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On Coenzyme Q Orientation in Membranes: A Linear Dichroism Study of Ubiquinones in a Model Bilayer

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Summary. A general approach is developed to interpret linear dichroism (LD) spectra of ubiquinones (Q_n) in host bilayers. Information is reported in terms of guest-host mutual orientation and localization. The overall orientational anisotropy of guest ubiquinone molecules is described by a basic set of limiting orientation/localization modes. Assignments of the UV transitions of the ubiquinone chromophore were obtained by the liquid crystal-linear dichroism technique and molecular orbital (CNDO/S) calculations. The LD spectra of Q_n in the bilayers provided by the lyotropic nematic mesophase exhibited by water solutions of potassium laurate and decanol were interpreted on the basis of the above assignments. The resulting experimental evidence showed a multisite distribution in the host bilayer for the aromatic heads of all the investigated Q_n derivatives except Q_0. The orientational distribution suggested by the LD spectra fits the solubilization model recently proposed by G. Lenaz [*J. Membrane Biol.* (1988) **104**:193–209] for ubiquinone in lipid membranes. Within this model Q_n molecules are located in the midplane and their headgroups oscillate transversally across the membrane. Q_0 instead has a single site location, close to the polar bilayer interface. Experimental evidence that the headgroup carbonyls tend to grasp the polar interface of the host bilayer was also obtained. Orientation and location distributions of Q_n guest molecules are therefore likely to result from the tendency of their aromatic heads to grasp the polar heads of the host bilayer and from the concurrent tendency of their chains to settle into the hydrocarbon host interior.

Key Words coenzyme Q · ubiquinone-orientation · ubiquinone-localization · linear dichroism · model membrane

Introduction

Ubiquinones (Q_n, *see* Fig. 1) and related lipophilic quinones are key redox compounds in energy-conserving membranes (Trumpower, 1983; Lenaz, 1985; Lenaz et al., 1990). They consist of a substituted benzoquinone ring with a long hydrophobic side chain usually ranging between 6 and 10 isoprenoid units (Crane & Barr, 1985). Although part of the ubiquinone content of the membranes appears to be bound to integral membrane enzymic complexes (Yu & Yu, 1981), the majority of the quinone molecules constitutes a free homogeneous pool dissolved in the lipid bilayer (Ragan & Cottingham, 1985). In spite of the great interest in the properties of ubiquinones and related compounds, even involved in energy conservation mechanisms (Mitchell, 1976), their localization in energy-conserving membranes is still controversial (Lenaz, Fato & Mandrioli, 1987; Rajarathnam et al., 1989). Several models have been proposed wherein Q_n molecules are in fully extended or in variously bent conformations (Lenaz & Degli Esposti, 1985). The quinone headgroup can be either buried in the interior of the bilayer (Fig. 1: *1a*, *2b* and *3a*) or faced with the polar heads of the membrane surface (Fig. 1: *1b*, *2a*, and *3b*). Also various distributions between these two different environments were proposed (Alonso et al., 1981; Degli Esposti et al., 1981; Kingsley & Feigenson 1981; Katsikas & Quinn, 1982*a*,*b*, 1983; Stidham, McIntosh & Siedow, 1984; Chatelier & Sayer, 1985; Ulrich et al., 1985; Battino, Fahmy & Lenaz, 1986; Fato et al., 1986). Segregation of ubiquinone molecules (Cornell et al., 1987) and phase separation into micellar clusters (Quinn, 1990) were also suggested.

Much effort has been expended to match appropriate physical techniques to simple model systems in order to reduce the ambiguity of the results so far reported in this field. Discrimination between the suggested models can be made by physical techniques providing order parameters of the guest quinone molecules with respect to the host bilayer: linear dichroism (LD), nuclear magnetic (NMR) and electron paramagnetic resonances (EPR).

LD in the UV-Vis was the technique chosen by us because of its very high sensitivity. The molar

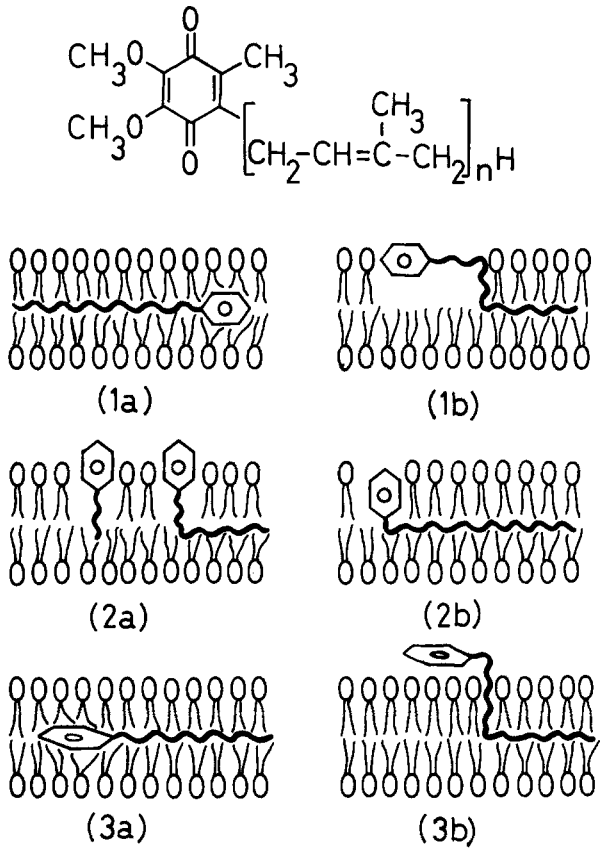

Fig. 1. Modes of ubiquinone localization and orientation within a lipid bilayer. The quinone headgroup is variously oriented either when it is buried into the hydrocarbonic interior (as in *1a*, *2b* and *3a*) or when it settles near the polar bilayer surface (as in *1b*, *2a* and *3b*).

ratio between ubiquinone and the host amphiphilic molecules should in fact be less than 1 : 50 in order to mimic physiological conditions (Lenaz, 1988) and to have it fully dissolved, thereby avoiding automicellization and segregation in a separate phase (Cornell et al., 1987; Quinn, 1990).

