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The Thermotropic Properties of Coenzyme Q_{10} and Its Lower Homologues

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Abstract

The thermotropic properties of coenzymes Q_{10}, Q_9, Q_8, and Q_7 have been examined by differential scanning calorimetry and wide-angle X-ray diffraction. Typical scanning calorimetry cooling curves of coenzyme Q from the liquid state exhibit a single exothermic phase transition into a crystalline state at a temperature that decreases as the length of the polyisoprenoid side-chain substituent decreases. Upon subsequent heating, the molecules undergo a series of thermal events which precede the main crystalline-to-liquid endothermic phase transition. The temperature of these transitions increases with increasing chain length. The crystallization phase transition temperature depends markedly on the rate at which the sample is cooled and increases with decreasing scan rate; the temperature of the melting endotherm is not markedly affected by the scan rate. Detailed calorimetric studies of coenzyme Q_{10} indicate that two crystalline states are formed, one at relatively high cooling rates to low temperatures and the other when preparations are cooled slowly from the liquid state to relatively high temperatures. Heating the crystalline phase formed by rapid cooling causes its transformation into the phase observed by cooling slowly. X-ray diffraction analysis confirmed the existence of these two crystal phases in coenzymes Q_9 and Q_{10} and the transformation from the rapidly crystallized form to the more ordered form associated with slower cooling rates. At body temperature (310 K) under equilibrium conditions coenzyme Q_{10} exists in an ordered crystalline phase; the implications of the thermotropic behavior of coenzyme Q_{10} on mitochondrial function *in vitro* and *in vivo* are discussed.

Key Words: coenzyme Q; ubiquinone; phase transitions.

Introduction

The importance of coenzyme Q as an essential redox component of certain-energy-transducing membranes is well recognized (Mitchell, 1976; Crane, 1977; Trumpower, 1981). Since the isolation, chemical characterization, and synthesis of coenzyme Q in the 1950's (Morton, 1958; Wolf *et al.*, 1958;

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Lester and Crane, 1959), a considerable knowledge of the physical properties of the naturally occurring homologues Q_{10} to Q_6 and shorter-chain derivatives Q_5 to Q_0 has accumulated (Isler *et al.*, 1961; Hatefi, 1963; Sommer and Kofler, 1966; Ramasarma, 1968). Studies of the thermotropic properties, however, have been restricted to a determination of crystalline-to-liquid melting points of coenzymes Q_{10} to Q_6, and there is some variation in the published values for the respective homologues (Lester *et al.*, 1959; Langemann and Isler, 1965).

Studies of the properties of coenzyme Q in monomolecular films of phospholipids have indicated only limited miscibility of the two lipid constituents (Maggio *et al.*, 1977) and the conclusion that, in the case of the long-chain homologues, the coenzyme is phase-separated from the phospholipid molecules aligned perpendicularly to the aqueous surface into a position on the surface of the film (Quinn and Esfahani, 1980). The surface pressure at which the phase separation is observed indicated that, in bilayer structures, coenzyme Q_{10} could be sandwiched between the two lipid lamellae (Quinn, 1980). A separate phase of aggregated coenzyme Q_{10} together with coenzyme molecules molecularly dispersed in phospholipid bilayers was subsequently recognized on the basis of differential scanning calorimetry (Katsikas and Quinn, 1981, 1982a) and X-ray diffraction (Katsikas and Quinn, 1982b). A coenzyme Q_{10} and Q_9-rich phase has also been distinguished in mixed dispersions with phospholipids on the basis of chemical shifts of the *O*-methyl protons of the benzoquinone ring derived from proton NMR studies (Kingsley and Feigenson, 1981).

The implications from these studies of model membrane systems is that coenzyme Q with long polyisoprenoid side chains may exist in at least two forms, one in an aggregated state and the other in molecularly dispersed form in the membrane phospholipid. Because the crystalline–liquid phase melting points of coenzymes Q_9 and Q_{10} are at or above the body temperature of typical homeothermic organisms, the physical state of aggregated coenzyme Q in energy-transducing membranes may be of considerable interest and important to our understanding of the mechanism of transmembrane hydrogen conduction. With this in mind we have undertaken a study of the thermotropic properties of coenzyme Q_{10} and its related homologues and have partially characterized the phase of the coenzyme at 310 K.

