Acta Chemica Scandinavica, 1997: 51: 233–241
Printed in UK – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

EPR, ENDOR and TRIPLE Resonance and MO Studies on Ubiquinones (Q-*n*): Comparison of Radical Anions and Cations of Coenzymes Q-10 and Q-6 with the Model Compounds Q-2 and Q-0†

Heikki Joela,* Seppo Kasa, Pekka Lehtovuori and Martin Bech‡

Department of Chemistry, University of Jyväskylä, FIN-40100 Jyväskylä, Finland

Joela, H., Kasa, S., Lehtovuori, P. and Bech, M., 1997. EPR, ENDOR and TRIPLE Resonance and MO Studies on Ubiquinones (Q-*n*): Comparison of Radical Anions and Cations of Coenzymes Q-10 and Q-6 with the Model Compounds Q-2 and Q-0. – Acta Chem. Scand. 51: 233–241. © Acta Chemica Scandinavica 1997.

Radical anions and cations of the biologically important coenzymes Q-6 and Q-10, which have 6 and 10 unsaturated isoprene units in their side chains, respectively, have been generated in various solvents, and the results compared with those obtained for Q-0, a ubiquinone with no isoprene units, and for decylubiquinone Q-2 which has a saturated side chain.

Hyperfine splitting constants (hfsc) of methyl and methoxy protons of the substituents in the quinone ring, and β and γ protons of the side chain were measured by EPR and ENDOR spectroscopy for both the radical anions and cations of Q-0, Q-6 and Q-10, and for the radical anion of Q-2. The relative signs of the hfsc were determined by general TRIPLE resonance spectroscopy. TRIPLE induced EPR (TIE) spectra were used for identification of the primary and secondary radicals of Q-10. The temperature dependence of the hfsc of the β protons of Q-2 was different from those of Q-6 and Q-10.

Fully optimised structures of Q-3 and Q-7 were obtained by performing semiempirical PM3 molecular orbital (MO) calculations for both neutral molecules and radical anions, neutral radicals and radical cations. Partial optimisation of the molecules was carried out for the side chain in a planar conformation. The folded conformation always had the minimum energy. Folding was so complete in the Q-7 series that the end of the side chain came into contact with the quinone ring, and small hfsc were detected in the PM3 calculations.

Ubiquinone compounds are currently the focus of much biomedical research. Ubiquinones with different numbers (*n*) of isoprene subunits in the side chain (Fig. 1) are widely found in the tissues of plants and animals. There are many ways in the literature in which the names of ubiquinones are abbreviated, e.g., UQ-*n*, UQ 5*xn*, Coenzyme Q_n, CoQ_n, and Q-*n*. We will use Q for a neutral ubiquinone molecule, because in this way various quinone species can be expressed in an elegant way as can be seen from Scheme 1. Quinones undergo an interesting set of electron and proton transfer reactions. Scheme 1 illustrates a stepwise reaction starting from the neutral quinone molecule and resulting in the neutral aromatic quinol molecule QH_2 having two hydroxy groups (not shown in Fig. 1).

$$Q \underset{-e^-}{\overset{e^-}{\rightleftharpoons}} Q^{\cdot-} \underset{-H^+}{\overset{H^+}{\rightleftharpoons}} QH^{\cdot} \underset{-H^+}{\overset{H^+}{\rightleftharpoons}} QH_2^{\cdot+} \underset{-e^-}{\overset{e^-}{\rightleftharpoons}} QH_2$$

Fig. 1a) Fig. 1d) Fig. 1e) or f) Fig. 1g)

Scheme 1.

The reactions shown in Scheme 1 are accepted to take place regardless of the length of the side chain. Also, the chemistry of the quinones Q should be very similar, e.g., radical anions can be generated in alkaline media, radical cations in strongly acidic media, and neutral radicals in about neutral solutions. Therefore when we discuss the results obtained for ubiquinones with different numbers of isoprene units in the side chain, we distinquish the species by the use of the abbreviation Q-*n*. There is considerable confusion in the literature regarding the results for radical ions, partly due to difficulties in the nomenclature of the various species. We want to emphasize that in Scheme 1, one can find a neutral aromatic quinol and its radical cation on the right. In principle the radical cation of the neutral quinone could be

† Lecture held at the 14th International Conference on Radical Ions, Uppsala, Sweden, July 1–5, 1996.

* To whom correspondence should be addressed.

‡ Present address: Department. of Chemistry, Aarhus University, DK-8000 Aarhus, Denmark.