Lyotropic nematic liquid crystals were chosen as model host systems: they provide amphiphilic bilayers able to dissolve and locally orient Q_n guest molecules. The linear anisotropy required to run LD spectra can be easily and reliably imparted to samples of these nematic solutions simply by putting them in a magnetic field.

To our knowledge no other method has so far been developed that is as comparatively effective and reliable in preparing anisotropic samples of lipid bilayers suitable for LD studies. The preparation of hydrated oriented multilayers of mitochondrial membranes was reported (Erecinska, Blasie & Wilson, 1977; Van Gurp et al., 1988). The "multisandwiches" technique for phospholipid orientation used for x-ray diffraction (Hentschel & Hosemann, 1983; Albertini et al., 1988) are not suitable for optical spectroscopy measurements. In any case the lipid molecules are homeotropically oriented in the films obtained by all these methods. Therefore, they are pseudo-isotropic and their LD spectra cannot be recorded without bringing into play the "tilted-angle" technique (den Engelsen, 1972; Erecinska et al., 1977; 1978). This nonclassical method of recording LD spectra makes the measurements much more complicated and less reliable, mostly from a quantitative point of view. A plethora of corrections are needed for accurate work (Norden, Lindblom & Ionàs, 1977; Johansson et al., 1978). We have been unsuccessful in applying phospholipid orientation by strong magnetic field (Seelig, Borle & Cross, 1985) to samples having a geometry compatible with optical absorbance spectroscopy. We were thus forced to set up our interpretative approach for LD spectra of ubiquinones in lipid membranes by using, for the moment, model bilayers built up by potassium laurate and decanol.

This approach now could be directly transferred to studies of Q_n in membranes if suitable LD spectra were available. A very preliminary account of this investigation has been published (Battino et al., 1990).

Materials and Methods

The lyotropic nematic host (potassium laurate/decanol/water: 27.9/6.7/65.4 wt%) was prepared by following the procedure described by Samorì & Mattivi (1986). The thermotropic solvents ZLI 1167 and ZLI 2359 are from E. Merck (Darmstadt).

The *Ubiquinone* homologs were kind gifts of Eisai Co., Tokyo, Japan. They were stored as solutions in absolute ethanol at −20°C at concentrations ranging between 10 and 40 mM, as determined spectrophotometrically at 275 nm with an extinction coefficient (oxidized minus reduced) of 12.5 mM^{-1} cm^{-1} (Degli Esposti et al., 1981).

The *LD spectra* were recorded by a modulated technique which enormously increases the sensitivity of the measurements (Davidsson & Norden, 1976; Jensen, Shellman & Troxell, 1978; Samorì, 1983; Dunlap et al., 1988; Schellman, 1988). The techniques of sample preparation and orientation are reported in Samorì & Mattivi (1986) for lyotropics and in Samorì (1988) for thermotropic samples. The cell polymeric coating for the orientation of the latter was formerly described by Kutty and Fisher (1983).

Molecular Orbital (MO) calculations on Q_0 and Q_1 molecules were carried out by using the CNDO/S method, implemented by configuration interaction of selected singly and doubly excited configurations. The data obtained, collected in the Table, refer to a calculation of 100 configurations. No sensible changes were observed increasing the size of the configuration interaction. The geometry of the benzoquinonic unit was taken from Jacques et al. (1981), whereas standard bond lengths and bond angles were adopted for the methoxyl, methyl and isoprenic substituents. The results reported in the Table were ob-

tained using the Ohno-Klopmans approximation for the Coulomb integrals (Ohno, 1964) and standard value for β (0) = −45 eV.

Abbreviations

AA = average absorption.
$OD_{\parallel}$, $OD_{\perp}$ = optical densities for plane polarized radiations parallel ($\parallel$) and perpendicular ($\perp$) to the sample optical axis.
Δ OD = $OD_{\parallel} - OD_{\perp}$.
EPR = electron paramagnetic resonance.
LC-LD = liquid crystal-linear dichroism.
LD = linear dichroism.
LD_r = reduced linear dichroism.
MO = molecular orbital.
N = nematic.
NMR = nuclear magnetic resonance.
S_{jj} = order parameters of the directions j of the transition moments of the guest chromophore.
S_{ii} = order parameters of the orientational axes i of the guest molecule with respect to the magnetic field.
S'_{ii} = order parameters of the axes i of the guest molecules with respect to the bilayer axis a.
S_{aa} = order parameters of the host bilayer axis a with respect to the orienting magnetic field.
$\theta_{j,i}$ = deflection angles between the directions j and the axes i.
O_i = optical factors of the i axis (*see* Eq. (A4)].
Q_n = ubiquinone whose isoprenoid chain contains n isoprenoid units.

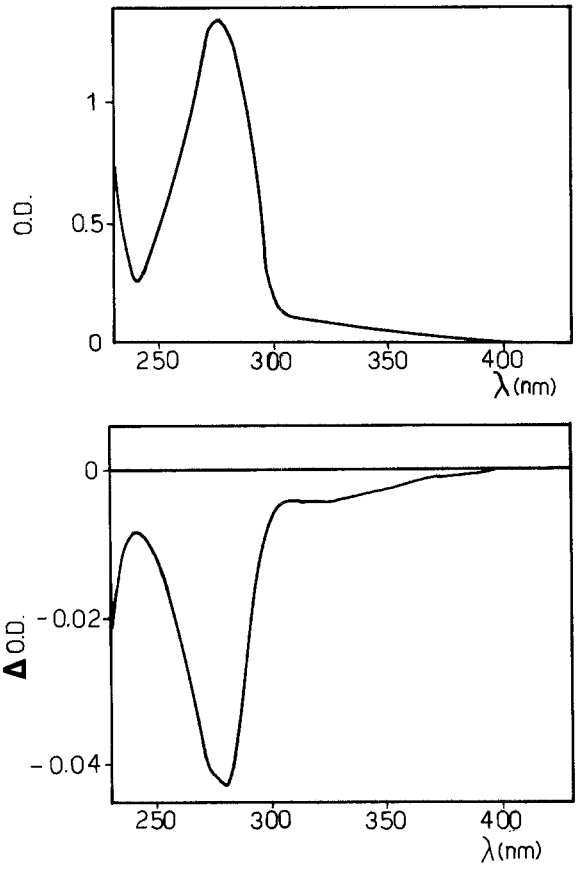

Fig. 2. Average absorption (upper) and linear dichroism (lower) spectra of Q_1 in the lyotropic solvent described in Materials and Methods.