Materials and Methods

Differential Scanning Calorimetry

Thermograms of pure coenzyme Q with polyisoprenoid chain lengths ranging from Q_6 to Q_{10} were obtained using a Perkin-Elmer DSC-2 differen-

tial scanning calorimeter. Samples were sealed in small aluminum pans for thermal analysis. The presence of water (5 μl) in the pans did not affect the thermal properties of the coenzyme Q samples under the conditions examined. To calculate heats of phase transitions, the contents of the pans were dissolved in absolute ethanol and the concentration of coenzyme determined from the absorbance at 275 nm using a molar absorption coefficient of 14500 M^{-1} cm^{-1} which was determined separately. This is close to published values of 14000 M^{-1} cm^{-1} (Lester *et al.*, 1959).

X-ray Diffraction Measurements

Preparations of coenzyme Q for X-ray diffraction were sealed under partial vacuum in glass capillary tubes of 1 mm diameter and 0.01 mm wall thickness. X-ray diffraction patterns were obtained with a standard powder camera fitted with a circulating water device and thermocouple to maintain the sample under specified temperature conditions.

Coenzyme Q homologues with polyisoprenoid chain lengths 6 to 10 were a gift from Eisai Co. Ltd., Tokyo, Japan, and were used without further purification. All of the thermal studies were performed on coenzyme in the fully oxidized state unless indicated otherwise.

Results

The thermal behavior of coenzyme Q homologues 7 to 10 is illustrated in Fig. 1. This shows differential scanning calorimetric heating (A) and cooling (B) thermograms of the pure compounds. It can be seen that heating the crystalline material causes a series of thermal events involving both the uptake and liberation of heat which precedes a major endothermic phase transition. Subsequent cooling of these preparations results in a liquid–crystal phase transition in which heat is liberated in an exothermic process. The temperature at which these thermotropic events are observed depends directly on the length of the polyisoprenoid substituent of the benzoquinone ring; the shorter the chain length, the lower the temperature of the corresponding thermal event. Attempts were made to investigate the thermotropic behavior of coenzyme Q_6, but once the preparation had been heated above the main endothermic transition prolonged storage at -20°C was required to recrystallize the compound; cooling in the calorimeter to -196°C did not produce any exothermic events associated with a crystallization process.

The temperatures and transition enthalpys of the main thermotropic phase transitions in coenzyme Q_7 to Q_{10} homologues are presented in Table I. This shows that the thermal behavior is dominated by the polyisoprenoid chain length since both the temperatures and transition enthalpies decrease

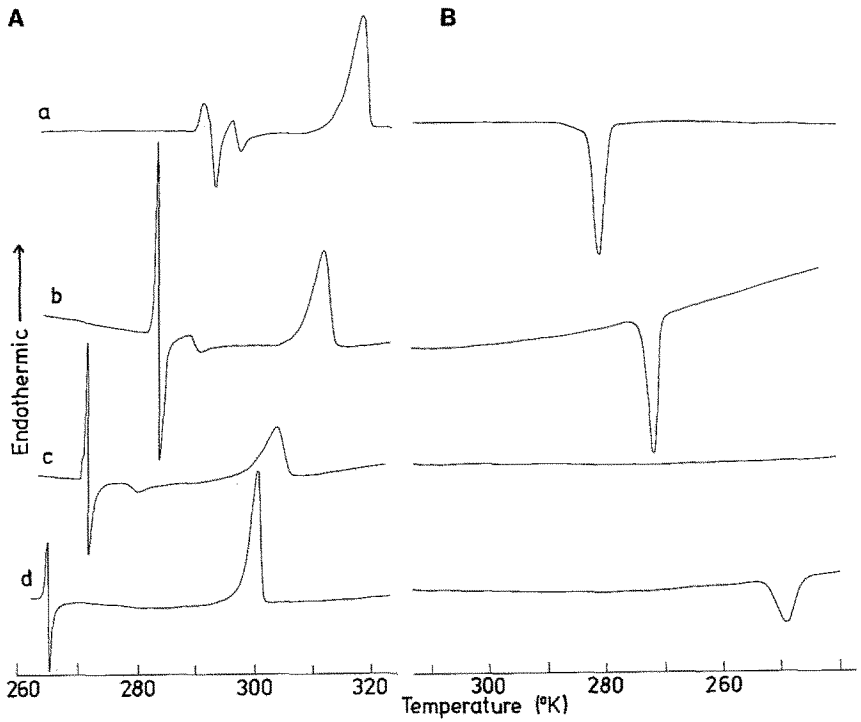

Fig. 1. Differential scanning calorimetric heating (A) and cooling (B) thermograms of: (a) coenzyme Q_{10}; (b) Q_9; (c) Q_8; (d) Q_7. All recordings were made at a scan rate of 5° min^{-1}.