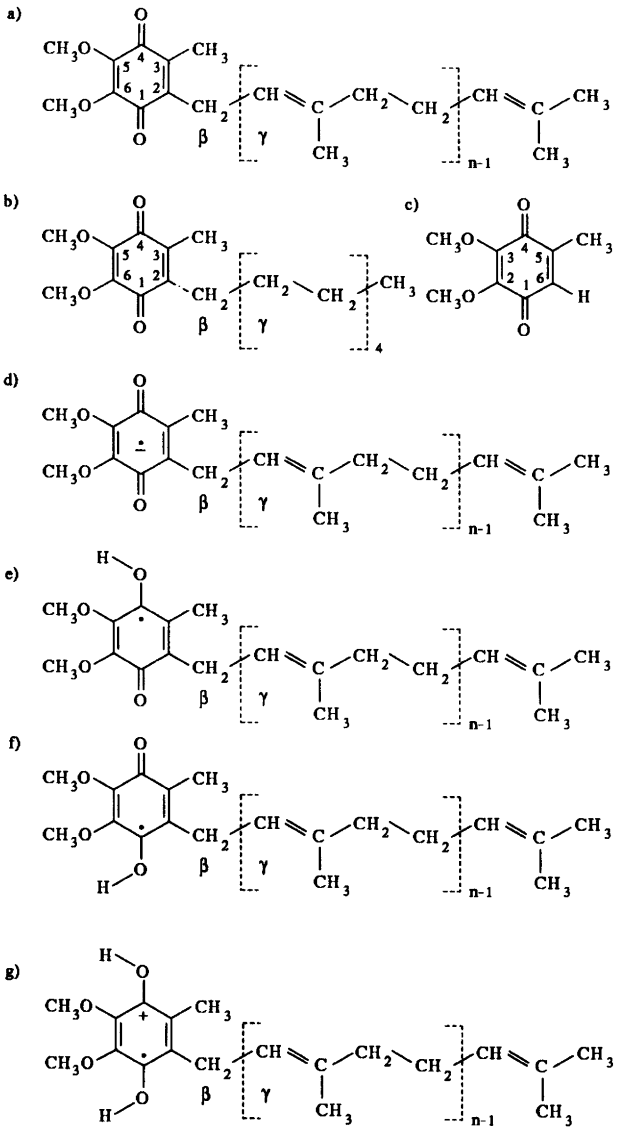

Fig. 1. (a) and (c) IUPAC numbering for the ubiquinone molecules Q-*n* and Q-0, respectively; (b) 5,6-dimethoxy-3-methyl-2-decyl-1,4-benzoquinone (decylubiquinone: Q-2 in this study); (d) radical anion; (e) and (f) two possible neutral radicals and (g) radical cation of quinol Q-*n*.

obtained by oxidizing the quinone on the left (not shown in Scheme 1), should it exist.

The function of coenzyme Q-10 in mammalian mitochondria has been closely studied, and it is thought to be responsible for the transport of hydrogen atoms through the bilayer membrane by a pendulum-type movement.[1] On the other hand, ubiquinone Q-7 is found in photosynthetic reaction centers (bRC) of *Rhodobacter sphaeroides*.[2] To the best of our knowledge only ubiquinones having 6–10 isoprene units are found in Nature as coenzymes, which suggests a certain minimum and maximum length for the side chain of biologically active coenzymes. In this study EPR and ENDOR experiments were carried out for a series of Q-0, Q-2, Q-6 and Q-10 radical ions to find out whether there is a limiting length of the side chain for which the EPR spectra or the dynamic behaviour[3] of the β protons will totally change. PM3 molecular orbital calculations were carried out to obtain fully optimised structures of carefully chosen ubiquinone molecules, Q-3 and Q-7, for comparison.

Das *et al.*[3] have reported that the EPR and ENDOR spectra of ubiquinone radical anions are temperature-dependent, and that this is due to the relative rotational motion of the aromatic ring and the long side chain around the single bond joining the side chain to the ring. They found that the β protons of the isoprenic side chain are non-equivalent at low temperatures, which causes an alternating linewidth effect. The effect was confirmed by Feher *et al*,[4] who also found new and smaller hfsc, which they suggested could be assigned to the methoxy-protons and the γ proton.

Kasa *et al.*[5] have measured hfsc for the methoxy groups in the 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q-0) radical anion by ENDOR. Their[5] UMNDO calculations indicated that the methoxy groups are twisted out of the quinone plane by about 80°. Kirste *et al.*[6] have measured and assigned the proton hfsc of the methoxy groups and the hfs of the γ proton of the radical anion of ubiquinone in 2-propanol. Samoilova *et al.*[7,8] have measured proton and ^{13}C couplings for the radical anion, neutral radical and radical cation of Q-0 and for the radical anion of Q-10.

Results

Ubiquinone Q-10. The EPR spectrum of the Q-10 radical anion shows a remarkable temperature-dependent alternating linewidth effect due to rotation of the aromatic ring relative to the attached methylene protons of the side chain.[3] Fig. 2 shows ENDOR spectra of the ubiquinone radical anion of the Q-10 sample recorded at different temperatures. In a mixture of chloroform–ethanol at about 250 K there was a clear coalescence point for the signals; two separate values of 3.48 MHz (0.124 mT) and 1.97 MHz (0.070 mT) were measured at temperatures below 250 K while only one value of 2.77 MHz (0.099 mT) was detected above it. The values of hfsc in various solvents are shown in Table 1.

By detecting different EPR transitions in the EPR spectrum of a Q-10 sample prepared in an alkaline mixture of chloroform–ethanol, we recorded two ENDOR spectra, indicating the presence of two different radical species. The ENDOR spectrum shown in Fig. 3(b) was obtained from the peak labeled with an asterisk (*) and exhibited hfsc of 3.96 MHz (0.141 mT), 1.71 MHz (0.061 mT) and 0.37 MHz (0.013 mT) at 298 K, while the other ENDOR spectrum [Fig. 3(c)] was obtained by saturating the EPR line labeled with a diamond (◇) and had hfsc of 5.71 MHz (0.204 mT) and 2.95 MHz (0.105 mT). The former ENDOR spectrum (*) originated from a secondary radical anion of Q-10 and the latter spectrum (◇) was confirmed as belonging to the ubiquinone radical anion.

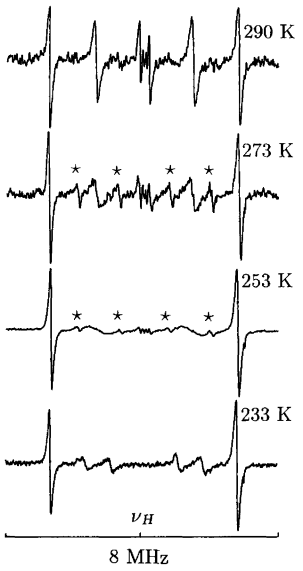

Fig. 2. ENDOR spectra of the radical anion of ubiquinone Q-10 recorded in an alkaline mixture of chloroform–ethanol (3:1 v/v) at different temperatures. Lines marked with an asterisk belong to the secondary radical (conversion factor: 0.03565 mT MHz^{-1}).

