interaction of this quadrupolar moment with the EFG causes the NMR spectrum of each ^2H to be split into two lines with a separation given by

$$\Delta\nu = {}^3/_4\nu_Q(3\cos^2\theta - 1)$$

where ν_Q is the quadrupole coupling constant associated with the ^2H–C bond and θ the angle between the principal component of the EFG (usually along the ^2H–C bond) and the magnetic field. For a powder of immobile atoms in which all orientations of the ^2H–C bond are present in a random distribution, all possible quadrupole splittings are superimposed, producing a characteristic powder spectrum (Mehring, 1983). The most easily identified feature of this spectrum is the central doublet with edge singularities arising from those orientations of the ^2H–C bond with $\theta = 90°$. In this case $\Delta\nu = {}^3/_4\nu_Q$. In the presence of fast (10^{-5} s) anisotropic reorientation of the molecule and its segments, the splitting of the edge singularities is reduced so that ν_Q is replaced by the averaged quadrupole splitting

$$\bar{\nu}_Q = \nu_Q\langle{}^3/_2\cos^2\theta_i - {}^1/_2\rangle = \nu_Q S$$

The order parameter, S, is a time average of the quantity in brackets, taken over all angles (θ_i) that the C–H_i bond direction assumes during the course of the NMR measurement.

A principal goal in the study of membrane structure by ^2H NMR is the interpretation of S in terms of a molecular model that accounts for the relative importance of the various motions that average the quadrupole interaction for a particular ^2H_i–C segment. These motions include intramolecular segmental motions, rigid-body reorientations of the molecule as a whole, and vesicle tumbling. In multilamellar preparations of phospholipids, vesicle tumbling is not significant in the time-averaging process. To account for the relative contributions of the other two processes, we make the valid approximation (Peterson & Chan, 1977; Oldfield et al., 1978) that the ordering is symmetrical about the director axis. This permits us to express $\langle{}^3/_2\cos^2\theta_i - {}^1/_2\rangle$ as the product of the conformational order parameter and molecular order parameter. That is

$$\langle{}^3/_2\cos^2\theta_i - {}^1/_2\rangle = a_i S_{zz} = \langle{}^3/_2\cos^2\beta - {}^1/_2\rangle\langle{}^3/_2\cos^2\theta_z - {}^1/_2\rangle$$

where β is the angle between the direction of the ^2H_i–C bond and the most ordered or principal molecular axis (z) and θ_z is the angle between the principal molecular axis and the director axis. As a first approximation, this axis can be assigned on the basis of the shape of the molecule.

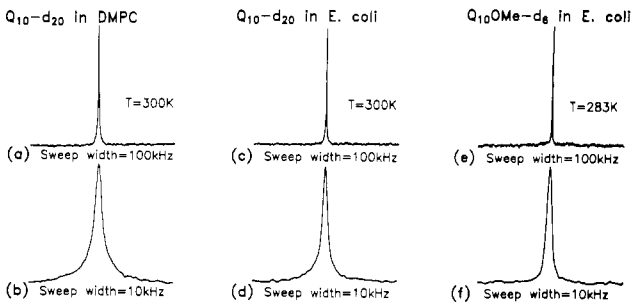

FIGURE 2: ^2H NMR spectra of the chain-labeled Q_{10}-d_{20} and ring-labeled Q_{10}-OMe-d_6 dispersed in DMPC or *E. coli* membranes: (a) 0.05 mol % Q_{10}-d_{20} in DMPC at 300 K, sweep width 100 kHz, solid echo sequence delay 40 μs, repetition delay 1 s, 40000 scans; (b) same spectrum as (a) on an expanded scale of 10 kHz; (c) 0.1 mol % Q_{10}-d_{20} in *E. coli* at 300 K and the same conditions as (a); (d) same spectrum as (c) on an expanded scale of 10 kHz; (e) 0.07 mol % Q_{10}-OMe-d_6 in *E. coli* at 283 K and the same conditions as (a); (f) same spectrum as (e) on an expanded scale of 10 kHz.