with decreasing length of the side chain. Despite repeated attempts with different preparations of coenzyme Q_8, no sharp exotherm was ever observed in the sample during cooling; transition to the crystalline state must nevertheless take place because characteristic thermal transitions are observed on reheating the preparation. The two most notable features with respect to the melting and crystallization of the different coenzyme Q homologues is the marked difference in the temperature between the melting endotherm and the

Table I. Temperatures and Transition Enthalpies Associated with Melting and Crystallization of Coenzyme Q Homologues with Polyisoprenoid Chain Lengths 7 to 10[a]

	Melting endotherm		Crystallization exotherm	
Compound	Temperature (K)	Enthalpy (kJ $mole^{-1}$)	Temperature (K)	Enthalpy (kJ $mole^{-1}$)
Q_{10}	316.8	87.2	282.7	64.6
Q_9	311.2	75.3	273.7	57.4
Q_8	302.0	58.1	—	—
Q_7	301.7	34.2	252.7	17.8

[a]Data obtained from differential scanning calorimetric curves recorded at scan rate of 5° min^{-1}.

crystallization exotherm and the greater enthalpy associated with the melting compared with the crystallization process. This difference in enthalpy, 22.6, 17.9, and 16.4 kJ mole^{-1} for coenzymes Q_{10}, Q_9, and Q_7, respectively, appears to be remarkedly consistent compared to the absolute enthalpy values observed upon decreasing the length of the polyisoprenoid side chain. It may

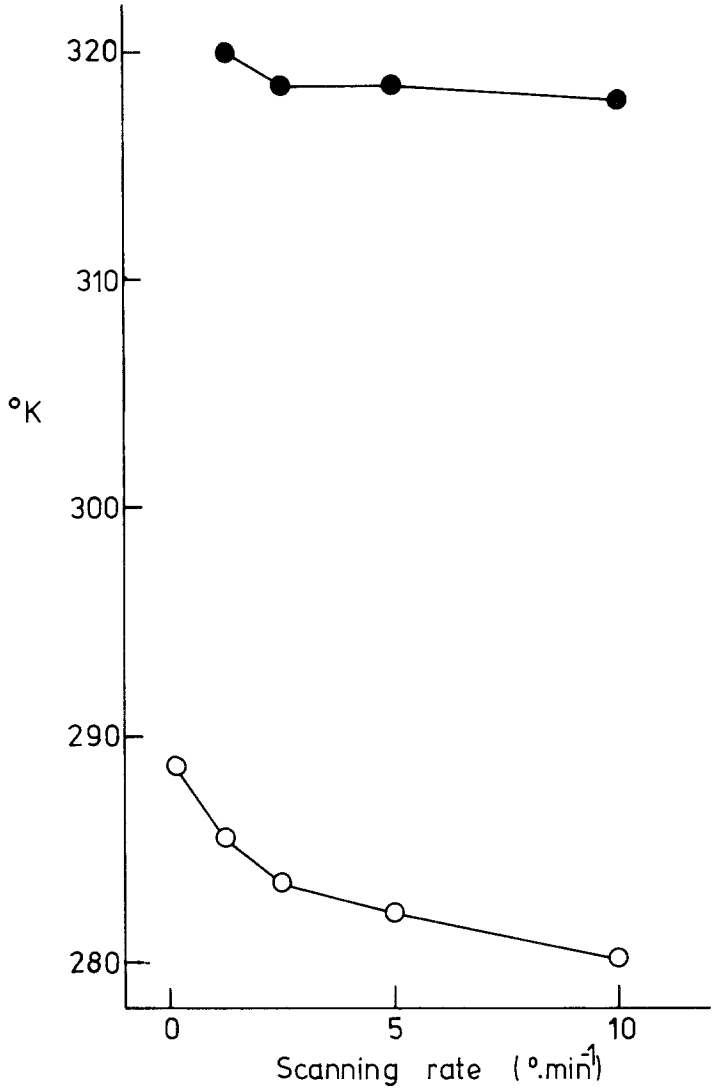

Fig. 2. Relationship between calorimetric scanning rate and the temperature of the main heating endotherm (●) and the cooling exotherm (○) of coenzyme Q_{10}.

be due in part to the temperature differential between the two phase changes and to the complex endo- and exothermic events that occur in the heating thermograms.

