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Phase behaviour of membrane lipids containing polyenoic acyl chains

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The low-temperature thermal behaviour of di-18:2 phosphatidylethanolamine (di-18:2 PE) is shown to be characterised by similar broad low-enthalpy transitions to those previously reported for polyenoic samples of phosphatidylcholines (Keough and Kariel (1987) Biochim. Biophys. Acta 902, 11–18), and monogalactosyldiacylglycerol (Sanderson and Williams (1992) Biochim. Biophys. Acta. 1107, 77–85). Real-time X-ray diffraction measurements indicate that these transitions correspond to transitions between the gel (L_β) and liquid-crystal (L_α) phases of the lipids. The gel phase of these lipids is, however, much more loosely packed than the corresponding phases of membrane lipids containing monoenoic or fully-saturated acyl chains. The low enthalpy and reduced co-operativity of the $L_\alpha \rightarrow L_\beta$ phase transitions of the polyenoic lipids is attributed to the reduced contribution of van der Waals interactions between their acyl chains in the gel-state of these lipids. Comparison with the earlier results obtained for MGDG suggest that the acyl chains of polyenoic lipids can form well-ordered lattices but require the additional energy input associated with the formation of a hydrogen bond network between the lipid headgroups in order to do so.

Introduction

Despite the fact that most lipids found in biological membranes are characterised by the presence of at least one polyunsaturated acyl chain, most studies on the phase behaviour of membrane lipids are performed on lipids containing fully saturated, or monoenoic, acyl chains. It has been recognised for some time that the introduction of the first double bond into the acyl chains of a membrane lipid leads to much more marked changes in its physical properties than the introduction of subsequent double bonds [1]. The limited availability, and the susceptibility to oxidation, of lipids containing polyenoic acyl chains has, however, meant that few studies on the phase behaviour of such lipids have been carried out.

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Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; L_α, liquid-crystal lamellar phase; L_β, gel lamellar phase; H_{II}, inverted hexagonal phase; L_c, crystal (sub-gel) lamellar phase.

The first detailed studies of the phase characteristics of related series of membrane lipids containing polyenoic acyl chains were performed by Keough and his collaborators [2,3]. They studied the phase behaviour of series of polyunsaturated hetero-acid phosphatidylcholines containing a saturated fatty acid in the *sn*-1 position and an unsaturated fatty acid in the *sn*-2 position. They showed that the introduction of a single double bond had a very much more marked effect on the thermal properties of the lipid than the introduction of additional double bonds. This work was subsequently extended to phosphatidylcholines containing two polyenoic chains [4,5]. The thermal behaviour of these latter lipids were found to be characterised by extremely broad, low enthalpy transitions, the temperatures of which again were only slightly influenced by the introduction of additional double bonds.

We have recently reported similar broad, weakly co-operative phase transitions for the chloroplast lipid monogalactosyldiacylglycerol (MGDG) [6], suggesting that these effects are not restricted to the phosphatidylcholines but are a general feature of polyenoic membrane lipids. Given the prevalence of such lipids in biological membranes (particularly those of plants,

fish and micro-organisms), it is clearly of interest to obtain a more detailed understanding of the ways in which their phase behaviour differs from that of the more saturated lipids commonly used in model systems.

In this investigation we report the results of an X-ray diffraction study of the low-temperature phase behaviour of di-18:2 PE, di-18:2 PC and the poly-unsaturated MGDG and DGDG of higher plant chloroplasts. These lipids, with the possible exception of DGDG which showed no detectable thermal transition, are characterised by low enthalpy, weakly co-operative transitions. Wide-angle X-ray diffraction measurements indicated that these transitions correspond to transitions between the liquid-crystal lamellar phase (L_α) and partially-ordered lamellar gel phases (L_β). The acyl chains of the polyenoics appear to be unable to pack into ordered arrays of the type characteristic of the gel-phases of lipids containing saturated and/or monoenoic acyl chains. Measurements performed on MGDG, which has the ability to form a well-ordered sub-gel or crystal phases (L_c), suggest that this lack of packing ability reflects the reduced contribution of van der Waals forces between neighbouring lipid chains rather than an inherent inability of polyenoic chains to organise into regular arrays.