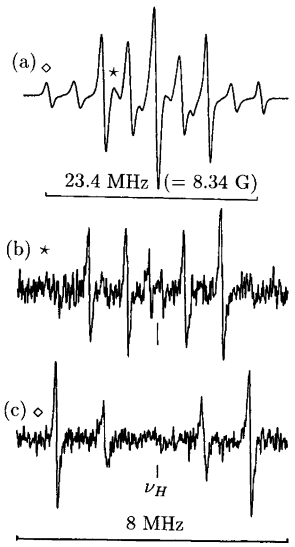

Fig. 3. EPR and ENDOR spectra recorded for the primary and secondary radical anions of Q-10 in a mixture of [$^2H_1$]ethanol–[^{2}H]chloroform–NaOD, D_2O (1:10:0.5) at room temperature: (a) overlapping EPR spectra, ENDOR spectra of (b) the secondary radical anion and (c) the Q-10 radical anion. Spectra were obtained by saturating the features marked by an asterisk (*) and by a diamond (◇) in the EPR spectrum (a), respectively.

A secondary radical anion of Q-10 and TRIPLE induced EPR (TIE) spectra. The appearance of ENDOR resonance signals of the secondary radical shown in Fig. 3(b) is interesting because they are close to the signals detected for the non-equivalent β protons of the ubiquinone radical anion at low temperature. The secondary radical was detected only at temperatures above 250 K. The reversible appearance and disappearance of the secondary radical depending on temperature may give some hints as to its structure. The secondary radical appeared when alkali-metal ions (from NaOH) were present in the sample. This may indicate the formation of a loose ion-pair and it may explain the influence on the unpaired spin density in the quinone ring.

ENDOR-induced EPR (EIE) spectroscopy is an important technique for obtaining an EPR spectrum of a single species from a mixture of radicals. We measured TIE spectra from both species, because if one detects the enhanced signal of the peak pair in the general TRIPLE experiment by scanning the magnetic field, one can get a better S/N ratio for the induced EPR spectrum. The absorption spectrum obtained was differentiated into the normal first derivative EPR spectrum as shown in Fig. 4. In connection with the analysis shown in Fig. 3 note that the ENDOR lines corresponding to the hfsc of 5.71 MHz (0.204 mT) and 2.95 MHz (0.105 mT) belong to the Q-10 radical anion. The TIE spectrum of Q-10 shown in Fig. 4(a) was obtained by pumping the lower field signal with the largest hfs appearing at ($\nu_H - 5.71/2$) MHz and detecting the respective line at ($\nu_H + 5.71/2$) MHz. On the other hand the TIE spectrum of the secondary radical shown in Fig. 4(b) was recorded by pumping the

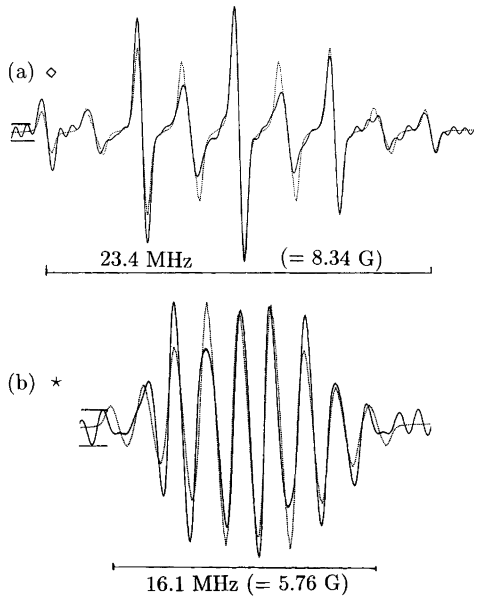

Fig. 4. TIE spectra of primary and secondary radical anions of Q-10 plotted as first derivative spectra. The solid line is the experimental and the dashed line is the simulated spectrum. The noise level of the recorded TIE spectra is estimated by the bars on the left-hand side.

ENDOR feature at ($\nu_H - 3.96/2$) MHz and detecting the feature at ($\nu_H + 3.96/2$) MHz.

According to a special TRIPLE resonance experiment, the two largest hfcs of the secondary radical showed a proton content ratio of 3:2, which would make the spectrum consistent with a methyl group and a methylene

Table 1. Hfsc of ubiquinone radical anions determined by ENDOR (in MHz[a]).