The difference in temperature between the melting and crystallization of coenzyme Q was found to depend on the rate of temperature change of the sample. This is illustrated for coenzyme Q_{10} in Fig. 2 in which the melting and crystallization temperature is plotted as a function of the scan rate. In contrast to the main endothermic melting temperature which is relatively independent of scan rate over the range 0.33–10° min^{-1}, the temperature of crystallization increases markedly as the scan speed is reduced.

Another interesting feature of the thermograms shown in Fig. 1 is the thermal events observed in the heating scans that precede the main endothermic transition. In coenzyme Q_{10} two separate endothermic transitions and two exothermic transitions can be distinguished in the temperature region 290 to 301 K. The temperature and enthalpy associated with the respective thermal events are presented in Table II. It can also be seen from the thermograms in Fig. 1 that as the isoprenoid side chain is shortened the endothermic and exothermic transitions 3 and 4 in this temperature region are progressively reduced or possibly become incorporated into the events associated with transitions 1 and 2. This is consistent with the absence of transitions 3 and 4 from the heating thermogram of coenzyme Q_7. The thermal properties of reduced coenzyme Q_{10} were also investigated (data not shown), and similar transitions to those seen in the oxidized form were observed; the melting endotherm was about 2° less in the reduced form.

The form of the transitions in the temperature region 290 to 301 K of the heating thermograms of coenzyme Q_{10} depended to some extent on the heating scan rate (Fig. 3A) and to a great extent on the rate at which the sample was cooled prior to heating (Fig. 3B). Thus there are slight changes in the relative proportions of the enthalpies associated with the respective transitions in preparations heated at rates between 10 and 2.5° min^{-1}, but all of the transitions can be observed. By contrast there were considerable variations in these transitions when coenzyme Q_{10} is cooled rapidly (80° min^{-1}), and they

Table II. Temperatures and Transition Enthalpies Associated with the Thermal Transitions Observed in Calorimetric Heating Scans of Coenzyme Q_{10} in the Temperature Region 290 to 301 K[a]

Transition	Temperature (K)	Enthalpy (kJ $mole^{-1}$)
1. Endotherm	293.4	1.96
2. Exotherm	294.5	7.85
3. Endotherm	298.9	11.15
4. Exotherm	300.8	13.31

[a]Data obtained from a differential scanning heating thermogram recorded at a scan rate of 5° min^{-1}.