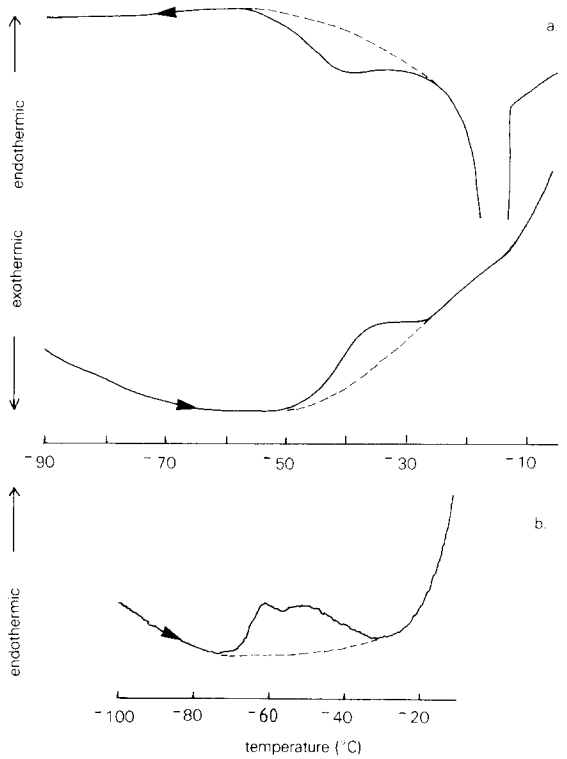

Fig. 1. Thermograms showing the low-temperature endotherms and exotherms of frozen dispersions of di-18:2 PC and di-18:2 PE. (a) heating and cooling thermograms for di-18:2 PE, (b) heating thermogram for di-18:2 PC.

Materials and Methods

Synthetic di-18:1 PE, di-18:2 PE, di-18:2 PC were purchased from Avanti (Alabaster, AL, USA). MGDG and DGDG, isolated from the leaves of *Vicia faba* (broad bean), were purchased from Lipid Products (Redhill, UK). Lipids were stored either in the dry form or in chloroform/ methanol (2:1, v/v) under nitrogen at −70°C. In the case of MGDG and DGDG, gas chromatography and TLC measurements were performed as described elsewhere [7,8]. The acyl chain compositions of the lipids are presented in Table I. Both lipids showed only a single spot when subjected to TLC. Lipid extracted from the TLC plates yielded identical thermograms to those of the lipid as purchased. The purchased material was, therefore, used without further purification.

Differential scanning calorimetry (DSC). Samples were prepared as described in the previous paper [9].

TABLE I

Fatty acid analyses of the MGDG and DGDG samples used in this study

	Fatty acid (weight%)				
	16:0	18:0	18:1	18:2	18:3
MGDG	5	4	2	5	84
DGDG	12	5	3	4	76

Care was taken to avoid lipid oxidation by hydrating with N_2-saturated water and limiting the exposure of the lipid to air wherever possible. Control TLC measurements showed no appreciable degradation of the lipids during the course of the measurements.

X-ray diffraction. Real-time X-ray diffraction was conducted at station 8.2 of the Daresbury Synchrotron Radiation Source as described in the previous paper [9].

Results

DSC measurements

Typical low-temperature heating thermograms for di-18:2 PC, di-18:2 PE are presented in Fig. 1. Both lipids are characterised by very broad, low-enthalpy endotherms. The endotherm for the di-18:2 PC sample appears to span a temperature range from about −70°C to about −30°C and to have a molar enthalpy value of about 0.8 kcal/mol. These values are in reasonable agreement with those previously reported for this lipid by Keough and his co-workers [4,5]. The corresponding temperature range for di-18:2 PE low-temperature endotherm is from about −52°C to −25°C and the molar enthalpy is about 0.4 kcal/mol. The corresponding values obtained on cooling (shown for di-18:2 PE only) were very similar emphasising the

fact that the breadth of the transitions reflects their lack of co-operativity rather than the occurrence of hysteresis.