Molecule	T/K	Solvent	Protons at positions 5, 6	3	2/β	2/γ
Coenzyme Q-10						
BioQinon	298	EtOD–$CHCl_3$–NaOD, D_2O	0.08	5.71	2.95	−0.29
	253	EtOH–$CHCl_3$		5.71	2.83	
	243	EtOH–$CHCl_3$		5.72	3.39, 2.15	
Super CoQ10	263	EtOH–$CHCl_3$	0.08	5.50	2.77	−0.29
	233	EtOH–$CHCl_3$		5.50	3.48, 1.97	
Q-10 (synthetic)	263	EtOH–$CHCl_3$–NH_3	0.08	5.77	2.92	
	223	EtOH–$CHCl_3$–NH_3	0.10	5.75	3.60, 2.04	
	203	EtOH–$CHCl_3$–NH_3		5.75	3.56, 1.94	
	263	EtOH		5.71	2.85	
	203	EtOH		5.86	3.56, 1.94	
Feher *et al.*[4]	270	1,2-DME		5.89	2.92	−0.27[b]
	205	1,2-DME	−0.06[b]	5.89	3.72, 2.06	−0.29[b]
Kirste *et al.*[6]	290	2-Propanol	0.09	5.78	2.94	−0.28
INDO[c] calc.			−0.08	7.97	5.22	−0.73
Coenzyme Q-6						
ENDOR	263	EtOH–$CHCl_3$	0.08	5.53	2.78	−0.28
ENDOR	233	EtOH–$CHCl_3$	0.10	5.52	3.56, 1.99	−0.36
Decylubiquinone						
EPR, ENDOR	203	liq. NH_3	0.07	5.59	2.69	−0.33
ENDOR	263	EtOH	0.07	5.36	2.75	−0.30
ENDOR	223	EtOH		5.40	2.67	
ENDOR	208	EtOH		5.41	2.62	
			2, 3	5[d]		
Coenzyme Q-0						
Kasa *et al.*[5]		MeOH	0.14	6.79		

[a] Conversion factor into mT is 0.03565 mT MHz^{-1}. The accuracy of ENDOR measurements was ±0.02 MHz. [b] The hfsc for the methoxy and γ proton(s) are reversed here. [c] Calculated with INDO (*QCPE*, program No. 141). [d] The IUPAC numbering is different for Q-0 and Q-*n*; see Fig. 1.

group. No exchangeable protons were detected in experiments in which deuteriated solvents were used. The simulation of the EPR spectrum shown in Fig. 4(b) was in excellent agreement with ENDOR-determined hfsc. On the basis of this analysis it looks as if, in the structure of the secondary radical, there are 3 H with an hfs of 3.84 MHz (0.137 mT), and 2 H with an hfs of 2.01 MHz (0.072 mT). There are also smaller hfs, one for one (γ) proton of 0.48 MHz (0.017 mT), and two very small ones for two groups of three (methoxy) protons of 0.09 MHz (0.003 mT) and 0.05 MHz (0.002 mT). This analysis does not demand a very dramatic change in the geometry of the ubiquinone molecule and it supports the earlier model of a secondary radical as a loose ion-pair comprising an alkali metal and radical anions of the quinone. Hfsc of secondary radical anions of ubiquinones in various solvents are shown in Table 2.

It has been suggested[8,9] that chromenols and chromanols may result from the action of a base or acid in solutions of ubiquinones. The hfsc determined in this study in alkaline conditions are different from those tabulated for chromanyloxyls.[10] This does not contradict our earlier hypothesis. On the other hand our MO results suggest that one possible structure for the secondary radical is a quinone structure, in which the folding of the side chain over the quinoidic oxygen causes a new distribution of spin density. This is supported by the fact that in the case of the Q-0 we could not find a secondary radical of this type.

Ubiquinone Q-6. As can be seen in Table 1, the hfsc of the radical anion of coenzyme Q-6, prepared in a mixture of ethanol–chloroform, were closely similar to those obtained for the coenzyme Q-10. Moreover, the temperature (250 K) at which the hfsc of the β protons became non-equivalent was the same. The same secondary radical anion found with Q-10 was also observed from the Q-6 sample.

Decylubiquinone (Q-2). The structure of decylubiquinone, labeled Q-2 in this study, is shown in Fig. 1(b). In

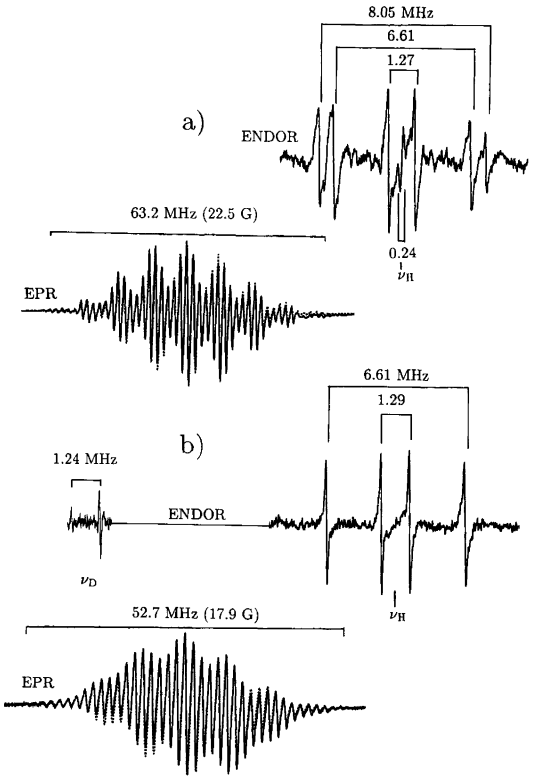

Fig. 5. ENDOR, experimental and simulated EPR spectra of the radical cation generated at 298 K from Q-10 (a) in trifluoromethanesulfonic acid (HTFMS) and (b) in deuteriated fuming sulfuric acid; allowing the assignment of OH protons.

ethanol or methanol solutions, both β protons of decylubiquinone have the same hfs, depending only slightly on temperature, as shown in Table 1. The relative signs of the hfsc were determined by general TRIPLE resonance spectroscopy. By assuming the positive sign for the hfs of the methyl group, recorded spectra suggested positive signs for the hfsc of the methoxy groups, in accordance with Kasa *et al.*[5] The important finding in the experiments of decylubiquinone was that the hfsc of the β protons remained equivalent even at temperatures as low as 200 K, indicating that the side chain does not behave in the same way as in the quinones Q-6 and Q-10. The β protons had the same hfs in all the solvent systems studied. A clear triplet could be detected in the outermost line pair in the EPR spectrum, showing the hfs of the γ protons, supporting the assignment.