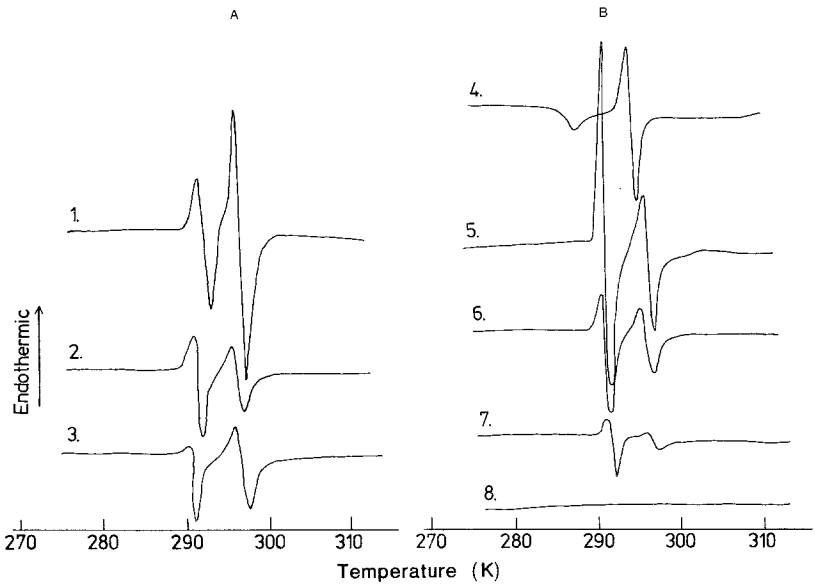

Fig. 3. The effect of heating scan rate (A) and cooling rate prior to heating at a scan rate of 5° min^{-1} (B) on the phase transitions of coenzyme Q_{10} in the temperature region 290–310 K. (A) Samples precooled from 320 to 260 at 5° min^{-1} and reheated at (1) 10° min^{-1}, (2) 5° min^{-1}, and (3) 2.5° min^{-1}. (B) Thermograms obtained at a heating scan rate of 5° min^{-1} of the same sample precooled from 320–260 at (4) 80° min^{-1}, (5) 10° min^{-1}, (6) 5° min^{-1}, (7) 2.5° min^{-1}, and (8) 1.25° min^{-1}.

were completely absent if the sample was crystallized by cooling at a sufficiently slow rate. This is illustrated in the experiment shown in Fig. 4A. A sample of coenzyme Q_{10} heated to 330 K was placed in the calorimeter and maintained at 300 K for 3 days. The sample was then cooled at 5° min^{-1} to 285 K and reheated at a scan rate of 5° min^{-1}. No thermal transitions were observed in the temperature range 290–310 K, but a major endothermic transition at about 316 K indicated that the coenzyme was in a crystalline form. The estimated enthalpy value indicated that the phase transition is identical to that seen in crystalline preparations of coenzyme Q_{10} scanned in the conventional way (Fig. 1) but showing phase transitions in the region 290–210 K. A second experiment (Fig. 4B) was undertaken in a similar way, but the equilibration was performed at body temperature for 6 days (310 K). Thermograms similar to those of samples equilibrated for 3 days at 300 K were obtained, but the enthalpy value of the main endothermic phase transition was only 20.4 kJ $mole^{-1}$. Assuming that this thermal event represents the same crystal-to-liquid phase transition seen in the fully crystallized sample (Fig. 4A), then after 6 days equilibration at 310 K only about 23% of the sample is in a crystalline phase and the remainder is liquid.