X-ray diffraction studies, as detailed in the previous paper [9], indicate that di-18:2 PE normally undergoes an $H_{II} \rightarrow L_{\alpha}$ transition between $-11°C$ and $-16°C$ on cooling and a $L_{\alpha} \rightarrow H_{II}$ transition on heating at about $-2°C$. No endotherms or exotherms corresponding to these transitions were observed in the DSC traces; probably because they were obscured by the prominent ice-peaks associated with the freezing of supercooled water and the melting of ice.

Similar DSC measurements were attempted using polyenoic DGDG isolated from broad bean leaves but no measurable endotherms or exotherms were identified. The corresponding measurements for MGDG have been reported in detail elsewhere [6]. Their main features are that on cooling the lipid undergoes a broad transition between about $-15°C$ and $-40°C$ with an ethalpy of about 1.0 kcal/mol. In the case of MGDG this is associated with an $H_{II} \rightarrow L_{\beta}$ rather than a $L_{\alpha} \rightarrow L_{\beta}$ transition. On re-heating, the lipid transforms via an exothermic transition centred at about $-5°C$, into a L_c phase which subsequently converts into an H_{II} phase at above approximately $-2°C$.

X-ray diffraction

Real-time X-ray diffraction measurements were used to investigate the origin of the thermal changes reported above in more detail. Small-angle diffraction measurements (not shown) confirmed that both the di-18:2 PE and di-18:2 PC formed lamellar phases at low temperatures. Small changes in *d*-spacing associated with the melting and freezing of ice at sub-zero temperatures, as discussed in the previous paper, were observed at temperatures above about $-30°C$ [9]. However, no significant changes in *d*-spacing were observed in the low-temperature region thought to be associated with $L_{\alpha} \rightarrow L_{\beta}$ transitions.

A series of selected frames showing the changes occurring in the wide-angle diffraction region of di-18:2 PE are presented in Fig. 2. Above about $-30°C$, the patterns show only the broad maximum centred at about 0.46 nm typical of lipids in the liquid-crystal state. Careful examination of the diffraction patterns reveals a slow shift in the position of the broad diffraction peak associated with the lipid chains to a value of about 0.435 nm on cooling below this temperature. The shift is gradual and the final peak is still much broader than the comparable peak associated with the formation of the L_{β} phase of lipids containing only fully-saturated and/or monoeoic chains. Nevertheless, there appears to be little doubt that a reorganisation of the lipid chains to an at least partially-ordered state is occurring.

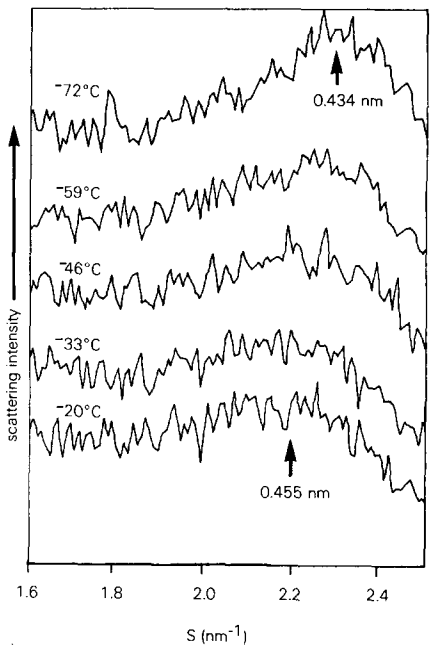

Fig. 2. A typical set of wide-angle diffraction patterns for di-18:2 PE samples showing the changes associated with the partial ordering of the lipid chains.

