Two-state and continuous phase transitions in lipid bilayers A time-resolved X-ray diffraction study

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Structural changes associated with transition from one phase to another have been examined in several lipid–water systems using time-resolved X-ray diffraction methods. Two types of transition mechanism can be recognized on the basis of scattering originating from the packing of the hydrocarbon chains. Two-state transitions are characterized by coexistence of the wide-angle scattering patterns of the initial and final phases throughout the transition region. Continuous transitions, on the other hand, take place through a series of intermediate states that are believed to arise from deformation of the hydrocarbon chain lattice as one phase transforms into another. Two-state processes are observed as subgel to liquid crystal transitions, and continuous transformations are typical of subgel to gel phase transitions. Examples of these transition types are presented and other transitions that do not appear to conform to either of these mechanisms are described.

1. Introduction

Many surfactants and lipids form bilayer phases in aqueous systems. These phases are characterized by orientation of the molecules in which the polar groups reside at the aqueous interface and the hydrocarbon chains extend into the interior of the structure from which water is excluded. A number of discrete bilayer phases can be distinguished from the mobility and order of the hydrocarbon chains [1–5]. Although molecular motion and order can be readily established under conditions of constant temperature, pressure, water concentration, etc., using conventional spectroscopic and diffraction methods, changes in these parameters during the transition from one phase to another is more difficult to access. Time-resolved X-ray diffraction methods which exploit the extreme brightness of synchrotron X-radiation have been used to examine the structural changes associated with different phase transitions [6–11]. Here we review data obtained from wide-angle diffraction measurements of some lipid–water systems and are able to discern different types of transition mechanism.

The hydrocarbon chains of many polar lipids that form a lamellar phase in aqueous systems exist in at least three types of packing arrangement. Equilibration of many phospholipids at low temperatures, for example, results in the formation of stable crystalline subgel bilayer phases (L_c). This type of chain packing gives rise to characteristic X-ray diffraction maxima consisting of at least two prominent wide-angle reflections [5, 12]. In the gel phase the hydrocarbon chains are arranged in an hexagonal subcell; the wide-angle diffraction pattern consists of a single symmetrical

Bragg reflection when the chains are perpendicular to the bilayer phase (L_β) and with a shoulder when they are inclined with respect to this plane ($L_{\beta'}$) [5]. A variant of the gel phase specific for dialkylphosphatidylcholines is the pretransition phase designated $P_{\beta'}$ in which the bilayers take on a rippled structure [5, 12]. Heating either crystalline or gel phases results in transformation to equilibrium liquid-crystalline phases characterized by a single broad diffraction band at 0·44–0·46 nm and arising from disordered or 'melted' chains [5]. A time-resolved examination of the changes in these typical wide-angle X-ray diffraction patterns during transition from one phase to another can provide information on the transition mechanism.

2.   Subgel to gel phase transitions

Transition from L_c to L_β phase is exemplified by the subtransition of L-dipalmitoylphosphatidylcholine at 18–19°C [13–15]. The formation of the L_c phase according to X-ray [15, 16], dilatometry [14] and calorimetric [13] measurements is accomplished after storage of the L_β phase for at least 3–4 days at about 0°C. The subtransition ($L_c \rightarrow L_\beta$), by contrast, has a much shorter transition time and is readily detected as an endothermic event in differential scanning calorimetry. The changes in acyl chain packing during the subtransition can be seen from the representative wide-angle X-ray scattering intensities recorded at 1·2 s intervals during a heating temperature scan at 5°C min^{-1} shown in figure 1 (a). Although the sharp spacing at 0·430 nm does not change in position but decreases progressively in intensity throughout the transition, the continuous shift to higher spacings of the broad maximum at 0·375 nm indicates a gradual expansion of the chain lattice in one direction until a hexagonal packing is achieved where the molecules are able to assume rotational disorder [8]. The same mechanism of transition was observed when the rate of change of temperature through the transition was increased to 6°C s^{-1}. This mechanism is consistent with the observed deformations that results from heating orthorhombic subcells where an expansion in one dimension is accompanied by a constant or slightly decreasing other dimensions of the unit cell until a hexagonal packing is reached [17].