Results and Discussion

Linear Dichroism of Ubiquinones in Lyotropic Bilayers

Nematic (N) phases seldom occur in the phase diagrams of lyotropic systems as precursors of lamellar or hexagonal phases (Charvolin, 1983). The building units of these N phases are in fact disc or rod-like aggregates which are pieces of bilayers. A magnetic field easily orients them, thus inducing the linear anisotropy which allows LD spectra of guest molecules to be recorded (Laurent & Samorì, 1987; Forni et al., 1989).

The host system chosen by us is a nematic (N) phase constituted by disc-like bilayer fragments (Hendrikx et al., 1983; Figueiredo Neto, Liebert & Galerne, 1985). It is transparent also to UV light down to 220 nm; the LD investigation can thus be extended to this spectral region, as required by the Q_n chromophore. The LD spectra of Q_0, Q_1, and Q_3, are reported in Figs. 2–4.

We recorded the LDs of all Q_n derivatives with $3 < n < 10$. All these LD spectra are characterized by two negative bands centered at about 320 and 275 nm. The "t.e.m. reduction procedure" was applied to these spectra. This approach was formerly described by Thulstrup, Michl and Eggers (1970) and further modified for the modulated techniques by Samorì, Mariani and Spada (1982). Figure 3 shows two significant steps of the reduction procedure carried out for Q_3. These two reduction curves provide the best evidence that the *LD bands at about 280 nm result from the overlapping of two oppositely signed quasi-degenerate bands*. By this t.e.m. approach, their absorption profiles were computed. This was made possible by having ruled out the presence of $n\pi^*$ transitions in this region (*see* infra). The lower energy absorption band was found to be almost twice as intense as the other, and the shift between the two maxima is about 2 nm. These two bands are likely to be determined by a multisite orientational distribution of the ubiquinone molecules. The absorption band centered at about 320 nm is too broad and weak to allow a t.e.m. reduction result as clear as that of the band at 280 nm.

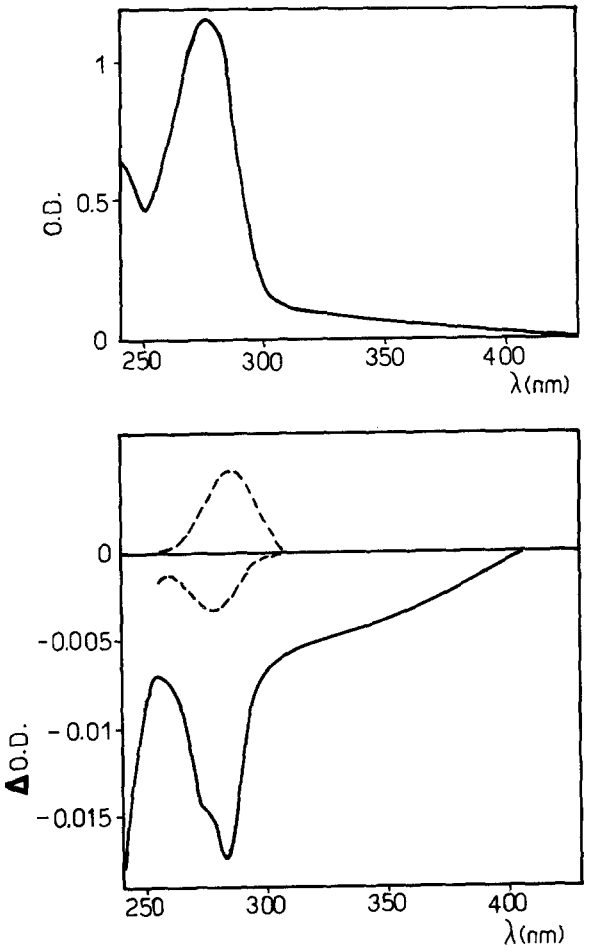

Fig. 3. Average absorption (upper) and linear dichroism (solid line, lower) spectra of Q_3 in the lyotropic medium described in Materials and Methods. Two significant steps of the "t.e.m. reduction procedure" which was applied to these spectra are also reported: the curves $[OD_{\parallel}(\lambda) - OD_{\perp}(\lambda)] - d'/2\ [OD_{\parallel}(\lambda) + OD_{\perp}(\lambda)]$ for $d' = -0.06$ (upper) and $d' = +0.02$ (lower) in arbitrary units. This reduction procedure (*see* text) shows that the negative linear dichroism band centered at about 285 nm results from overlapping and strong elision between two oppositely signed and quasi-degenerate bands (dashed lines).