Radical cations of Q-10 and Q-6 in comparison with Q-0. The radical cation of Q-10 was generated in various solvents. The ENDOR spectrum was recorded in HTFMS acid at room temperature (298 K) and four hfsc of 8.05 MHz (0.287 mT), 6.61 MHz (0.236 mT), 1.27 MHz (0.045 mT) and 0.24 MHz (0.009 mT), could be detected as shown in Fig. 5(a). Simulation of the EPR spectrum [Fig. 5(a)] was carried out using hfsc with the assignment shown in Table 3, because otherwise the spectral width of 63.2 MHz (2.25 mT) could not be obtained. Three methyl protons and two hydroxy protons having hfsc of 6.61 MHz (0.236 mT) and 8.05 MHz (0.287 mT), were not enough, and two additional hfsc of 6.61 MHz (0.236 mT), and 1.27 MHz (0.045 mT), corresponding to three (β+γ) and six protons (OCH_3), respectively, had to be added for a successful simulation. The hfs of 8.05 MHz (0.287 mT), was assigned to hydroxy protons by using DTFMS acid and deuteriated fuming sulfuric acid in the substitution of OH by OD; the disappearance of the respective ENDOR signals is shown in Fig. 5(b). This accidental equivalency of hfsc does not sound reliable, and it is possible that the generated species is not the aromatic ubiquinol radical cation of Q-10. A quite similar set of hfsc was used for the simulation shown in Fig. 5(b), except that a deuterium hfs of 1.24 MHz (0.044 mT), obtained from the ENDOR spectrum, replaced the respective hfs of the OH protons.

The ^1H and ^{13}C hfsc of the radical cation of Q-0 have previously been determined at room temperature.[7,8] We also measured a large value, 13.13 MHz (0.468 mT), for methyl protons. The radical cation of Q-0 did not have an average hfs for methyl protons (position 5) and for the single ring-proton (position 6), as was found for the radical anion of Q-0 in liquid ammonia.[5]

MO results for ubiquinones (Q-n). Fully optimised theoretical structures for the ubiquinone molecules Q-3 and Q-7 were obtained by performing semiempirical PM3 molecular orbital calculations by means of the GAUSSIAN 94[11] program package for both the neutral molecules Q and QH_2 and the radical anions, neutral radicals and radical cations shown in Scheme 1 (and in Fig. 1). Ubiquinones Q-3 and Q-7 were selected for the following reasons. According to our EPR results obtained at temperatures under 250 K for the radical anions of Q-6 and Q-10, the hfsc of the β protons were unequal, whereas the hfsc of the β protons were the same at any temperature for the radical anion of Q-2. PM3 results will provide a solution to the question, what kind of conformational change forces these β protons to be in unsymmetrical positions? Calculations for Q-10 would need a lot more computer time than for Q-6. Q-7 was chosen instead of Q-6 because Chang *et al.*,[2,12] reported that after adding the co-ordinates of Q-7 to the structural data of photosynthetic reaction center from *Rhodobacter sphaeroides* R-26 they were able to refine the protein and pigment atoms together. Because the electron density for the isoprenoid tails of Q_A and Q_B allowed the positioning of the first four isoprene units of the quinone tail,[2] Q-3 was chosen to represent the flexibility of shorter quinones. PM3 results for fully or partially optimised molecules are shown in Table 4. Partial optimisation was carried out for a planar conformation, where only the torsion angles were constrained to be 180° forcing the side chain to be planar, i.e., all other parameters were allowed to relax. Optimised geometries of Q-3 and Q-7 are shown in Figs. 6 and 7. The side view of the neutral ubiquinone

Table 2. Hfsc of secondary radical anions of ubiquinones Q-2, Q-6 and Q-10 determined by ENDOR in alkaline solvents (in MHz[a]).

Molecule	*T*/K	Solvent–alkali	Protons at positions[b] 5, 6	3	2
Coenzyme Q-10					
BioQinon	298	CH_3CH_2OD–$CDCl_3$–NaOD, D_2O	0.37	3.96	1.71
Super CoQ10	298	CH_3CH_2OH–$CHCl_3$–KOH	0.37	3.96	1.71
Coenzyme Q-6					
Q-6 (*Saccharomyces*)	260	CH_3CH_2OH–$CHCl_3$–NaOH	0.29	3.95	1.46
Decylubiquinone Q-2					
Q-2	298	CH_3CH_2OH–$CHCl_3$–KOH	0.38	3.76	1.58
Q-2	298	CH_3OH–$CHCl_3$–KOH	0.36	3.65	1.57

Hfsc obtained from the simulated EPR spectra differed less than ± 0.03 MHz from the ENDOR-determined values. [a]Conversion factor into mT is 0.03565 mT MHz^{-1}. [b]Numbering was assigned on the assumption that the atomic structure of ubiquinone has not been changed.

Q-3 molecule shown in Fig. 6(b) reveal that when the side chain is planar the quinone ring is in a boat conformation. On the other hand, in all anion and cation radicals the quinone rings are strictly planar. One methoxy group in position 5 is planar, and the other is in an out-of-plane conformation in the cation radical of Q-3, as shown in Fig. 6(g). In our previous study[5] of the alkoxylation reaction of the Q-0 anion radical, we pointed out that there are many possible conformations very close in energy and with methoxy groups in either planar or out-of-plane conformations. In out-of-plane conformations there is no possiblity of hyperconjugation, and only very small hfsc from the methyl protons of the methoxy group could be measured by ENDOR.[5] On the other hand, the hfsc of methoxy groups in the cation radicals were easily detectable. The electronic structure of the carbon atoms in the quinone ring in the cation radicals is aromatic, and a redistribution of the charge density has taken place. Therefore one can expect hyperconjugation effects to induce unpaired spin density in the planar methoxy group(s). Solvent molecules are probably loosely hydrogen-bonded to the radicals, causing deviations from the out-of-plane conformation.