Another example of a subtransition is that of aqueous dispersions of a dipalmitoylphosphatidylcholine : palmitic acid, 1 : 2 molar ratio mixture. The changes observed in the wide-angle X-ray diffraction patterns recorded during heating through this transition at 38°C at a rate of 5°C min^{-1} are shown in figure 1 (b). As in the case of the subtransition in L-dipalmitoylphosphatidylcholine, the subtransition in the dipalmitoylphosphatidylcholine : palmitic acid mixture can be characterized as a continuous process with a progressive conversion of the unit cell type of the L_c phase into that of the L_β phase through a series of intermediate states [9]. These states possess wide-angle diffraction spacings that are intermediate between those of the initial and final phases.

3.   Subgel to liquid-crystal phase transitions

A completely different transition mechanism is observed in the transformation of lamellar crystal phases (L_c) to liquid crystal (α) phases. Two examples are illustrated in figure 2 and comprise aqueous dispersions of dipalmitoylphosphatidylethanolamines and palmitic acid. Unlike the unsaturated phosphatidylcholines, there are no recognizable L_β phase intermediates between the L_c and the α-phases of these lipids [9, 18–21]. Changes in the wide-angle X-ray scattering region of an aqueous dispersion of L-dipalmitoylphosphatidylethanolamine equilibrated in the L_c phase during heating
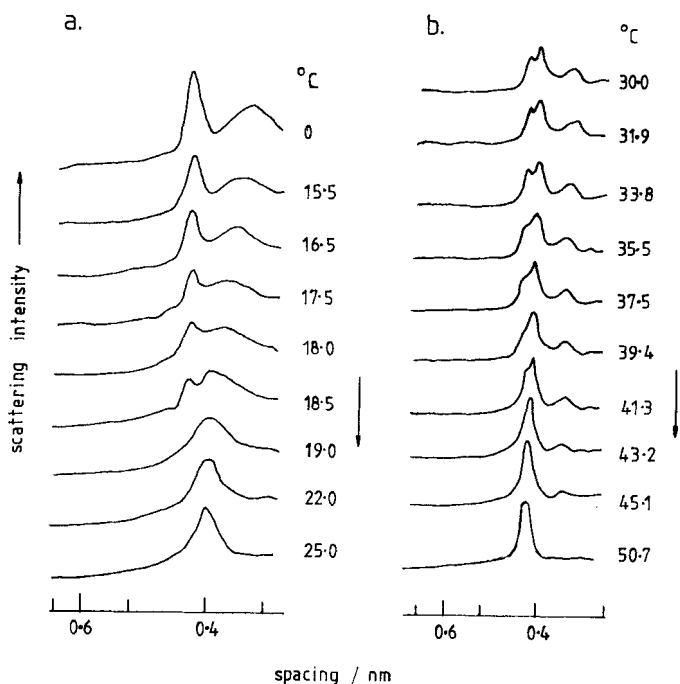
Figure 1. Selected wide-angle scattering profiles during heating at 5°C min^{-1} of (*a*) L-dipal-mitoylphosphatidylcholine dispersed in 50 mM HEPES buffer (pH 7·2) (25 per cent (w/v)) equilibrated for 4 days at 0°C [8] and (*b*) dipalmitoylphosphatidylcholine : palmitic acid (1 : 2 molar ratio) dispersed in water (20 per cent (w/v)) [9]. The scattering intensities in 1·2 and 2·4 s, respectively, are shown at the average temperature at which the patterns were recorded. The experiments were performed on the Synchrotron Radiation Source at the S.E.R.C. Daresbury Laboratory, and details of the methods used have been published elsewhere [7, 23].

through the L_c–L_α phase transitions is shown in figure 2 (*a*) [10]. The diffraction pattern recorded at 49·5°C is typical of the L_c phase and consists of two intense and at least two weaker scattering maxima. Heating the dispersion through the phase transition temperature (66°C) shows a synchronous monotonic decrease in intensity of all the wide-angle diffraction peaks indexing the L_c phase until they finally disappear; there is no apparent broadening or shift in the spacing of these maxima during the transition. At the same time there is an increase in intensity of the broad scattering band which characterizes the liquid-crystalline phase. There was no evidence for structures intermediate between the L_c and the L_α phases. The experiment was repeated with an aqueous dispersion of DL-dipalmitoylphospha-tidylethanolamine under identical conditions and the same changes in diffraction patterns were recorded; the transition in this case was observed at a temperature of 80°C [22].