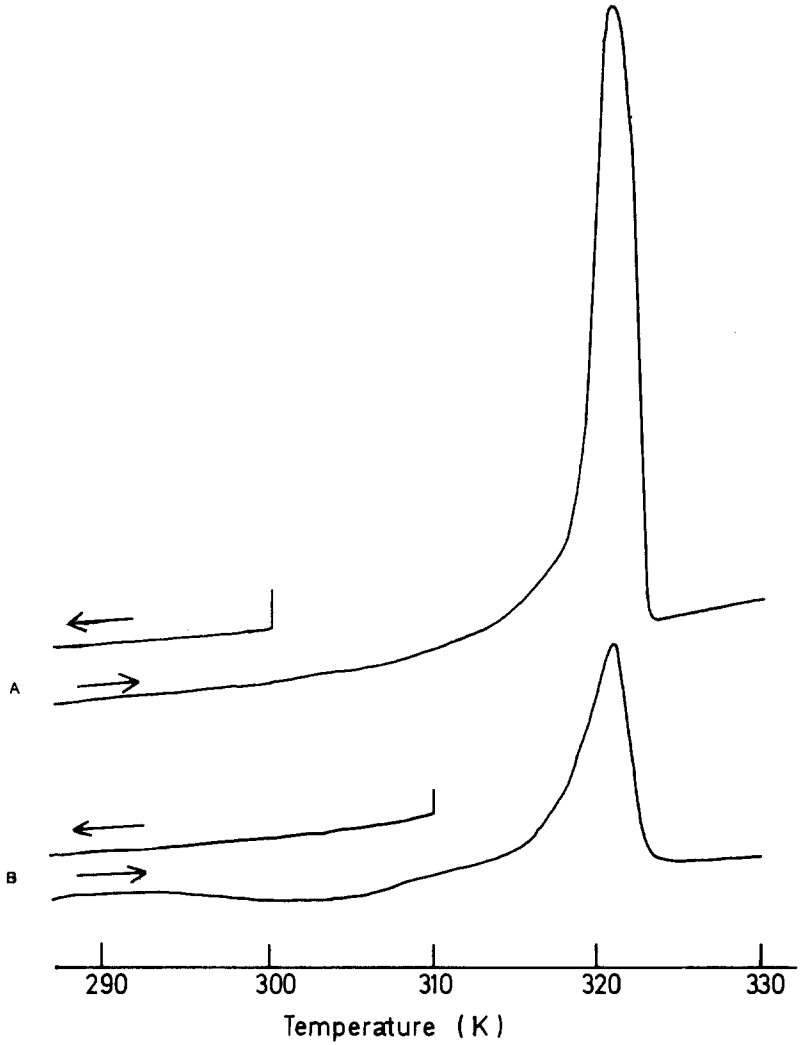

Fig. 4. Differential scanning calorimetric curves of a sample of coenzyme Q_{10} thermally equilibrated for 3 days at 300 K (A) and 6 days at 310 K (B). Heating and cooling scans indicated by the direction of the arrows; all scans recorded at a rate of 5° min^{-1}. In both experiments the preparation was heated to 330 K prior to cooling to the equilibration temperature.

Other studies of this sample showed no crystallization after 3 days equilibration at 310 K; longer equilibration times were not examined.

The experiment shown in Fig. 4 indicates that coenzyme Q_{10} exists in at least two crystalline forms. The first, designated Q^{C1}, is created by cooling the compound from a liquid state above 320 to 260 K, in which case an exothermic

event appears to accompany the liquid-to-crystalline phase transition. Reheating this crystalline phase transforms it through a complex series of thermotropic events to another crystalline phase designated Q^{C2}. This form also appears to be formed directly from the liquid phase if the rate of cooling is sufficiently slow. The formation of two distinct crystal phases was also indicated in an examination using wide-angle X-ray diffraction methods. The results are presented in Fig. 5. When coenzyme Q_{10} is in the liquid state (Q^L), as expected no sharp X-ray reflections are observed (Fig. 5a) and only a diffuse reflection centered at 0.461 nm can be seen which is typical of a disordered hydrocarbon. If the preparation is cooled rapidly to 275 K to form Q^{C1}, the crystal structure (Fig. 5b) is dominated by two sharp reflections at 0.480 and 0.395 nm with weaker reflections at other spacings. A list of X-ray spacings is presented in Table III. If the preparation is crystallized slowly at 300 K (Fig. 5c), additional sharp reflections are seen at 0.330 and 0.786 nm. This suggests that crystallization at slow rates of cooling produces a structure which is similar to that formed at high cooling rates but has more long-range order in the lattice. If the preparation shown in Fig. 5b is heated to 300 K to produce Q^{C2}, a diffraction pattern identical to that shown in Fig. 5c is obtained. It is possible that the phase transitions seen in the heating thermograms in the temperature region 290–310 K are associated with the creation of a more ordered crystal lattice, i.e., the Q^{C1} to Q^{C2} transition. Figures 5d and 5e show that coenzyme Q_9 also exists in two crystalline states which are formed and transformed in the same manner as coenzyme Q_{10}.

Discussion

Coenzyme Q with polyisoprenoid substituents 7 to 10 units in length have been shown, on the basis of differential scanning calorimetry, to exist in at least three physical states, two crystalline forms and a melt. The temperatures of the transition between the crystalline and liquid state as well as between the two crystalline forms all depend on the number of isoprenoid units attached to the benzoquinone ring. Thus increasing polyisoprenoid chain length results in a shift of all corresponding transitions to greater temperature. Although the melting point appeared to be characteristic of the particular Q homologue, the crystallization temperature and the type of crystal structure formed depended markedly on the thermal history of the sample. Interconversions between the different phases of the higher homologues of coenzyme Q are illustrated in diagrammatic form in Fig. 6. This shows that Q^{C1} can only be formed from Q^L by relatively fast rates of cooling, i.e., 2.5° min^{-1} or greater. We have implied that Q^{C1} is a single crystalline state, but at the highest cooling rate we used (80° min^{-1}) the Q^{C1}/Q^{C2} phase transition was different from that observed at the slower rates of cooling (Fig. 3B), suggesting that Q^{C1} may not be a