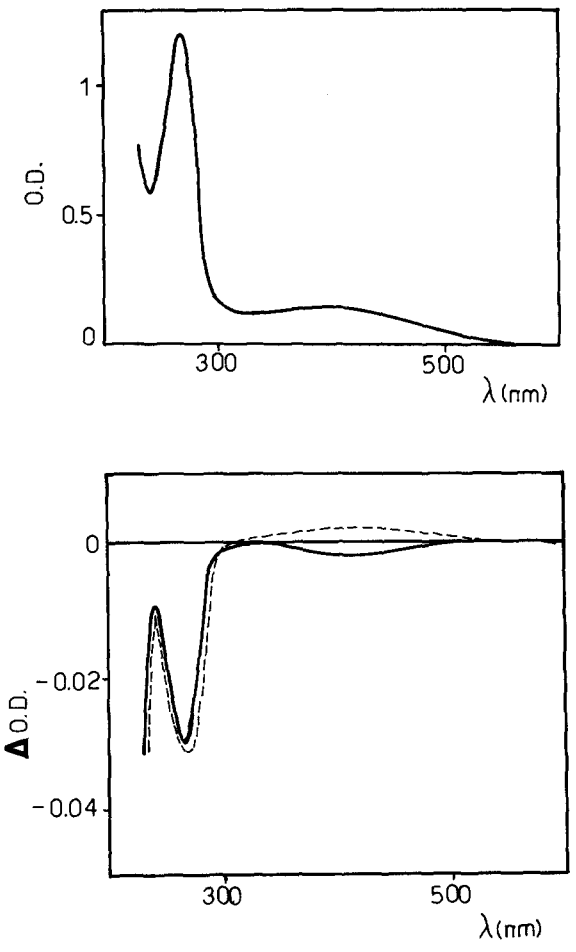

Fig. 4. Average absorption (upper) and linear dichroism (lower) of Q_0 in the lyotropic solvent described in Materials and Methods. The linear dichroism spectrum of Q_0 in a different nematic host medium (potassium laurate/potassium chloride/water: 35.28/2.3/62.10 wt%) (*see* Samorì & Mattivi, 1986), is also reported (dashed line).

In order to get a stereochemical interpretation of LD spectra of Q_n as in Fig. 2–4 the approach described in the papers by Samorì and Mattivi (1986) and Laurent and Samorì (1987) was employed.

A General Approach to Interpretation of LD Spectra of Guest Molecules in Membranes or Model Membranes

The overall orientational anisotropy of Q_n molecules in the investigated sample is determined by both the average orientation of the host bilayer under the magnetic field and by the restrictions imposed on their diffusion properties within all their location sites. In this way our guest-host system can be split into two uncorrelated and uniaxial sub-systems: that of the bilayer plane with respect to the magnetic field (along the $\parallel$ direction) and that of the orientational $i = x, y, z$ axes of the guest molecule with respect to the bilayer axis (a) (*see* Fig. 5 and Appendix). An S_a order parameter (*see* Appendix Eq. (A4)] can thus describe in the oriented samples the average orientation achieved by the host axis (a) with respect to the orienting magnetic field. An S'_{ii} order parameter instead describes in the latter subsystem the average orientation of the guest i axes with respect to the axis (a).

The anisotropic distribution of Q_n guest molecules in the host frame can be described by a basic set of six limiting orientations (Fig. 5). Because of the uniaxiality of the system, they actually reduce

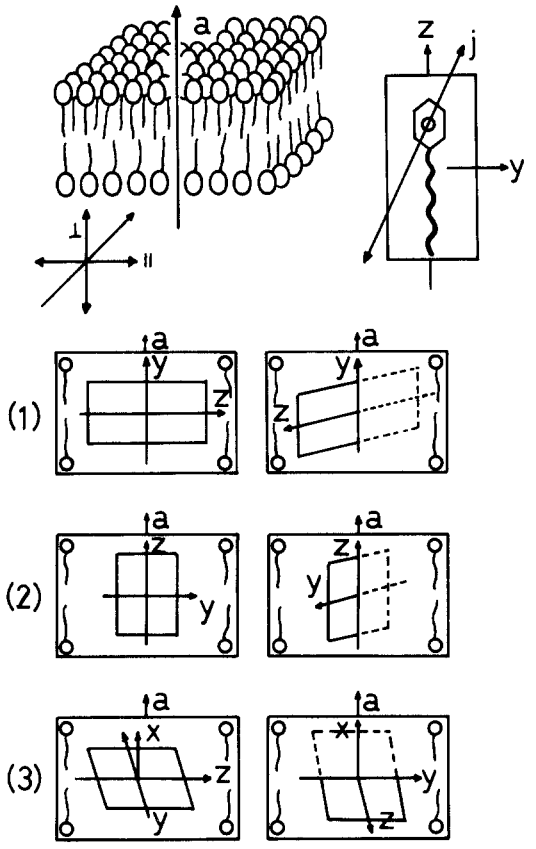

Fig. 5. Limiting orientations of a ubiquinone guest molecule in a uniaxial host bilayer. A direct correspondence between these theoretical modes and those of Fig. 1 was settled.

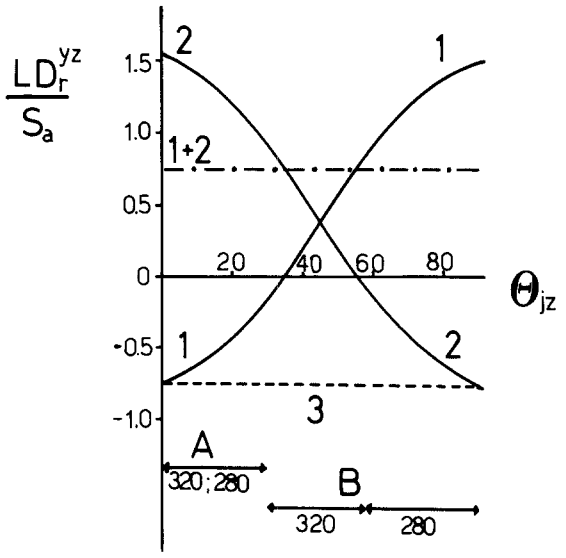

Fig. 6. Plots by Eqs. (A6)–(A8) of the reduced linear dichroism (LD_r) normalized by S_a for modes (*1*)–(*3*) of Fig. 5 *vs.* the deflection angle θ_{jz} between the transition polarization *j* and the orientational axis *z*. N.B. The order parameter S_a of the bilayer axis is negative, therefore experimental positive LD_r values correspond to negative LD_r/S_a value in this plot. The conformations *A* and *B*, sketched in Fig. 7, lead to different θ_{jz} values for the transitions at 280 and 320 nm. The angular ranges in which the transition moments *j* defined in Fig. 10 should lie in the different cases, are reported on the bottom of this figure.

to three couples, (1)–(3), of orientation modes. They are obtained by aligning in turn each orientational $i = x, y, z$ axis to the bilayer *a* axis. These (1)–(3) modes directly correspond to the (1)–(3) orientations/localizations of Fig. 1. The LD signals relative to each orientation should therefore depend on the direction of the *j* transition moment within the frame of the orientational axes of Q_n molecules.