Changes in the wide-angle diffraction patterns recorded during heating of a sample of palmitic acid in excess water are shown in figure 2 (*b*) [9]. The low temperature equilibrium phase is a typical L_c phase with sharp wide-angle reflections from the packing of the fatty acid chains. The intensity of these diffraction peaks decreases during the phase transition with no change in position, whereas the broad diffuse diffraction band centred at 0·46 nm shows a corresponding increase in intensity.
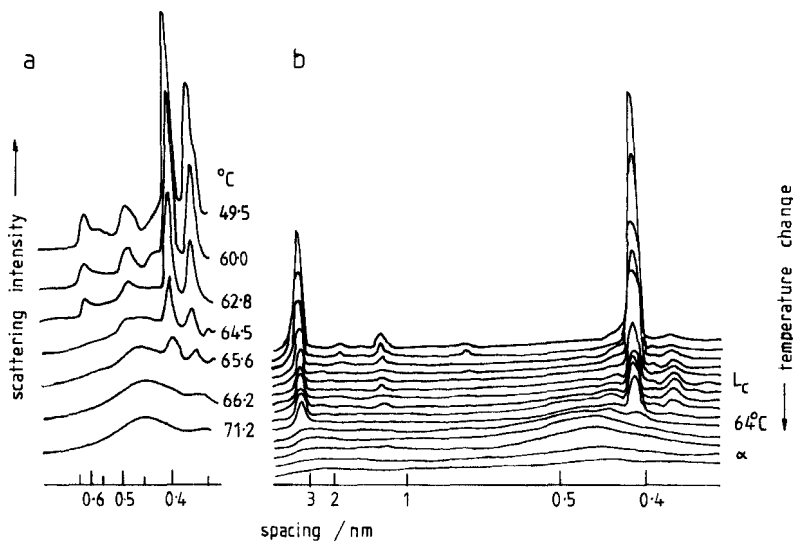
Figure 2. Selected wide-angle scattering profiles recorded during heating of (*a*) L-dipal-
mitoylphosphatidylethanolamine dispersed in 50 mM borate buffer (pH 8) (25 per cent
(w/v)) equilibrated in the L_c phase at a rate of 10°C min^{-1} [10] and (*b*) palmitic acid
dispersed in water (20 per cent (w/v)) through the melting phase transition at 5°C min^{-1}
[9]. Scattering intensity recorded at 3·5 s at average temperatures shown in (*a*) and
successive 2·4 s accumulations are shown in (*b*). Details of the experiment are the same
as those in figure 1.

Low-angle scattering is consistent with a transition from an L_c to an isotropic phase
with concomitant chain melting.

The changes observed during the transition from L_c to α-phases in dipal-
mitoylphosphatidylethanolamines and palmitic acid imply a two-state mechanism in
which there is coexistence of the initial and final phases during the transition and
direct conversion of the former into the latter. Intermediate structures, if formed,
could not be detected at the resolution of the method.

Time-resolved X-ray experiments summarized here have all been performed at heat-
ing rates of at leat 5°C min^{-1}. At such relatively high heating rates the systems studied
would be driven out of equilibrium during the phase transformations and the possibility
that the observed structural rearrangements have been influenced by kinetic effects
cannot be excluded. Clearly more studies are required to assess the importance of
kinetic factors due to slow response times and to verify the validity of the proposed
structural classification of the phase transitions in lipid bilayers. Similarly, a number
of other transitions have been observed which do not appear to conform to either two-
state or continuous transitions, but instead combine features of both types of transition.
These include lamellar gel to lamellar liquid-crystalline transitions of dipalmitoyl-
phosphatidylethanolamine and freeze-dried dispersions of L-dipalmitoylphosphatidyl-
choline and trehalose [11]. Whether these transitions are truly of an intermediate type
or can be categorized into either two-state or continuous transitions requires more
detailed studies such as observations under quasistatic heating conditions.

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