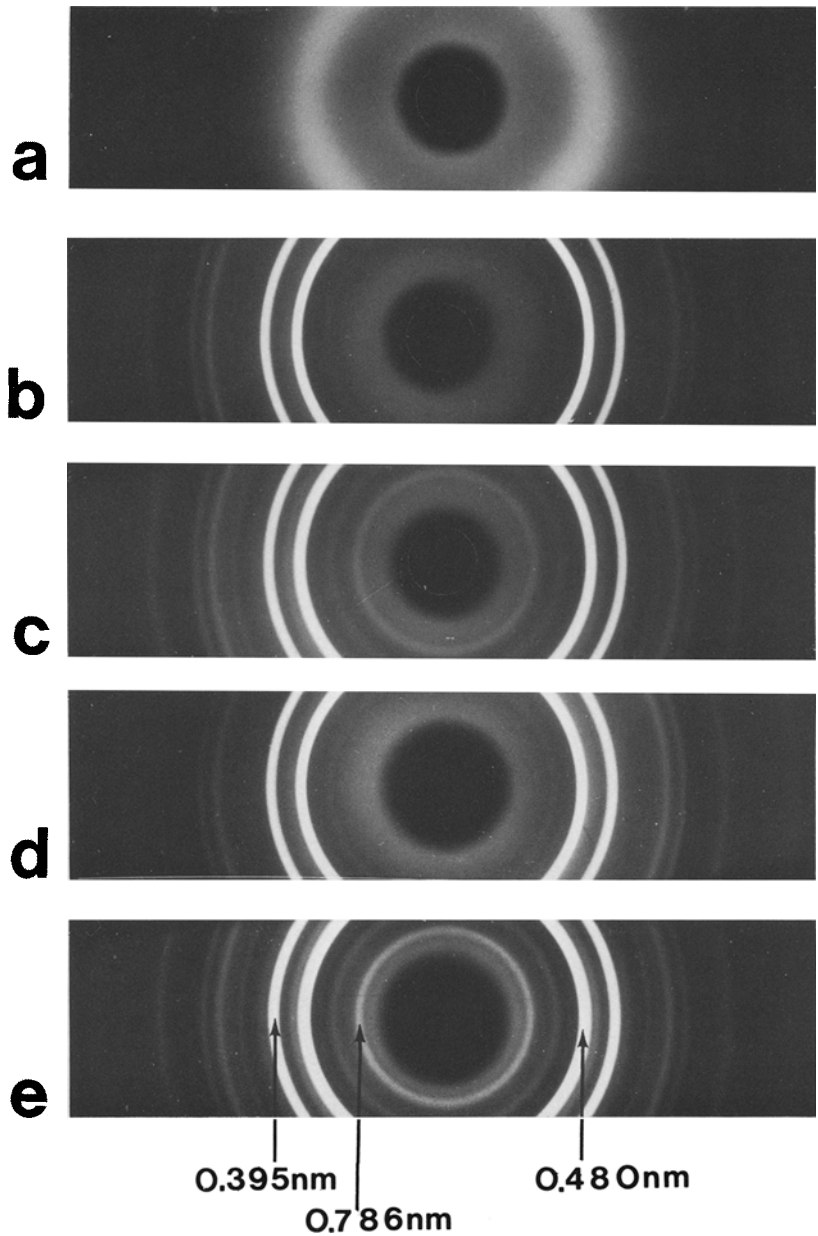

Fig. 5. X-ray diffraction patterns from coenzyme Q_{10} and Q_9. (a) Coenzyme Q_{10} at 320 K; (b) coenzyme Q_{10} cooled rapidly from 320 to 275 K; (c) coenzyme Q_{10} crystallized by cooling slowly from 320 to 300 K; (d) coenzyme Q_9 cooled rapidly from 320 to 270 K; (e) coenzyme Q_9 crystallized by cooling slowly from 320 to 295 K.

Table III. Wide-Angle X-ray Diffraction Spacings of Coenzyme Q_9 and Coenzyme Q_{10} Obtained from Samples Crystallized at Different Cooling Rates

Sample and crystalline state		Diffraction spacing (nm)							
		1	2	3[a]	4[a]	5	6	7	8
Coenzyme	Q_9 (Q^{C1})	—	0.601	0.480	0.395	—	0.303	0.282	0.240
	(Q^{C2})	0.786	0.601	0.480	0.395	0.330	0.303	0.282	0.240
Coenzyme	Q_{10} (Q^{C1})	—	0.601	0.480	0.395	—	0.303	0.282	0.240
	(Q^{C2})	0.786	0.601	0.480	0.395	0.330	0.303	0.282	0.240
	(Q^{L})	0.461							

[a]Two reflections of greatest intensity.