To emphasise this point, typical wide-angle diffraction patterns obtained for di-18:0, di-18:1 and di-18:2 PE in the gel phase measured using the same X-ray camera configuration are presented in Fig. 3. Even

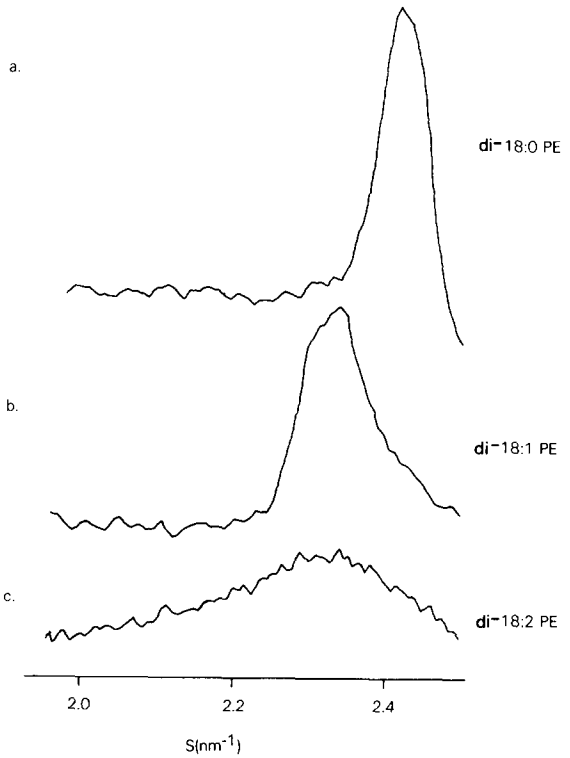

Fig. 3. Comparison of the wide-angle diffraction maxima associated with the acyl chain packing of (a) di-18:0 PE (b) di-18:1 PE and (c) di-18:2 PE. Measurements were performed at 50°C, $-25°C$ and $-70°C$, respectively.

allowing for the fact that the measurements were carried out at different temperatures, there is a clear shift in the position, and a broadening, of the wide-angle diffraction maximum on going from di-18:0 PE to di-18:1 PE. The broadening effect is even more marked in the case of di-18:2 PE and it becomes extremely difficult to identify a true diffraction maximum.

Similar wide angle data (not shown) were obtained for di-18:2 PC and the unsaturated DGDG sample. It is difficult to determine the precise temperature ranges over which these wide-angle diffraction changes occur but in the cases of di-18:2 PE and di-18:2 PC they do seem to coincide reasonably well with those of the low-enthalpy transitions shown in Fig. 1.

Typical diffraction spectra of MGDG collected for lipid in the liquid-crystal phase (H_{II}), the putative L_β phase and the L_c phase are presented in Fig. 4. Again there is a small but significant decrease in the breadth of the wide-angle diffraction maximum on going from the liquid-crystal to the 'gel' state. The lipid chains in the low-temperature state are, however, clearly still only at best partially organised. The maxima at 0.39 and 0.37 nm, it should be emphasised correspond to the two most prominent of the three peaks associated with hexagonal ice [10].

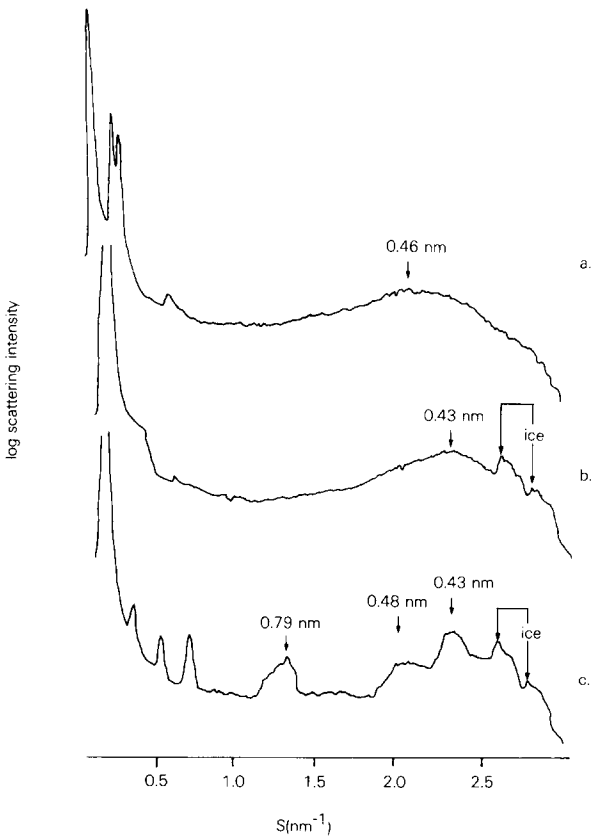

Fig. 4. Typical X-ray diffraction patterns of MGDG dispersions showing the differences in the wide-angle diffraction region of lipid in the (a) liquid-crystal (H_{II}) phase, (b) the gel lamellar phase (L_β) and (c) the crystalline sub-gel phase (L_c). The patterns were measured at 20°C, −40°C and −30°C, respectively.