Furthermore, both OH hydrogens are in planar *cis* conformations and are hydrogen-bonded to methoxy oxygen. Unequal hfsc were indeed detected for the methoxy protons and these are listed in Table 3. Molecules having either a twisted (in Q-3) or the folded conformation (in Q-7) of the isoprenyl side chain always had the minimum energy. Folding is so complete in the Q-7 series, that the tip of the side-chain comes into contact with the quinone ring. We emphasise that it was not possible to get the fully optimised structures by means of the PM3 Hamiltonian in the GAUSSIAN 94[11] program for all Q-7 molecules; as is indicated by footnote *c* in Table 4. The calculated energies started to oscillate and no self consistency was obtained, when, e.g., the fully optimised structure of the neutral quinol Q-7 was used as a starting geometry to get the optimised structure for the cation radical. Therefore a damping factor of 0.1 eV (shifts the virtual energy levels up 0.1 eV) was defined for PM3 calculations carried out for the cation radical by MOPAC 7.00[13] and a self-consistent field was achieved. A single-point calculation was then carried out by GAUSSIAN 94. Because the gradient test was not completely passed, there being no way to reduce the gradient in these cases, the structures obtained are the best ones possible.

Energies of the neutral radicals of Q-7 (also for Q-3) are almost the same indicating that both oxygen atoms in the quinone radical anions are equally susceptible to proton attack. Taking this into account, suggestions[14] that two neutral radicals would disproportionate in the reaction center, generating a neutral quinone and an aromatic quinol is consistent with the data. The tabulated $\langle S^2 \rangle$ values are reasonably small for all the radicals except the neutral ones. The semiempirical UHF level of theory is not good enough for the neutral radicals. The energies of the radical cations of the aromatic quinols Q-3 and Q-7 differ from the energies of the other molecules shown in Table 4. The energy of the folded Q-7 radical cation is so high that it might not be involved in the energy transfer processes taking place in the reaction centers. However, the folded Q-7 radical cation is surprisingly stable compared with the corresponding Q-3 radical cation.

Discussion

X-Ray results[2,12] have shown that the side chains of ubiquinones are flexible, but how flexible are they really? How much can they move inside the reaction center, and what conformation can they take on? The PM3 results obtained in this study (gas-phase and 0 K) indicate that the side chains can fold so completely that the ends are over the ring part. The calculated Fermi contact interactions (PM3, UHF) show the presence of small hfsc of the two closest methyl groups and the vinylic proton on

Table 3. Hfsc[a] of radical cations generated from Q-*n* (in MHz) at 298 K.

		Protons at positions[b]				
Molecule	Solvent	1, 4	5, 6	3	2/β	2/γ
Coenzyme Q-10						
Q-10 (synthetic)	HTFMS[c]	8.05	1.23	6.53	6.53	6.53
	DTFMS[c]	1.24[d]	1.29	6.65	6.65	6.65
	H_2SO_4	7.94	1.28	6.59	6.59	6.59
	D_2SO_4	1.24[d]	1.29	6.62	6.62	6.62
Coenzyme Q-6						
Q-6 (*Saccharomyces*)	HTFMS	7.93	1.26	6.51	6.51	6.51
		1, 4	2, 3	5	6[e]	
Coenzyme Q-0						
	HTFMS	8.22, 7.53	2.25, 0.90	13.12	2.25	
	DTFMS	1.46[d], 0.98[d]	2.25, 0.90	13.12	2.25	
	D_2SO_4	9.67, 7.76	1.90, 1.18	13.65	1.90	
Samoilova *et al.*[7]	HTFMS	8.19, 7.32	2.10, 0.95	13.02	2.10	

[a] Hfsc obtained by EPR iterations and ENDOR experiments differed by less than ±0.1 MHz. Conversion factor into mT is 0.03565 mT MHz^{-1}. [b] Numbering was assigned on the assumption that the cation radical of Q-*n* was formed. [c] Trifluoromethanesulfonic acid is abreviated to HTFMS and the deuteriated acid to DTFMS. [d] Deuterium coupling. [e] The IUPAC numbering is different for Q-0 and for Q-*n*; see Fig. 1.

the tip of the side chain. For example, for the radical anion of Q-7 we obtained hfsc of −0.043 MHz (0.002 mT) and −0.037 MHz (0.001 mT) for the methyl protons, but −0.443 MHz (0.016 mT) for the single vinylic proton. Is this a possible new route for electron transfer processes?