homogeneous structure. The existence of other intermediate crystalline forms is also consistent with the complexity of the thermal events associated with the Q^{C1}/Q^{C2} phase transition. There was no evidence from the conditions examined here that Q^{C1} is transformed directly to the liquid phase, but upon heating the more ordered (lower entropy) structure was formed prior to melting. The formation of other crystalline states is consistent with the modified Q^{C1}/Q^{C2} transition in the thermograms of coenzyme Q_8 and more especially coenzyme Q_7. Furthermore, the crystal structures of these shorter-chain homologues appear to be different particularly with respect to the lattice spacings of 0.5 nm or greater (Rüegg *et al.*, 1959). These authors recognized only one crystal form, probably Q^{C1}, but the reflection at about 0.48 nm that we have shown as a single spacing most likely consists of two closely spaced reflections; there was some evidence for two lines in our preparations that had been exposed only briefly to the X-ray beam. The detailed crystal structures of Q^{C1} and Q^{C2} have yet to be determined; however, one possibility that may be considered is that one form is a ubiquinone and the other a ubichromenol configuration. Such structures have been shown to be interconvertible (Langemann and Isler, 1965). Calculations of Breen (1975) based on molecular orbital theory have

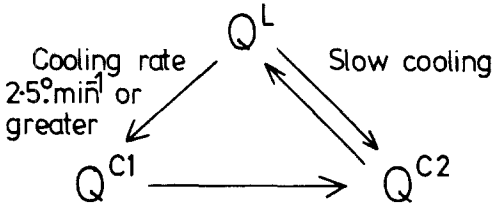

Fig. 6. Diagrammatic representation of the phases of coenzyme Q and their thermal interconversions. Q^L = liquid phase; Q^{C1} = crystalline phase resulting from rapid cooling; Q^{C2} = crystalline phase observed on slow cooling.

indicated that the conformational stability of ubiquinone may be increased from between 60 to 300 kJ $mole^{-1}$, depending on the geometry of the methoxy groups, by the creation of a chromenol configuration in the isoprene unit adjacent to the benzoquinone ring. The structure of the polyisoprene chain also influences the types of motion in this part of the molecule. Thus the molecular configuration of the chain permits only limited flexibility because of the constraints imposed on internal rotation about both single and double bonds. The presence of up to 10 *trans* double bonds at intervals along the chain introduces a periodic rigidity and prevents an increased precessional radius as the terminal end of the side chain is precessed around a proximal single bond. Rotation of the isoprenoid chain about single bonds is also restricted by the vicinal methyl groups which protrude from the chain axis and are subject to steric hindrance.

One factor that has considerable biochemical significance is the fact that, at body temperature (310 K), coenzyme Q_{10} that is phase separated from membrane lipids will tend to assume a crystalline configuration of the Q^{C2} type unless it is stabilized in some micellar form. In the crystalline state it is unlikely that coenzyme Q could act as a mobile carrier of hydrogen atoms as envisaged by the chemiosmotic hypothesis. The possibility of protons and electrons hopping through a crystal of coenzyme Q (Hauska, 1977) cannot be entirely excluded, however, especially if there are mutual orientations of molecules in the crystal. It would seem reasonable to expect that coenzyme Q which is molecularly dispersed in the phospholipid (Katsikas, 1982a) would be the most likely candidate to conduct protons and electrons across the membrane. The nature of this form of coenzyme Q is presently unknown, but it does not appear to melt or crystallize in a manner typified by bulk coenzyme Q, which supports the notion that it is dispersed as monomers sandwiched between leaflets of phospholipid bilayers. Proton NMR studies (unpublished observations), however, indicate that there is a close proximity of the benzoquinone ring substituents in this dispersed coenzyme Q which also undergoes rapid isotropic motion even in gel-phase phospholipid, providing clear evidence for a micellar arrangement of the molecules. Tumbling of a coenzyme Q micelle, which may have dimensions approaching that of the length of a coenzyme Q molecule (approximately 5.4 nm), could provide a mechanism of hydrogen transfer across biological membranes. Whether or not hydrogen translocation can occur by diffusion of coenzymes Q monomers along a central plane of the membrane is problematical and would depend on the equilibrium between crystalline–liquid bulk coenzyme Q and the free form of the coenzyme. Obviously temperature will have a marked effect on this equilibrium, and the implications of this with regard to *in vitro* studies of mitochondrial function in organelles isolated at temperatures below the Q^{L}–Q^{C} phase transition may not have been fully appreciated.

Acknowledgments

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