It follows then that the order of a solute molecule such as Q_{10}, which is incorporated into a bilayer, may be deduced from the separation of the 90° edge singularities in the ^2H NMR spectrum of a deuteron label on the Q_{10}. If many sites are labeled, a variety of splittings will result, their number and value depending on the relative order and conformation of the labeled site. If a zero quadrupole splitting is observed at a given site, it can arise from two sources, namely, a_i or $S_{zz} = 0$. To eliminate the first possibility, in which case the solute molecule may be highly ordered ($a_i = 0$), it is necessary to label several sites. It is highly improbable that a_i will be zero at all sites. The other situation is that $S_{zz} = 0$, which corresponds to a deuteriated Q_{10} being incorporated into a phase that is undergoing rapid isotropic tumbling. Other factors such as relaxation will determine the width of the residual unresolved peak.

RESULTS

(A) Deuterium Nuclear Magnetic Resonance. Parts a and c of Figure 2 show the ^2H NMR spectra for Q_{10}-d_{20} in the L_α phase of DMPC and hydrated *E. coli* membranes. As the Q_{10}-d_{20} is labeled with ^2H on the chain, we show for comparison, in Figure 2e, the spectrum of the ring-labeled Q_{10}-OMe-d_6 in hydrated *E. coli* membranes. The spectra in all cases possessed no resolvable quadrupolar splitting and as seen on the expanded sweep scale of Figure 2b,d,f had a half-height half-width of approximately 500 Hz. The spectrum of Q_{10}-OMe-d_6 dispersed in the L_α phase of DMPC was essentially the same, as were the spectra from Q_{10}-d_{20} and Q_{10}-OMe-d_6 in DMPC at low temperature (274 K) and in beet mitochondrial membranes, their lipid extracts, or dispersions with egg yolk lecithin at room temperature. Very different effects were seen when QC-13-d_{27} was incorporated in the L_α phase of DMPC, in *E. coli* (Figures 3a,b), and in mitochondrial membranes. In all three cases a broad quadrupole splitting is seen, which in the DMPC dispersion was resolved into a series of individual doublets reminiscent of the axially symmetric spectra derived from ^2H-labeled phospholipids (Seelig & Seelig, 1974). The spectra from the *E. coli* and the mitochondrial dispersions appear to be a superposition of a broad (40 kHz) component and a narrower component, the latter being similar to that obtained from the labeled Q_{10} compounds. This, together with the loss of the resolved

Table I: Restoration of Electron-Transfer Activity to *E. coli* Membranes Assessed by O_2 Uptake and NADH Oxidase Activity

	NADH oxidized [nmol min^{-1} (mg of protein)$^{-1}$]			O_2 uptake [nmol min^{-1} (mg of protein)$^{-1}$]		
	control	depleted	restored	control	depleted	restored
E. coli membranes	210 ± 15	6 ± 4		250 ± 20	0 ± 5	
Q_{10}			75 ± 10			80 ± 10
Q_{10}-d_{20}			73 ± 10			80 ± 10
Q_{10}-OMe-d_6			66 ± 5			90 ± 20
QC-13-d_{27}			72 ± 7			70 ± 15
Q_0			10 ± 5			20 ± 10
mutant *E. coli* membranes	19 ± 2			10 ± 5		
Q_{10}			78 ± 5			60 ± 15
Q_{10}-d_{20}			80 ± 10			60 ± 10
Q_{10}-OMe-d_6			66 ± 5			50 ± 10
QC-13-d_{27}			70 ± 5			55 ± 10
Q_0			22 ± 3			10 ± 10

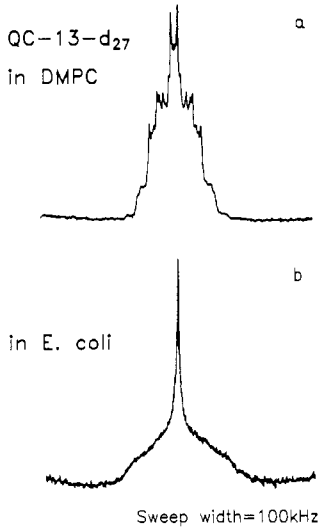

FIGURE 3: ^2H NMR spectra of the short-chain-labeled QC-13-d_{27} in DMPC at 300 K and *E. coli* membranes at 285 K. The same conditions were employed as in Figure 2a. (a) 3 mol % dispersion of QC-13-d_{27} in DMPC; (b) 0.4 mol % dispersion of QC-13-d_{27} in *E. coli*.