In bRC, light-induced electron transfer takes place along the A-branch to the quinones Q_A and Q_B where both Q_A and Q_B are Q-10 ubiquinones. According to Okamura *et al.*,[15] Q_A can accept only one electron but cannot receive any protons H^+, while Q_B can be doubly reduced and protonated to form a neutral aromatic quinol molecule as shown in Scheme 1. The protein environment therefore has a strong effect on the electronic structure of the quinones, and probably Nature has modified their geometry to optimise the efficiency of the electron and proton transfer processes as discussed by Okamura and Feher[16] and Shinkarev and Wraight.[17] Isaacson *et al.*,[18] determined the magnitude and orientation of the electronic *g*-tensor of the radical anion of Q_A in single crystals of zinc-substituted *Rhodobacter sphaeroides* R-26 by EPR spectroscopy. The orientation of the radical anion as determined by the *g*-tensor axes deviated by only a few degrees from the orientation of the neutral Q_A molecule obtained from an average of four different X-ray structures. They do not consider the deviation to be significant. According to our MO calculations the neutral ubiquinone molecules Q did differ structurally substantially from the corresponding radical anion, because the boat conformation changed to a planar one. However, no big conformational changes took place in the side chains of the quinones. To the best of our knowledge there are no fully optimised theoretical structures for ubiquinones of this size. In any case, *ab initio* calculations are needed. The side chain of bioactive ubiquinones appears to have only limited freedom to move in the protein network, as if the tail is wagging the dog; i.e., it is the quinone ring that moves the most. Since the side chains of ubiquinones are situated on the cytoplasmic side of the reaction center, with the side

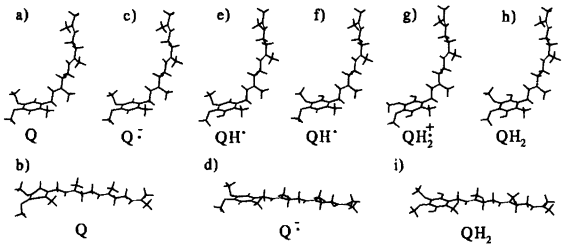

Fig. 6. (a), (c), (e), (f), (g) and (h) illustrate the fully optimised; PM3, HF and UHF, GAUSSIAN 94, and (b), (d) and (i) the partially optimized planar structures of quinones and quinols of Q-3.

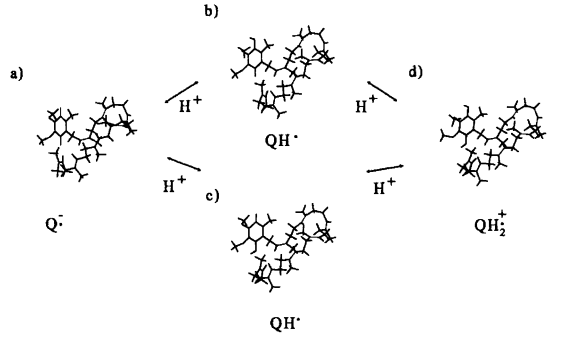

Fig. 7. (a) The fully optimised structure of Q-7 anion radical; PM3, UHF, GAUSSIAN 94, and (b), (c) and (d) the almost fully optimised (see the text) structures of neutral and cation radicals of ubiquinols Q-7; PM3, UHF, MOPAC 7.00 and GAUSSIAN 94.

Table 4. PM3 results (GAUSSIAN 94, HF and UHF) for both fully optimised and a planar conformation of ubiquinones Q-3 and Q-7.

Molecule	E/kJ mol^{-1} $\langle S^2 \rangle$	Q full opt.	Q planar	$Q^{\cdot -}$ full opt.	$Q^{\cdot -}$ planar	$QH^{\cdot}$ full opt.[a]	$QH^{\cdot}$ full opt.[b]	$QH_2^{\cdot +}$ full opt.	$QH_2^{\cdot +}$ planar	QH_2 full opt.	QH_2 planar
Q-3	Energy	−522.057	−478.083	−743.410	−690.693	−575.819	−575.798	+89.097	+142.728	−661.692	−602.059
	$\langle S^2 \rangle$	0.0	0.0	0.824	0.823	1.411	1.409	0.8859	0.8767	0.0	0.0
Q-7	Energy	−611.326	−506.584	−843.408	−719.36	−661.944[c]	−661.100[c]	−1.591[c]		−750.736	
	$\langle S^2 \rangle$	0.0	0.0	0.849	0.823	1.134	1.137	0.807		0.0	

[a] OH-group between methyl and methoxy groups; see Fig. 1(e). [b] OH-group between side chain and methoxy group; see Fig. 1(f). [c] Optimised first by MOPAC 7.00, then a single-point calculation by GAUSSIAN 94; see the text.

chain parallel to the membrane and the quinone plane at the centre of the protein, the occasional immobility of the quinone plane of the radical would be of considerable importance for the electron transfer.

Experimental

Materials. Super CoQ10 or Q-10 (Livsenergi, 30 mg/capsule) was obtained from Vitamex AB, Sweden; BioQinon or Q-10 (30 mg/capsule) from Pharma Nord, Denmark; synthetic coenzyme Q-10, decylubiquinone (98%) and coenzyme Q-6, or 2,3-dimethoxy-5-methyl-6-[all *trans*]farnesylfarnesyl-1,4-benzoquinone, separated from the yeast *Saccharomyces*, were from Sigma. KOH (*pro analysi*), methanol, [^{2}H]chloroform, and NH_3 (99.99%) were obtained from Merck. Ethanol (99.9%, AaS) was from Alko Oy, Finland, chloroform from Riedel-de-Haën (freshly distilled), and solid sodium metal from Baker. CH_3CH_2OD and NaOD (40% in D_2O, 99.5%) were Merck products. Fuming sulfuric acid was a Riedel-de-Haën product and $[^2H_1]H_2SO_4$ was from Merck. Trifluoromethanesulfonic acid (HTFMS) and [^{2}H]TFMS (DTFMS) were Fluka products and were obtained in sealed ampoules.

Equipment and MO calculations. Spectra were recorded on a Bruker ER 200 D-SRC spectrometer equipped with a Varian E-12 magnet and a Bruker ER 033 M FF lock. EPR spectra were simulated either with a Bruker program or iteratively with an EPRFT program developed by Kirste.[19] SYBYL 6.0[20] was run on a Sun SPARC 10 workstation. Semiempirical PM3 molecular orbital calculations were carried out by means of GAUSSIAN 94[11] and MOPAC 7.00[13] program packages, run on Digital Alpha (OSF/1) and Silicon Graphics Power Onyx computers.