The appendix shows how the LD intensities normalized by S_a can be analytically obtained for each orientation mode 1–3 as a function of θ_{jz}, the angular deflection from the *z* axis of the *j* polarization direction of the investigated transition. This result is graphically reported in Fig. 6.

In order to apply this interpretative approach to LD spectra of Q_n as in Figs. 2–4, we must therefore find the θ_{jz} values of the investigated transitions. The polarization directions of these transitions can be obtained by a specific version of the LD technique which is called liquid crystal-linear dichroism (LC-LD) (Sackman & Mohwald, 1973; Samorì et al., 1982; Samorì, 1983; 1988). The ubiquinone chromophore must be oriented by a liquid crystalline matrix in which the orientation it assumes can be predicted. If the preferential orientation of the chromophore is known, its LD spectrum provides information about the polarization of the investigated transition.

Polarizations of the UV Bands of the Ubiquinone Chromophore by the LC-LD Technique and MO Calculations

Thermotropic liquid crystalline solvents are very flexible and effective tools to order molecules with predictable orientations (Michl & Thulstrup, 1987, 1988; Samorì & Mattivi, 1986). A strongly elongated guest molecule, such as Q_n ($n \neq 0$) is obliged by collisions with the host liquid crystalline molecules to align its axis (*z*) of major elongation parallel to the sample director and to assume the least order-perturbing conformations (Samorì, 1979; Johansson et al., 1987.) Q_n is therefore likely to take on in this thermotropic solvent lath-like structures such as those depicted in Fig. 7 and labeled *A* and *B*. *B* is obtained from *A* simply by rotation about the exocyclic C–C bond which connects the isoprenoid chain to the ring.

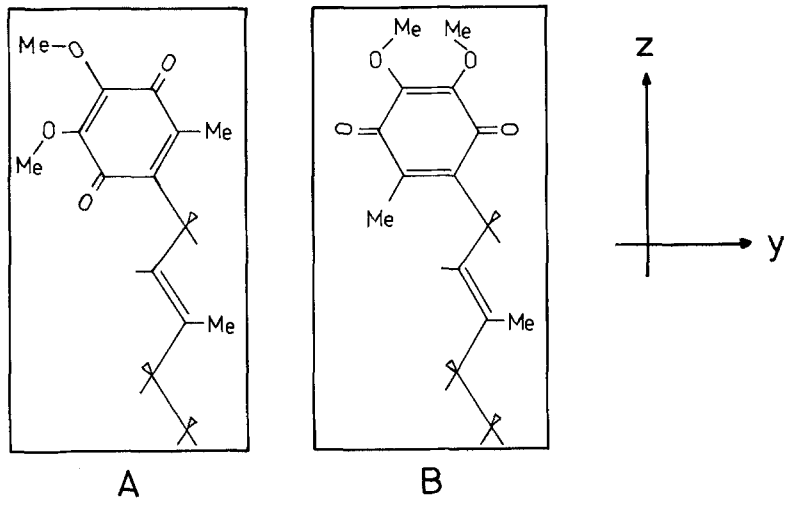

Fig. 7. Rotation of the quinone headgroup around the exocyclic bond which connects with the isoprenoid chain, leads to *A*-like and *B*-like conformers. They are expected to orient in a mesomorphic ordered system as lath-shaped objects, whose x, y, z orientational axes are defined.

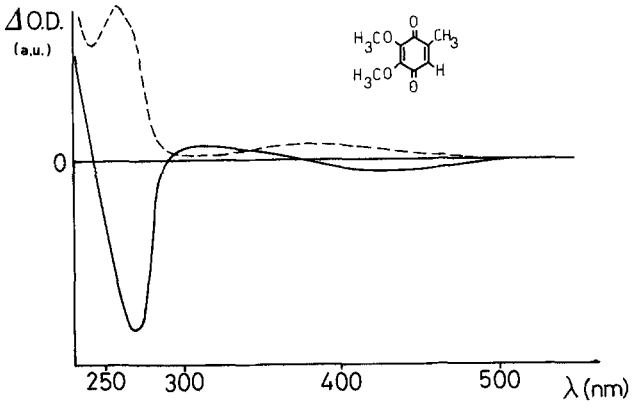

Fig. 8. Profiles of the liquid crystal-linear dichroism spectra (arbitrary units) of Q_0 in thermotropic ZLI 2359 (dashed line) and ZLI 1167 (solid line).

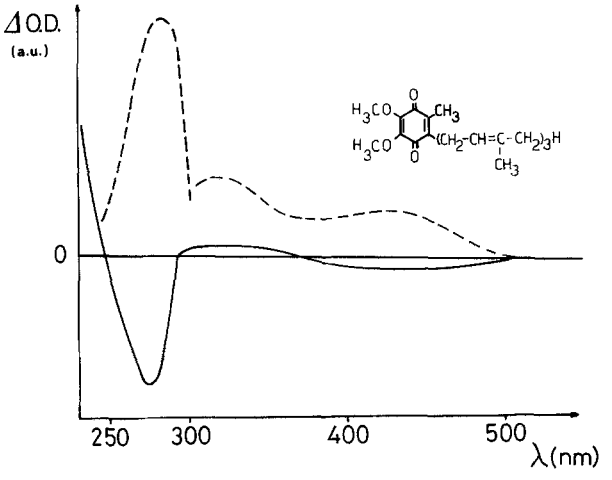

Fig. 9. Profiles of the average absorption (dashed line) and Linear Dichroism (full line) of Q_3 in ZLI 1167 (arbitrary units).

