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Real-Time Characterization of Thermotropic Liquid Crystal Phase Transitions in Coenzyme Q

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The phases and transitions observed for anhydrous coenzyme Q_{10} as a function of temperature were characterized using x-ray diffraction techniques at the Daresbury Synchrotron Laboratory. Four phases consisting of two crystalline bilayers (LC_1 and LC_2), one liquid crystalline bilayer (L_C) and one isotropic liquid (I_L) phase were identified. Hysteresis was observed upon cooling for the I_L to LC_1 transition which resulted in the formation of the mestastable LC_2 and L_C phases. The phase transition from L_C to LC_2 upon heating involves an ordering of the molecular packing within the phase. This disorder to order transition is a representation of the exothermic requirements for the formation of the LC_2 phase. The delay in the observation of the LC_2 phase during the initial cooling from the isotropic liquid is characteristic of a nucleation phenomenon. The influence of these transitions on the phase states of biological membranes is also discussed.

INTRODUCTION

Coenzyme Q has been postulated to act as a mobile energy carrier in the chemiosmotic hypothesis of energy coupling in mitochondria.[1] Reconstitution[2] and kinetic studies[3–6] have indicated that coenzyme Q acts as a redox carrier of the respiratory chain in the inner mitochondrial membrane. The arrangement of coenzyme Q within a phospholipid membrane, however, is not easily deduced since coenzyme Q consists of long polyisoprenoid chains which could span the bilayer if fully extended. A variety of physical studies[7–11] have deduced that coenzyme Q molecules with an isoprenoid chain length of 10 have a low solubility within a phospholipid bilayer, and form aggregates as the proportion of coenzyme Q to phospholipid increases. These aggregates display the typical melting and crystallization behavior of coenzyme Q_{10}.[11] A general model for coenzyme Q_{10}/phospholipid

mixtures would need to infer a phase separation between these species, even within the lipid bilayer. It becomes important to characterize the thermal and structural properties of coenzyme Q_{10} in the uncomplexed state in order to interpret the functionality observed in membranes and the aggregation of coenzyme Q_{10} within the membrane.

It has recently been shown[12] that coenzyme Q is a thermotropic liquid crystal with phase transitions which are extremely dependent on thermal history. When the system is allowed to crystallize before examination, then two phase transitions are observed at 9.7° and 43.8°C leading to an eventual isotropic liquid phase. Supercooling is observed in the transformation from the isotropic liquid structure, which is a clear function of the rate at which heat is extracted. The faster the rate of cooling, the lower the observed phase transition temperature. Wide angle x-ray diffraction patterns have been recorded as the system is cooled. An initial diffraction peak assigned to a disordered subcell packing of 0.46 nm is transformed upon cooling into two diffraction peaks of 0.49 and 0.395 nm, spacings characteristic of an orthorhombic subcell. Additional diffraction peaks at 0.33 and 0.786 nm are observed when the sample was allowed to isothermally equilibrate at 27°C *or* when the sample was reheated to below the transition temperature leading to the isotropic liquid phase.

Time-resolved x-ray diffraction techniques have proven useful in examining systems that are influenced by the thermal history of the sample.[13-15] In this report, we describe results obtained while coenzyme Q was subjected to a variety of heating and cooling cycles, and consecutive x-ray diffraction patterns were recorded. High resolution x-ray diffraction patterns were recorded to enable us to classify the phases as well as to describe the kinetics and mechanisms of the phase transition processes.

MATERIALS AND METHODS

Coenzyme Q_{10} was obtained from Sigma Chemical Company (St. Louis, MO) and used without further purification. This lipid was loaded directly into the x-ray sample holder for examination.

X-ray experiments were carried out using a monochromatic (0.150 nm) focussed x-ray beam at station 7.25 of the Daresbury Synchrotron Laboratory as previously described.[16] A cylindrically bent single crystal of Ge,[17] and a long float glass mirror were used for monochromatization and horizontal focussing providing 2×10^9 photons$\cdot s^{-1}$ down a 0.2 mm collimator at 2.0 GeV and 200–300 mA of electron beam current. A Keele flat plate camera was used with a linear detector constructed at the Daresbury Laboratory. The dead time between data acquisition frames was 50 μsec. X-ray scattering has been plotted as a function of reciprocal spacing, $s = 2 \sin \theta/\lambda$, using teflon (0.48 nm) as a calibration standard.[18] All mesophase and subcell spacings were calculated using Bragg's Law.[19]

Temperature scans and jumps were produced by water baths connected internally to the sample mount of the x-ray camera. The temperature of the sample was monitored internally using a thermocouple placed adjacent to the sample in the

x-ray sample holder. We expect that the thermal diffusion through our samples was approximately the same as that reported by Caffrey[20] for a similar sample thickness of 1 mm.