Sample preparation. Anion radicals were prepared under high-vacuum conditions in alkaline ethanol or in liquid ammonia, with solid sodium as the reducing agent. Ammonia was distilled into a sample tube under an atmosphere of nitrogen and degassed with freeze–pump–thaw cycles under conditions of high vacuum. The CoQ10 sample was prepared in chloroform and ethanol (3:1 v/v) and by adding a tiny fragment of dry KOH pellet. The BioQinon sample was prepared in a mixture of $CDCl_3$–CH_3CH_2OD–NaOD (1:10:0.5 v/v). Radical cations of ubiquinones Q-*n* were prepared by reaction with sodium dithionite ($Na_2S_2O_2$) in trifluoromethanesulfonic acid and in a mixture of fuming sulfuric acid–sulfuric acid (in various ratios) or in the respective deuteriated acids.

Acknowledgements. M.B. gratefully acknowledges the financial support of the *Nordisk Forskerutdannings Akademi's (NorFA)* ENDOR Network project (1993) for co-operation between the universities of Linköping, Oslo, Aarhus and Jyväskylä.

References

1. Tyler, D. D. *The Mitochondrion in Health and Disease*, VCH, New York 1992, pp. 284–323.
2. Chang, C. H., El-Kabbani, O., Tiede, D., Norris, J. and Schiffer, M. *Biochemistry 30* (1991) 5352.
3. Das, M. R., Connor, H. D., Leniart, D. S. and Freed, J. H. *J. Am. Chem. Soc. 92* (1970) 2258.
4. Feher, G., Isaacson, R. A., Okamura, M. Y. and Lubitz, W. In: Michel-Beyerle, M. E., Ed., *Antennas and Reaction Centers of Photosynthetic Bacteria, Structure, Interactions and Dynamics*, Springer 1985, Series in Chemical Physics, Vol. 42, p. 174.
5. Kasa, S., Mäkelä, R., Salo, E., Hannonen, K. and Joela, H. *J. Chem. Soc., Faraday Trans. 87* (1991) 3163.
6. Kirste, B., Niethammer, D., Tian, P. and Kurreck, H. *Appl. Magn. Reson. 3* (1992) 1.
7. Samoilova, R. I, van Liemt, W., Steggerda, W. F., Lugtenburg, J., Hoff, A. J., Spoyalov, A. P., Tyryshkin, A. M., Gritzan, N. P. and Tsvetkov, Y. D. *J. Chem. Soc., Perkin Trans. 2* (1994) 609.
8. Samoilova, R. I, Gritzan, N. P., Hoff, A. J., van Liemt, W., Lugtenburg, J., Spoyalov, A. P. and Tsvetkov, Y. D. *J. Chem. Soc., Perkin Trans. 2* (1995) 2063.
9. Bowman, M. K., Thurnauer, M. C., Norris, J. R., Dikanov, S. A., Gulin, V. I., Tytyshkin, A. M., Samoilova, R. I. and Tsvetkov, Y. D. *Appl. Magn. Reson. 3* (1992) 353.
10. Pedersen, J. A. *CRC Handbook of EPR Spectra from Quinones and Quinols*, CRC Press, Boca Raton 1985, p. 211.
11. GAUSSIAN 94, Revision B.1, Frisch, M. J., Trucks, G. W., Schlegel, H. B., Gill, P. M. W., Johnson, B. G., Robb, M. A., Cheeseman, J. R., Keith, T., Petersson, G. A., Montgomery, J. A., Raghavachari, K., Al-Laham, M. A., Zakrzewski, V. G., Ortiz, J. V., Foresman, J. B., Cioslowski, J., Stefanov, B. B., Nanayakkara, A., Challacombe, M., Peng, C. Y., Ayala, P. Y., Chen, W., Wong, M. W., Andres, J. L., Replogle, E. S., Gomperts, R., Martin, R. L., Fox, D. J., Binkley, J. S., Defrees, D. J., Baker, J., Stewart, J. P., Head-Gordon, M., Gonzales, C. and Pople, J. A. Gaussian, Inc., Pittsburgh PA, USA. 1995.
12. Protein Data Bank, Brookhaven National Laboratory, Upton, New York 11973, USA.
13. Stewart, J. J. P., MOPAC 7.00, Public Domain Copy, Frank J. Seiler Research Laboratory, US Air Force Academy, Colorado Springs, CO 80840, USA.
14. McComb, J. C., Stein, R. R. and Wraight, J. C. *Biochim. Biophys. Acta 1015* (1990) 156.
15. Okamura, M. Y., Debus, R. J., Kleinfield, D. and Feher, G. In: Trumpower, B. L., Ed., *Function of Quinones in Energy Conserving Systems*, Academic Press, New York 1982, pp. 299–317.
16. Okamura, M. Y. and Feher, G. *Ann. Rev. Biochem. 61* (1992) 861.
17. Shinkarev, V. P. and Wraight, C. A. In: Deisenhofer, J. and Norris, J. R., Eds., *The Photosynthetic Reaction*, Academic Press, San Diego 1993, Vol. 1, pp. 193–225.
18. Isaacson, R. A., Lendzian, F., Abresch, E.C., Lubitz, W. and Feher, G. *Biophys. J. 69* (1995) 311.
19. Kirste, B. *Anal. Chim. Acta 265* (1992) 191.
20. SYBYL 6.0, Tripos Associates, Inc., Hanley Road, St. Louis, MO 63144, USA.

Received July 1, 1996.