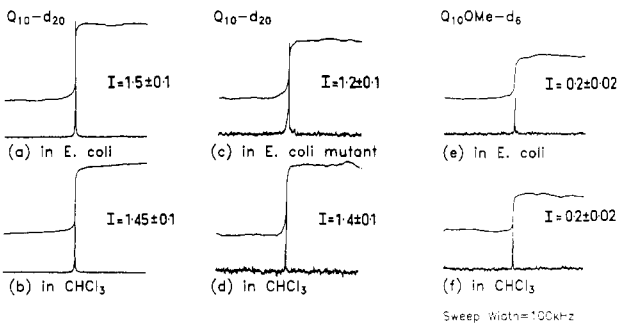

FIGURE 4: Integrals of ^2H NMR spectra of chain-labeled Q_{10}-d_{20} and ring-labeled Q_{10}-OMe-d_6 in hydrated dispersions of *E. coli* and *E. coli* mutant membranes at 285 K or in $CHCl_3$ solutions. The reconstituted quinones were in the range 0.05–0.13 mol % relative to the membrane lipid. Acquisition conditions are the same as in Figure 2a. (a) Q_{10}-d_{20} in *E. coli* membrane dispersions; (b) quinone from the same sample following extraction and solvation in $CHCl_3$; (c) Q_{10}-d_{20} in mutant *E. coli* membrane dispersions; (d) quinone from the same sample following extraction and solvation in $CHCl_3$; (e) Q_{10}-OMe-d_6 in *E. coli* membrane dispersions; (f) quinone from the same sample following extraction and solvation in $CHCl_3$.

doublets seen in Figure 3a, indicates a heterogeneity of order in the *E. coli* membranes which exceeds that of a DMPC dispersion. A similar heterogeneity was seen in mitochondrial membranes. A further source of the poor resolution seen in *E. coli* (Figure 3b) and also in the mitochondria (not shown) is possibly the shorter transverse relaxation times known to exist in biomembranes relative to their lipid extracts (Deese et al., 1981; Cornell et al., 1983). The value of the maximum quadrupolar splitting obtained from both *E. coli* and mitochondrial dispersions is close to the maximum splitting obtained in DMPC, suggesting that both arise from the common effect of the QC-13-d_{27} intercalating between the lipid chains of the membrane.

In order to determine the relative intensity of the signals in the ^2H NMR spectra of Q_{10}-d_{20} and Q_{10}-OMe-d_6, their integrals were compared with those obtained from a $CHCl_3$ solution of the quinones extracted from the same samples. These data are shown in parts a and e of Figure 4 for the hydrated dispersions of Q_{10}-d_{20} and Q_{10}-OMe-d_6 in *E. coli* and in parts b and f of Figure 4 for the extracted quinones in $CHCl_3$. Within an experimental precision of 10%, there was no detectable loss of signal intensity when these labels were incorporated into the hydrated dispersions.

A further unsuccessful attempt to demonstrate the intercalation of Q_{10}-d_{20} into *E. coli* membranes was to use a mutant strain of *E. coli* in which there was no ubiquinone activity. It has been shown that the electron-transfer activity of the strain is increased 4-fold when Q_{10}-d_{20} is added. As with the native strains of *E. coli*, there was no detectable orientationally restricted component to the ^2H NMR spectrum. The results of using this strain of *E. coli* are shown in Figure 4c,d. Within an experimental precision of 10%, all the potential ^2H NMR signal was visible.

The effect of incorporation of the shorter chain analogue QC-13-d_{27} in the native *E. coli* is seen in Figure 5a,b. The apparent signal loss seen in Figure 5a demonstrates the sensitivity of the technique in detecting the association of the analogues with the dispersions. The orientational restrictions suffered by QC-13-d_{27} when intercalated into the bilayer produced a dramatic reduction in T_2 and thus an order of magnitude reduction in the signal intensity. As discussed earlier this effect arises from the use of a solid echo sequence in the acquisition of the ^2H NMR signal.

(*B*) *Electron-Transfer Activity.* The restoration of NADH oxidase and O_2 uptake activities in Q_8-depleted membranes from native *E. coli* and from the mutant *E. coli* are summarized in Table I. Both assays indicate a similar ability to restore electron transfer, suggesting that the quinone analogues were functioning in the respiratory chain. The compounds Q_{10}, Q_{10}-d_{20}, Q_{10}-OMe-d_6, and QC-13-d_{27} were equally effective in restoring activity. A comparison with Q_0 shows that although there is no difference in activity between Q_{10} and QC-13-d_{27}, the side-chain length becomes important to electron transfer between a chain length of C_{13} and C_0. This is con-