RESULTS AND DISCUSSION

Examination of the thermotropic liquid crystal phase transitions in anhydrous coenzyme Q_{10}, resulted in x-ray patterns characteristic of the four previously identified phases[12] being obtained. The crystalline phase (LC_1) that was representative of the supplied coenzyme Q_{10} powder was characterized (Figure 1) as a lamellar array

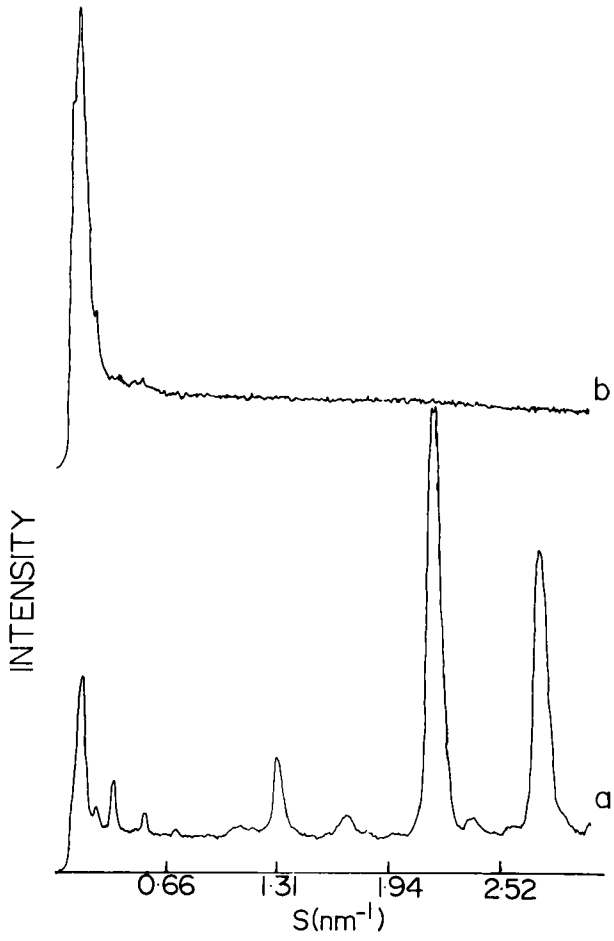


FIGURE 1 X-ray diffraction patterns for coenzyme Q at (a) 3°C and (b) 60°C. Patterns were collected over 100 s.

with four orders of a 6.27 nm spacing, and five wide angle scattering peaks at 0.76, 0.59, 0.46, 0.42 and 0.37 nm. This phase was similar to that previously reported[12] upon reheating a sample cooled to below the isotropic liquid phase, or by isothermal equilibration at 27°C. The isotropic liquid phase (I_L) was characterized (Figure 1) by a single broad small angle scattering peak which could be indexed to 7.07 nm and a broad wide angle scattering peak which was inferred to be indexed at 0.461 nm while the liquid crystalline phase (L_C) observed upon cooling from the isotropic liquid was characterized (Figure 3 insert) by the same small angle and wide angle peaks first observed in the preceding liquid crystalline phase except that the 0.46 nm peak has increased dramatically in intensity.