Figures 8 and 9 report the profiles of the LC-LD and AA (Samorì, 1988) spectra of Q_0 and Q_3 in thermotropic, hydrocarbon orienting solvents. In ZLI 1167 the profiles of the LD spectra of Q_0, Q_3 (Figs. 8 and 9) and other Q_n compounds with $n > 3$ (*unpublished results*) are very similar. This implies, first of all, that all those molecules orient as rod-like objects. The major elongation axis of Q_0 is in the zy plane the same as that of Q_n molecules when the latter assume a *B*-like conformation (Fig. 7). This conformation, being more elongated than the *A*-like, is actually expected to be favored by the structural organization of the liquid crystalline host.

On this basis the negative LD of the lowest energy band at about 400 nm is thus assigned to typical $n\pi^*$ out-of plane transitions of the quinone chromophore (Jacques et al., 1981), while the two bands at about 320 and 275 nm are $\pi\pi^*$ transitions. This $\pi\pi^*$ assignment fits their red shift upon increasing solvent polarity. It is strongly supported also by the LD spectra of Q_0 recorded in ZLI 2359 (*see* Fig. 8). The orienting ability of this solvent is much lower than that of ZLI 1167; no discrimination between the orientations of y and z axes in the chromophoric plane of Q_n molecules is thus possible in this medium. Q_0 thus behaves in this solvent as a disc-like molecule and all its $\pi\pi^*$ in-plane polarized transitions therefore exhibit positive LD bands (Samorì et al., 1982).

Because of the low symmetry of Q_n chromophore these LC-LD spectra alone are not sufficient to define precisely the polarization directions j of the in-plane polarized bands at 320 and 275 nm. The

Table. Calculated energies and components of the transition moments (M in Å) along the i axes for the low-lying states of Q_0 and Q_1.

Q_0	E(nm)	M_x	M_y	M_z
S_1	451	−0.0062	0.	0.
S_2	435	0.0028	0.	0.
S_3	362	0.0	0.1711	−0.0788
S_4	240	0.0	1.1345	0.0170
S_5	221	−0.0062	0.0	0.0
S_6	215	−0.0383	0.0	0.0
S_7	204	−0.0128	0.0	0.0
S_8	201	0.0	0.4004	−0.2969
Q_1				
S_1	429	0.0617	0.0	0.0
S_2	385	0.0013	0.0	0.0
S_3	350	0.0	0.006	0.0786
S_4	309	0.0037	0.0	0.0
S_5	288	−0.1285	0.0	0.0
S_6	250	0.0	−1.0643	0.3056
S_7	223	0.0	0.3680	0.3408
S_8	213	0.0	0.4877	−0.9941

S_{jj} order parameter of the j axes with respect to the magnetic field must thus be expressed in terms of the order parameters S_{yy} and S_{zz} of the orientational axes z and y of ubiquinone molecules

$$S_{jj} = S_{yy} \sin^2 \theta_{jz} + S_{zz} \cos^2 \theta_{jz} \quad (1)$$

where θ_{jz} is the deflection angle between the j polarization axis and z orientation axis of the molecule. On this basis it can be easily inferred that these *LD spectra of Figs. 8 and 9 can fit θ_{jz} values ranging between 0° and 60° for the 280 nm band and between 60° and 90° for that at 320 nm.*

This very large indetermination can be reduced by combining the LC-LD approach to *Molecular Orbitals (CNDO/S) computations* on Q_0 and Q_1 (Table).

The transition pattern of Q_0 in terms of energies and oscillator strengths is very similar to those of the basic chromophore *p*-benzoquinone (Jacques et al., 1981), thereby confirming that the alkyl and methoxyl substitution affects the lowest electronic states only as a weak perturber through mesomeric and inductive effects (Salem, 1966). On the other hand, the substituents affect the directions of the transition moments.

In Q_0 the first two states are of $n\pi^*$ nature, out-of-plane (x) polarized. They correspond to the split n^+ and n^- states of benzoquinone, of B_{1g} and A_u symmetry, respectively. The first sizeable in plane transition moments (S_3 and S_4) were computed at 362 and 240 nm, and are likely to be responsible for the two bands which occur at about 320 and 280 nm.

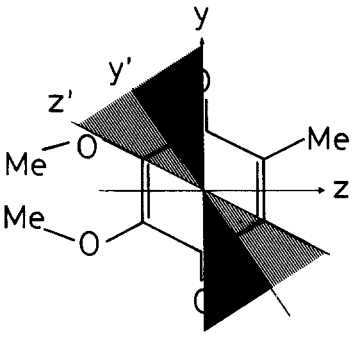

Fig. 10. Assignments of the polarizations of the ubiquinone chromophore transitions at 320 and 280 nm. The polarization direction of the former band is most safely expected to lie between z' and y' and of the latter between y and y'.

The insertion of the isoprenoid chain in Q_1 results in an enhancement of the computed moments of the transitions to the lowest energy $n\pi^*$ states, and in a new (S_5) out-of-plane polarized state. It appears in the computations between the $\pi\pi^*$ states S_3 and S_4, which were recognized as characterizing the spectrum of Q_0. This S_5 state is not localized on the isoprenic chromophoric unit. No spectroscopic evidence of this x-polarized band was found either in absorption or in LD spectra in thermotropic solvents (Fig. 9). It is not hidden below the strong band centered at 280 nm, unless its intensity is too low to be revealed by the "t.e.m." reduction procedures. In aqueous lyotropic environment this state, with some degree of $n\pi^*$ nature, is certainly shifted to higher energy regions, and its insertion in the area of the two lowest energy $\pi\pi^*$ states can thus be reasonably ruled out.

We carried out the same CNDO/S calculation also on different conformations of both Q_0 and Q_1, and on different derivatives substituted by a saturated short alkyl chain (as a methyl or an ethyl) instead of the isoprenoid one. This was done in order to gain greater insight into the ability of this computational method to provide a reliable description of this chromophoric system.