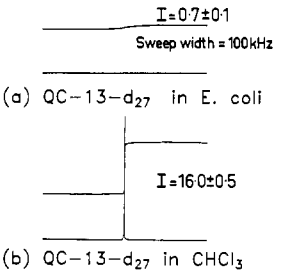

FIGURE 5: Integrals of ^2H NMR spectra of the short-chain-labeled QC-13-d_{27} *E. coli* membranes as hydrated dispersions at 285 K and as a solution in $CHCl_3$: (a) 0.4 mol % QC-13-d_{27} in hydrated *E. coli* membrane dispersions; (b) QC-13-d_{27} from the sample following extraction and solvation in $CHCl_3$.

sistent with a recent study by Yu et al. (1985), who demonstrated that the electron-transfer activity of ubiquinone in mitochondrial succinate–cytochrome *c* reductase is dependent on the length of the ubiquinone side chain. For ubiquinone acting as either a donor or an acceptor of electrons, they report a sigmoidal dependence of the electron-transfer activity, which achieves 100% activity between 7 and 10 carbons in the chain and possesses 50% activity between 4 and 6 carbons. It is possibly because the very short chains do not partition sufficiently into the nonaqueous phase.

The transmembrane function of the ubiquinone after reconstitution was indicated by two kinds of evidence. First, the oxidation of NADH in reconstituted *E. coli* membranes was inhibited by cyanide, suggesting that the electrons were passing from NADH to the cytochrome oxidase (or oxidases). For example, in an experiment where the average NADH oxidation in untreated membranes was 219 nmol min^{-1} (mg of protein)$^{-1}$, the addition of 1.9 mM cyanide (incubated with the membranes for 10 min to allow penetration) reduced the rate to 15 nmol min^{-1} (mg of protein)$^{-1}$, i.e., a 93% inhibition. After reconstitution with Q_{10}, the NADH oxidation rate was 149 nmol min^{-1} (mg of protein)$^{-1}$ and was decreased to 6 nmol min^{-1} (mg of protein)$^{-1}$ by 1.9 mM cyanide, i.e., 96% inhibition.

The second check on the transmembrane function of the ubiquinone was carried out by following the pH shift during oxidation as protons were transfered to the interior of leaky vesicles formed by the membranes; these vesicles are inside out; i.e., what was the inside of the cell membranes is the outside of the vesicles, so the direction of proton translocation is inward. Both undepleted and reconstituted membranes showed a loss of protons from the external solution.

Discussion

Our results show that the short ubiquinone analogue, QC-13-d_{27}, can intercalate into lipid bilayers but that the long-chain Q_{10} analogues segregate into a phase which possesses isotropic motion and is not constrained by the ordered chains of the membrane lipid. However, these compounds restore electron-transfer activity to the same extent.

The conclusion of this work is that despite the difference in the physical interactions of the Q_{10}-d_{20} and QC-13-d_{27} quinones with lipid multilayers and biological membranes, the electron-transfer activity is unaltered. Since a disordered environment is not present in the QC-13-d_{27}-enriched membranes of *E. coli*, we suggest that the bulk of the phase-separated population of long-chain ubiquinones (i.e., that in the disordered state) does not have a direct function in electron-transporting activity.

Green (1962) first proposed that electron transfer requires the solvation of ubiquinone within the interior of a membrane. This view is widely accepted, although the location of the ubiquinone relative to the membrane is unknown. Several studies have now shown that Q_8–Q_{10} forms a phase which is physically separated from the bilayer lipid in mixed ubiquinone–lipid dispersions (Katsikas & Quinn, 1981; Degli Esposti et al., 1981; Stidham et al., 1984; Fato et al., 1986).

By observing the effects of NMR shift reagents, Kingsley and Feigensen (1981) have reported that Q_{10} is in rapid exchange across the bilayer walls of unilamellar vesicles. However, sonicated aqueous dispersions of ubiquinone can form metastable aggregates that are sufficiently small and mobile to yield high-resolution proton NMR spectra. (Post, Cornell, and Hiller, unpublished results). In the presence of phospholipid vesicle solutions containing external NMR shift reagents, this ubiquinone resonance may be mistaken for ubiquinone that is rapidly migrating across the bilayer.

On the basis of further ^1H NMR measurements, Ulrich et al. (1985) argued against the possibility of rapid flip-flop motion of the Q_{10} and proposed that a large fraction of Q_{10} is located in a mobile pool near the center of the lipid bilayer.