The structural changes that occur upon heating powdered/crystalline coenzyme Q_{10} at $10°C \cdot min^{-1}$ were determined from the three-dimensional plot of the diffraction patterns beginning at ~8°C and presented as a function of temperature in Figure 2. A phase transition was observed at ~50°C which consists of a transformation from the initial LC_1 crystalline phase to the I_L isotropic liquid phase. The transition was described by a decrease in intensity of the LC_1 diffraction pattern until the I_L diffraction pattern is observed. We could not distinguish if the process involves the coexistence of the two phases because of the relatively large differences in the diffraction intensities in the two phases. Upon subsequent cooling of the sample, a phase transition was indicated at ~4°C which consists of a transformation from the isotropic liquid to a liquid crystalline phase. Although additional wide angle scattering peaks are observed for the L_C phase, the mesophase phase structure is the same as that for the isotropic liquid. It was thus concluded that the structure characterized by the single small angle scattering peak at 7.07 nm is stable for long periods after being induced by the transformation to the isotropic liquid. Previous studies[12] had shown that the phase structure represented by the wide angle scattering peaks of the LC_1 phase was eventually induced, but did not have the low angle scattering resolution to determine if the mesophase structure returns to the LC_1 phase with unit cell spacing of ~6.0 nm.

The sample was then reheated from 6°C at $10°C \cdot min^{-1}$ with diffraction patterns being recorded continuously during the temperature change. Two phase transitions were observed as determined from changes in the character of the recorded diffraction patterns at ~37° and ~43°C. The first transition involved the transformation from the L_C to LC_2 phase, while the second transition involved the transformation from the LC_2 to I_L phase. This transition sequence was unusual in that the transformation from the L_C to the LC_2 phase involved an ordering of the molecular packing; this was recorded as a dramatic increase in the intensity of the 0.46 nm diffraction peak. The LC_2 to I_L transition represents the usual first order, or order–disorder type of transition. A previous study[12] had shown that the LC_2 phase eventually transformed to the LC_1 phase if allowed to equilibrate isothermically at 27°C. One can speculate that the reordering in the L_C to LC_2 transition involves a thermally induced process during which the supercooled L_C phase requires additional energy in order to facilitate the nucleation of the LC_2 phase in the isotropic mesophase (first observed when coenzyme Q_{10} was transformed into a liquid). Under longer equilibrium times, it would be expected that the LC_2 phase could be directly nucleated from the I_L phase.