By using the transition moment components of the Table together with the whole bulk of these computation results, the θ_{jz} ranges previously inferred from the LC-LD spectra of Figs. 8 and 9 could be reduced and the polarizations of the bands at 320 and 280 nm could be ascribed as in Fig. 10. We define just two angular sectors in which the two transition moments lie. We did not try to define these directions of polarization more precisely because of the conformational freedom of Q_n molecules and because of the impossibility of applying in-parallel also IR-LD techniques (Michl & Thulstrup, 1988) to liquid crystalline samples.

This level of definition, as shown in the following, is anyway sufficient to allow the LD spectra in Figs. 2–4 to be effectively interpreted in terms of guest-host mutual orientations and localizations, which is the aim of this investigation. The LD spectra of Figs. 2–4 can thus be analyzed on the basis of the general plots of Fig. 6 and the assignments of Fig. 10.

Stereochemical Interpretation of the LD Spectra of Ubiquinones in Lyotropic Bilayers

We have seen that the LD spectra of Q_n molecules in the investigated bilayer are characterized by two negative bands centered at about 320 and 275 nm; t.e.m. reduction procedures showed that the latter, stronger LD band clearly results from the overlapping and elision between two oppositely signed, quasi-degenerate bands. The LD spectra could therefore be interpreted in terms of an equilibrium of guest Q_n molecules between two types of localizations and orientations.

The *positive LD band* revealed by the "t.e.m. reduction" at about 275 nm is blue shifted with respect to the negative one, which was found at about 280 nm. The positive band is therefore determined by Q_n molecules located in environments less polar than those that can be associated to the negative one. The sites associated with the positive band are likely to be found in the hydrocarbon bilayer interior, whereas the sites associated with the negative band are likely much closer to the polar bilayer-water interface.

The LD is linked to S_{jj} by Eqs. (A1)–(A3) of the Appendix. The signs of the LD signals associated to the limiting modes 1–3 of our approach, being the same of those of the relative S_{jj}, can be directly obtained by the general plot of Fig. 10. Being S_a negative, a positive LD sign can be associated to mode 3, for any θ_{jz} value, but also to either mode 1 or 2, according to the θ_{jz} values that the guest molecule assumes within its localization site. Sign inversions take place at $\theta = 35°26'$ or $54°73'$ for mode 1 or 2, respectively. The ranges of θ_{jz}, besides being determined by the polarizations of the transition moments within the chromophoric head group (*see* Fig. 10), depend also upon the average orientation of this headgroup with respect to the isoprenoid chain (*see*, for instance, conformations *A* and *B* in Fig. 7). For the moment, without entering this conformational problem (as we will do infra) just on the basis of its sign and its shift, this positive band can be ascribed to the localizations/orientations of the headgroup in the hydrocarbon bilayer interior sketched by modes 1*a*, 2*b* and 3*a* (*see* Fig. 1).

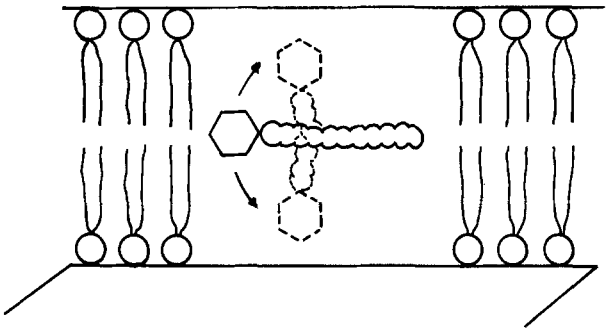

Fig. 11. Model of ubiquinone localization in lipid bilayers formerly suggested by Lenaz (1988).

The *negative* component can be accounted for only by modes 1 and 2. The plot in Fig. 6 shows that the latter mode provides negative LD signals when $0° < \theta_{jz} < 35°26'$, the former when $54°73' < \theta_{jz} < 90°$. Its red shift makes this component associated to Q_n molecules whose aromatic headgroup should be located at the polar bilayer surface: only modes 1*b* and 2*a* can therefore account for this negative band. Actually the former is expected to be far less populated than the latter. In fact, guest molecules dissolved in a liquid crystalline ordered system as a membrane are thus obliged to take on orientations and conformations which increase as much as possible their structural similarity to the solvent molecules (Samorì, 1979; Johansson et al., 1987). Therefore deformations of the isoprenoid chains away from the extended conformation are very unlikely to occur close to the polar interface as it should be in 1*b*. They can instead more easily take place in the central part of the bilayer, as in 2*a*. In fact the orientational disorder of the segment chains increases as does the distance from the polar heads (Charvolin & Hendrikx, 1985). This negative LD band at about 280 nm is thus likely to be determined by Q_n molecules located and oriented as in *2a*.

A combination of a 2a localization with 1a, 3a and 2b modes can therefore account for the LD spectra in this 280 nm region. The mode *2a* settles the quinonic head groups at the polar surface of the bilayer. The other modes instead bury them into the host hydrocarbon interior. This picture of Q_n orientational distribution well matches the localization model in lipid bilayers suggested by Lenaz (1988). Within this model the ubiquinone molecule is located in the membrane mid-plane, with the headgroup oscillating transversally across the membrane between localizations at the water interface and inside the hydrocarbon host interior (*see also* Battino et al., 1986) (Fig. 11).

This picture was so far obtained without putting

any restriction on θ_{jz} values. Free rotation of the aromatic head around the exocyclic bond to the isoprenoid chain would lead to a continuous distribution of θ_{jz} values. By giving some definition of θ_{jz} and by bringing into play also the assignments in Fig. 10 the LD spectra in Figs. 2 and 3 become sources of further information. In fact both the strong negative LD signals in the 320 nm regions and the assignment of the negative band at 280 nm to a *2a* mode imply that $0° < \theta_j < 35°26'$. The polarization assignments in Fig. 10 make this restriction of the θ_{jz} values compatible only with *A*-like conformation (*see* Fig. 6). If this restriction in conformation holds, as it should, also for a Q_n molecule located in the bilayer interior, then mode *2* and in particular *2b*, can be ruled out. Only modes *1a* and *3a* would thus account for the positive LD signals of the absorption component at higher energy.