TABLE II

Collected values for the onset temperatures, the transition span and the molar enthalpy values for the $L_\alpha \rightarrow L_\beta$ transitions for the polyenoic lipids studied in this and earlier studies and the corresponding data for other relevant di-monenoic and fully saturated lipids

Lipid	T_m (°C) *	Span (°C) **	$-H$ (kcal/mol)
Di-18:0 PC	58 [a]	3	10.7
Di-18:1 PC	−22 [a]	5	7.6
Di-18:2 PC	−73	42	0.8
	−70 [b]	32–29	1.4
	−70 [c]	28	1–3
Di-18:3 PC	−73 [c]	30–40	1
Di-18:0 PE	74 [d]	3	9.6
Di-18:1 PE			
(water)	−10	2	6.1
(ice)	−6.5	2	8.5
Di-18:2 PE	−52	27	0.4

* Values taken from heating thermograms.
** Ranges refer to combined values from different samples.
[a,b,c,d] Values taken from Refs. 15, 4, 5 and 16, respectively.

The lipid chains of the L_c phase, in contrast, are well organised giving rise to clearly identifiable maxima at spacings of 0.48 and 0.43 nm. The diffraction peak centred at 0.79 nm is thought to arise from an ordering of the galactose headgroups brought about by the mutual hydrogen bonding of the sugars [11].

Discussion

The results reported in this investigation confirm and extend earlier reports [1–4] that the phase behaviour of lipids containing polyenoic acyl chains is significantly different from that of lipids containing fully-saturated or monoenoic chains.

A summary of the characteristic temperatures, the temperature span and molar enthalpy values for the $L_\alpha \rightarrow L_\beta$ transitions of the lipids studied in this investigation is presented in Table II. Values obtained in earlier investigations, and the corresponding values for relevant monoenoic and fully-saturated lipids, are included for comparison. Two important points emerge from this comparison. Firstly, the results confirm that the relatively small effect of adding a second (or a third) double bond into the acyl chains of the lipids on the onset temperatures of the $L_\alpha \rightarrow L_\beta$ transitions is a general feature of membrane lipids. Secondly, they emphasise the large difference in temperature spans and enthalpy values between the polyenoic lipids on the one hand and the corresponding values for di-monoenoic and fully-saturated lipids on the other.

The low enthalpy and lack of co-operativity of the $L_\alpha \rightarrow L_\beta$ transitions of polyenoic lipids is clearly not a

simple reflection of the fact that they take place at extremely low temperatures. Van Dijk et al. [12] have shown that the $L_\alpha \rightarrow L_\beta$ transitions of lipids such as di-16:1 PC ($T_c = -36°C$) are similar in terms of peak half-width and enthalpy to transitions in lipids containing fully-saturated or monoeoic chains taking place at much higher temperatures.

The measurements of $L_\alpha \rightarrow L_\beta$ transitions in polyenoic lipids reported here were all made in frozen samples. Comparative measurements of $L_\alpha \rightarrow L_\beta$ transitions occurring in supercooled and frozen dispersions of di-18:1 PE, reported in the previous paper [9], revealed that the presence of ice raised the transition temperature from $-10°C$ to $-6.5°C$ and increased its molar enthalpy from 6.1 to 8.5 kcal/mol but had little effect on the co-operativity of the transition. These effects were attributed to changes in lipid hydration. The amount of unfrozen water present in lipid dispersions decreases with decreasing temperature [7,13,14] and this will also undoubtedly influence the energetics of lipid phase transitions.