Using fluorescence techniques, Chatelier and Sawyer (1985) devised a two-site model for the location of ubiquinone in rat liver mitochondrial membranes. One of the sites was in the interior of the membrane and the other near its hydrocarbon–water interface. However, the results of their experiments might be interpreted differently: the probes may not lie within the body of the membrane and might be in such a position as to come into contact with the phase-separated ubiquinone.

Our results show that the Q_{10} is not intercalated in the bilayer lipids but exists in a disordered state in *E. coli* and beet mitochondrial membranes, presumably in a separate phase. The physiologically active Q_{10} reaching the reaction site may be only a small fraction of the total separate ubiquinone, a fraction that is below the level of detection of the present NMR equipment. However, these data do not support any model of Q_{10} action that includes predominant intercalation of the long isoprenoid chain in lipid. The whereabouts of the separate phase are unknown.

Thus free movement of ubiquinones shown by the NMR technique does not necessarily mean rapid diffusion across the bilayer since it may be movement in a separate phase; however, diffusion from this phase to the reactive sites may be sufficient to maintain the electron transport chains' activity.

Acknowledgments

We thank Professor F. Gibson, John Curtin School of Medical Research, Australian National University, for helpful comments, Frances Separovic for NMR spectroscopy, and Sutin Horvath for excellent technical assistance.

Registry No. I, 303-98-0; II, 110971-01-2; III, 110971-02-3; IV, 110970-99-5; V, 13699-48-4; Q_0, 605-94-7; QC-13, 110971-00-1; $Me(CH_2)_{12}CO_2H$, 544-63-8; $[Me(CH_2)_{12}CO]_2O$, 626-29-9; $NaOCD_3$, 6552-73-4; $DOCD_3$, 811-98-3; pyrogallol, 87-66-1.

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DNA Structure in Which an Adenine–Cytosine Mismatch Pair Forms an Integral Part of the Double Helix†

Mukti H. Sarma, Goutam Gupta, and Ramaswamy H. Sarma*
Institute of Biomolecular Stereodynamics, State University of New York at Albany, Albany, New York 12222
Rolf Bald, Ute Engelke, Shiao Li Oei, Reinhard Gessner, and Volker A. Erdmann
Institut für Biochemie, Fachbereich Chemie, Freie Universität Berlin, 1000 Berlin 33 (Dahlem), FRG
Received April 13, 1987; Revised Manuscript Received July 1, 1987

ABSTRACT: Extensive studies using one- and two-dimensional ^{1}H NMR at 500 MHz revealed that the oligonucleotide d(CGCCGCAGC) in solution at 5 °C forms a double helix under conditions of high salt (500 mM in NaCl, 1 mM sodium phosphate), low pH (pH 4.5), and high DNA concentration (4 mM in duplex). The presence of very strong nuclear Overhauser effects (NOEs) from base H8/H6 to sugar H2′,H2″ and the absence of NOE from base H8/H6 to sugar H3′ suggested that the oligomer under these solution conditions forms a right-handed B-DNA double helix. The following lines of experimental evidence were used to conclude that C4 and A7 form an integral part of the duplex: (i) the presence of a NOESY cross-peak involving H8 of A7 and H8 of G8, (ii) the presence of a two-dimensional NOE (NOESY) cross-peak between H6 of C3 and H6 of C4, (iii) base protons belonging to C4 and A7 forming a part of the H8/H6---H1′ cross-connectivity route, and (iv) the pattern of H8/H6---H2′,H2″ NOESY cross-connectivity based upon a B-DNA model requiring that both C4 and A7 form an integral part of the duplex. The possibility of an A-C pair involving H bonds was also examined. Two possible structural models of the duplex at pH 4.5 are proposed: in one model A-C pairing involves two H bonds, and in the other A-C pairing involves a single H bond.

Until very recently, structural studies on short DNA duplexes were restricted to self-complementary DNA sequences. By single-crystal and solution studies it has been shown that self-complementary DNA sequences can exhibit a wide variety of secondary structures while still retaining the Watson–Crick base-pairing schemes. Structural morphologies of the A-, B-, and Z-DNA have been understood in terms of the nucleotide geometries (Conner et al., 1982; Drew et al., 1980; Shakked

† This research is supported by a grant from the National Institutes of Health (GM29787), the Deutsche Forschungsgemeinschaft (DFG-SFBg-B5), the Fonds der Chemischen Industrie e.V., and a contract from the National Foundation for Cancer Research.

0006-2960/87/0426-7707$01.50/0