When the aromatic headgroup of a Q_n molecule is located at the bilayer surface as in *2a*, this conformational restriction to *A*-like structures makes one of the two quinonic carbonyls to face the host bilayer polar heads. This is likely to be determined by the *tendency, within this localization site, of the carbonyls to specifically grasp the polar surface of the host bilayer.*

This finding was strongly confirmed by the LD spectrum of Q_0 (Fig. 4). Its profile, being the same as that of the absorption spectrum, suggests a single-site location of this guest molecule. This profile provides clearer evidence of the specific grasping of the carbonyls. On the basis of the approach reported by Samorì and Mattivi (1986), Q_0 molecules reveal their tendency to be intercalated within their single host site between the hydrocarbon host chains and to take on (following the notations of that paper) a *C*-like orientational mode in K laurate/decanol/H_2O or an *A*-like one in K laurate/KCl/H_2O (*see* Fig. 4).

Both *C*- and *A*-like orientational distributions clearly locate one quinone carbonyl of the aromatic head at the polar interface of the bilayer. This is the same interpretative approach that demonstrated the same type of strong specific grasping of heterocyclic nitrogens of guest aromatic molecules to the polar surface of host bilayers (Samorì & Mattivi, 1986).

In conclusion this study provides experimental evidence of a multisite distribution of the aromatic head groups of guest Q_n molecules (for $n > 0$) in host lyotropic bilayers. This distribution matches well the solubilization model suggested by G. Lenaz (1988) for ubiquinone in lipid membranes. Within this model Q_n molecules are located in the membrane midplane and their headgroups oscillate transversally across the membrane. Q_0 takes instead a single site location, close to the polar bilayer interface. Experimental evidence of the tendency of the headgroup carbonyls to grasp the polar interface of the host bilayer was also attained.

These results seem to suggest that Q_n orientation and location distributions are the result of the tendency of the heads to grasp the polar heads of the host bilayer and of the concurrent tendency of the chains to settle into the hydrocarbon host interior. A thermodynamic analysis of the solubilization processes of ubiquinone in membrane bilayers is in the process of publication by the same authors.

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Appendix

The signs and the intensities of an LD band that is determined by a *j*-polarized transition provide information about the orientational distribution of the molecular *j* axis itself within the oriented sample under investigation. This LD, which can be also expressed in terms of "reduced LD" (LD_r), is linked to an S_{jj} function by the following equations:

$$LD = [OD_{\parallel}(\lambda) - OD_{\perp}(\lambda)]/[OD_{\parallel}(\lambda) + OD_{\perp}(\lambda)] = 3S_{jj}/(2 + S_{jj}) \quad \text{(A1)}$$

$$LD_r = 3[OD_{\parallel}(\lambda) - OD_{\perp}(\lambda)]/[OD_{\parallel}(\lambda) + 2OD_{\perp}(\lambda)] = 3S_{jj} \quad \text{(A2)}$$

where

$$S_{jj} = \tfrac{1}{2} < 3\cos^2\beta - 1> \quad \text{(A3)}$$

is an order parameter of rank two which is averaged over the distribution of the deflexions β's of the transition moment *j* directions of all the guest absorbing molecules from the sample director, or, as chosen in this investigation, from the magnetic field direction.

S_{jj} of our guest-host system can be factorized by the order parameters S_a and S'_{ii} describing the average orientations of the two uncorrelated subsystems: That of the bilayer host axis (*a*) with respect to the magnetic field and that of the $i = x, y, z$ axes of the guest molecule with respect to the bilayer axis (*a*) (*see* Fig. 5 and text).

The *j* directions of the transition moments of Q_n lie at a nonvanishing angle $\theta_{j,i}$ to the orientational axes *i*, they must therefore be related to S'_{ii} by the optical factors $O_i = \cos^2\theta_{j,i}$. The S_{jj} tensor and LD_r become (Norden, 1980; Laurent & Samorì, 1987):

$$S_{jj} = S_a(S'_{xx}O_x + S'_{yy}O_y + S'_{zz}O_z) \quad \text{(A4)}$$

$$LD_r = 3S_a \Sigma S'_{ii}O_i \qquad \text{for } i = x, y, z \quad \text{(A5)}$$

S_a is always negative and takes the maximum value of $-\frac{1}{2}$ for limiting perfect ordering of the bilayer in the magnetic field.

The information about guest-host mutual orientation we are looking for is contained in the S'_{ii} values. A very effective and simple geometrical method capable of extracting this information was used.

Equations (A4) and (A5) take very simple forms for the orientational modes 1–3. For transition polarized in the *yz* planes LD_r is given by

$$(LD_r)_1^{yz} = \tfrac{3}{4}S_a(2 - 3\cos^2\theta_{jz}) \quad \text{(A6)}$$

$$(LD_r)_2^{yz} = \tfrac{3}{4}S_a(3\cos^2\theta_{jz} - 1) \quad \text{(A7)}$$

$$(LD_r)_3^{yz} = -\tfrac{3}{4}S_a \quad \text{(A8)}$$

and for transitions out-of-plane (*x*) polarized

$$(LD_r)_1^{x} = (LD_r)_2^{x} = -\tfrac{3}{4}S_a \quad \text{(A9)}$$

$$(LD_r)_3^{x} = +\tfrac{1}{2}S_a. \quad \text{(A10)}$$

The dependence upon $\theta_{j,z}$ disappears, as expected, in Eqs. (A8) and (A10) because of the free molecular rotation around the *x* axis allowed by mode 3. It disappears also in Eq. (A9) because free rotations of *y* and *z* are expected in modes 1 and 2, respectively.

Plots of Eqs. (A6), (A7) and (A8) *vs.* θ_{jz} are reported in Fig 6.