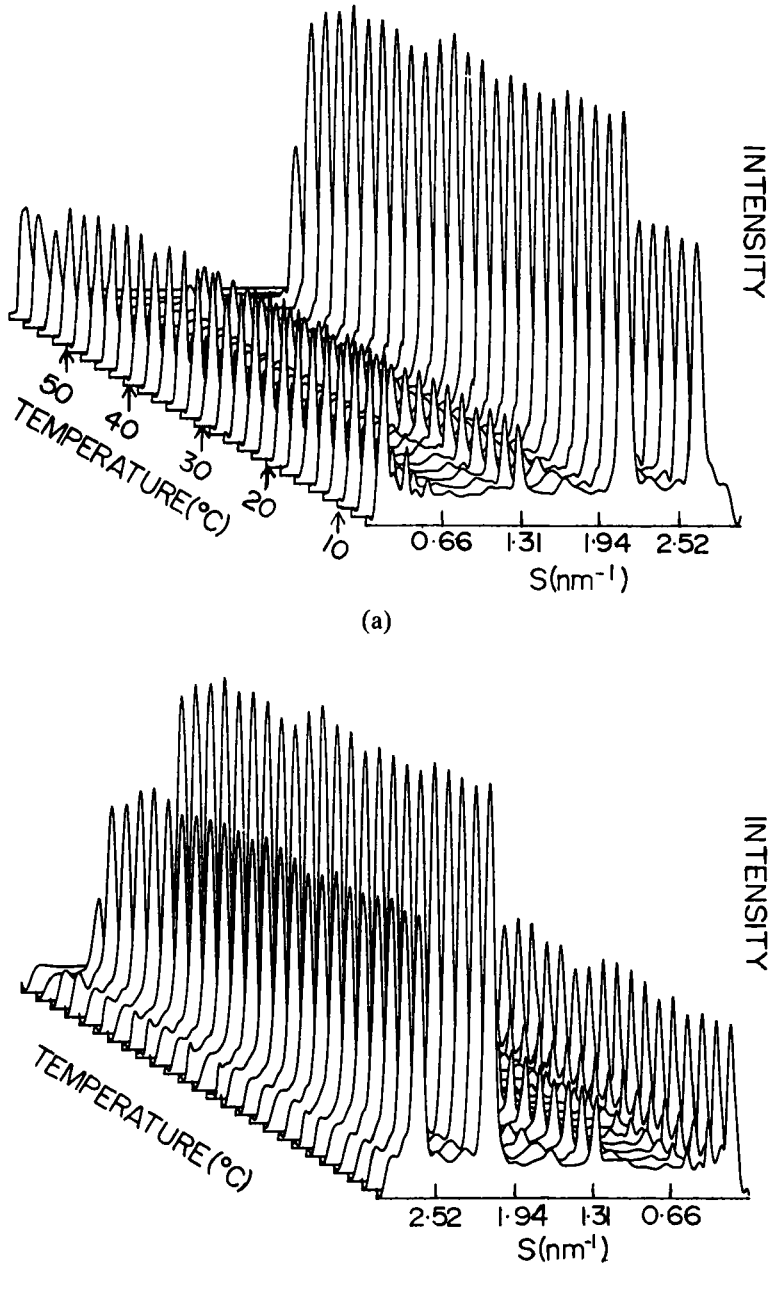


FIGURE 2 Three-dimensional plot of x-ray scattering intensity versus reciprocal spacing as a function of temperature for anhydrous coenzyme Q. The sample was heated from 0°C at a rate of 10°C·min^{-1}. Diffraction patterns were accumulated over 1.2 s. Every tenth frame from the total data set of 255 frames is shown. Two perspectives of this plot are shown.

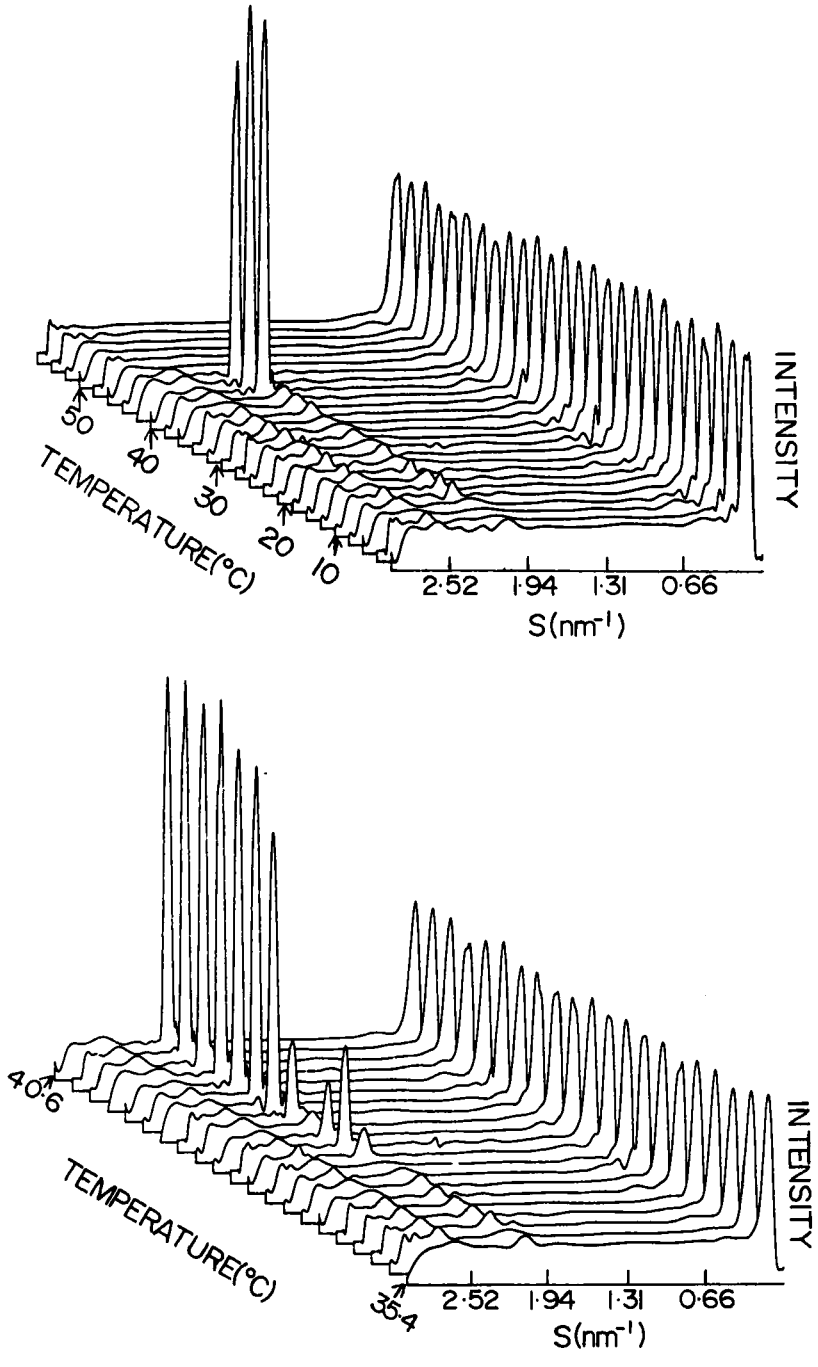
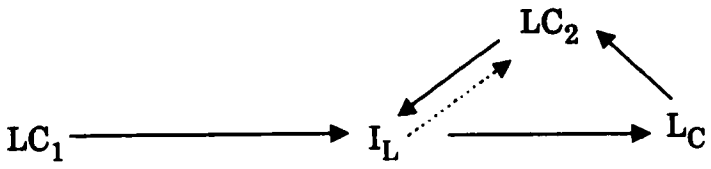