Keough and his co-workers [2–5] have suggested that the difference between the polyenoic lipids and the di-monenoic lipids lies in the relative ability of their acyl chains to pack in the ordered state characteristic of the gel state. The presence of two (or more) *cis*-double bonds in these chains is clearly likely to influence their ease of packing. The wide-angle X-ray diffraction data presented in Figs. 2 and 3 strongly confirm that the main difference between the polyenoic lipids and their fully saturated and di-monoenoic counterparts lies in the limited ability of the polyenoic lipids to form highly-ordered gel phases. This decrease in packing density inevitably leads to decreased van der Waal interactions between the chains which could account for the low enthalpy values associated with their $L_\alpha \rightarrow L_\beta$ transitions. It must be emphasised that the acyl chains of polyenoic lipids are inherently capable of packing into ordered arrays under appropriate circumstances. Efficient packing of the acyl chains in polyenoic lipids is possible as demonstrated in the diffraction pattern for the L_c phase of MGDG presented in Fig. 4. It seems, however, that the extra driving force provided by headgroup interactions is required to bring about the necessary acyl chain reorganisation. This view is supported by the fact that the diffraction peak centred at 0.79 nm associated with the ordering of the galactose units is only seen in the diffraction pattern of the L_c phase (Fig. 4). Futher support comes from the comparison of the collected molar enthalpies for the $L_c \rightarrow H_{II}$ and $L_\beta \rightarrow H_{II}$ transitions of a series of MGDG samples of differing saturation presented in Fig. 5. In each case, the molar enthalpy of the $L_c \rightarrow H_{II}$ transition is about 6 kcal/mol greater than the corresponding value for the $L_\beta \rightarrow H_{II}$ transition. If this rule holds equally well for the highly unsaturated lipids, this

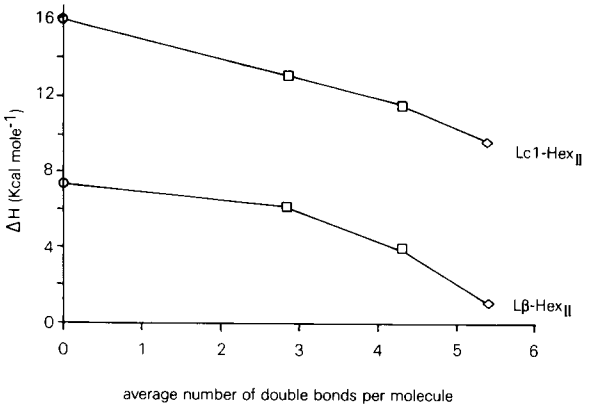

Fig. 5. Plot showing the variation of molar enthalpy of the $Hex_{II} \rightarrow L_c$ and $Hex_{II} \rightarrow L_\beta$ transitions of MGDG as a function of the average number of double bonds per molecule (given by Σ[fatty acid (mole%) × number of double bonds per molecule] × 0.02). Data from (○) Sen et al. [17], (□) Gounaris et al. [8] and (◇) Sanderson and Williams [6].

means that the true value for the molar enthalpy of the $L_\beta \rightarrow H_{II}$ transition of MGDG, at least, is likely to be about 1.0 kcal/mol. Given that the molar enthalpies of the $L_\alpha \rightarrow L_\beta$ transitions of the PC and PE samples show similar variations with saturation to the $L_\beta \rightarrow H_{II}$ transition of MGDG, this gives us a good deal of confidence that the very low measured molar enthalpy values for the polyenoic lipids are realistic estimates of the true enthalpy values for these transitions.

The results obtained in this investigation are thus consistent with the view that the very low enthalpy and weak co-operativity of the $L_\alpha \rightarrow L_\beta$ phase transitions of polyenoic lipids is associated with the low contribution of van der Waal interactions between neighbouring lipid chains. This appears to be a direct reflection of the reduced ability of hydrocarbon chains of the polyenoic lipids to form a regular closely packed lattice in the gel phase. The very small enthalpy contribution associated with chain packing means that there is insufficient energy to drive a fully co-operative $L_\alpha \rightarrow L_\beta$ phase transition in these systems.

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