FIGURE 3 Three-dimensional plot of x-ray scattering intensity versus reciprocal spacing as a function of temperature for anhydrous coenzyme Q. The sample was re-heated from 6°C at a rate of 10°C·min^{-1}. Diffraction patterns were accumulated over 1.2 s. Every tenth frame from the total data set of 255 frames is shown. Insert: Plot of representative diffraction peaks for anhydrous coenzyme Q during re-heating from the supercooled isotropic liquid crystal phase to the isotropic liquid phase.

CONCLUSIONS

The following phase transition sequence can be deduced from the above results for the thermotropic liquid crystal phase transitions in coenzyme Q:



The appearance of the metastable supercooled L_C and LC_2 phases can be related to the time required for thermal energy to rearrange molecular packing within the mesophase array thereby allowing the crystalline phase to form. One could speculate that if sufficiently long times were allowed for equilibrium to occur as one cools from the I_L phase, then the crystalline phases could form without the appearance of the L_C phase. In either situation, the transition sequence for anhydrous coenzyme Q_{10} is hysteretic. Speculation on the relationship of the transition sequence and the function of coenzyme Q within a membrane would need to focus on the metastable, supercooled L_C phase and its influence on the packing of the acyl chains in the membrane. The transformation of coenzyme Q_{10} to the I_L phase would clearly disrupt the order and packing within a phospholipid multi-lamellar array if it partitioned into the acyl chain region of the bilayer.

The effect of 25 mol% coenzyme Q_{10} on the dipalmitoylphosphatidylcholine bilayer structures and transitions was subsequently examined using resolved x-ray diffraction. Above ~60°C, the x-ray patterns from the DPPC/coenzyme Q_{10} bilayers became very broad. This was indicative of the mesophase structural disorder caused by the presence of the isotropic liquid phase of coenzyme Q_{10}. The mesophase d-spacing of the bilayer at ~70°C was found to be ~7.1 nm which was significantly greater than that usually observed for fully hydrated DPPC bilayers in the L_α phase thus indicating changes in the interbilayer equilibrium. It can be inferred that coenzyme Q must reside to some extent within the hydrocarbon chain region of the bilayer in order to cause disruption of the interbilayer repeat. It is less likely that portions of Q_{10} reside at the bilayer interface but rather produce a secondary change in the interbilayer separation due to an expansion of the area per lipid head groups at the bilayer interface.

In general, the real-time or non-equilibrium phenomenon described in this report can be related to previously reported static or equilibrium phase characterizations[11,12] of this system. However, it cannot be expected that this will occur in all systems that may be studied. Indeed, we have previously reported a difference in the real time versus equilibrium phase sequence for dipalmitoylphosphatidylcholine in water.[15] This "difference" was used to deduce how the equilibrium phase formed and how long was required for the process to occur. Real time structural characterizations are also useful if comparisons are to be made to other real time measurements, such as scanning calorimetry, or if processes involving such changes are proposed for biological processes. It is well known from thermodynamics that it is more

likely for rapid changes in a system to occur in a non-equilibrium manner rather than as a collection of equilibrium phases.

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