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Biochimica et Biophysica Acta 1285 (1996) 109–122

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

New phases induced by sucrose in saturated phosphatidylethanolamines: an expanded lamellar gel phase and a cubic phase

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Received 23 April 1996; revised 9 July 1996; accepted 11 July 1996

Abstract

A new lamellar gel phase (L_{β}') with expanded lamellar period was found at low temperatures in dihexadecylphosphatidylethanolamine (DHPE) and dipalmitoylphosphatidylethanolamine (DPPE) dispersions in concentrated sucrose solutions (1–2.4 M). It forms via a cooperative, relatively broad transition upon cooling of the L_{β} gel phase of these lipids. According to the X-ray data, the transformation between L_{β} and L_{β}' is reversible, with a temperature hysteresis of 6–10°C and a transition width of about 10°C. No specific volume changes and a very small heat absorption of about 0.05 kcal/mol accompany this transition. The L_{β}'–L_{β} transition temperature strongly depends on the disaccharide concentration. From a value of about 10°C below the melting transition of DHPE, it drops by 25°C with decrease of sucrose concentration from 2.4 M to 1 M. The low-temperature gel phase L_{β}' has a repeat spacing by 8–10 Å larger than that of the L_{β} gel phase and a single symmetric 4.2 Å wide-angle peak. It has been observed in 1, 1.25, 1.5 and 2.4 M solutions of sucrose, but not in 0.5 M of sucrose. The data clearly indicate that the expanded lamellar period of the L_{β}' phase results from a cooperative, reversible with the temperature, increase of the interlamellar space of the L_{β} gel phase. Other sugars (trehalose, maltose, fructose, glucose) induce similar expanded low-temperature gel phases in DHPE and DPPE. The L_{β}' phase is osmotically insensitive. Its lamellar period does not depend on the sucrose concentration, while the lattice spacings of the L_{α}, L_{β} and H_{II} phases decrease linearly with increase of sucrose concentration. Another notable sugar effect is the induction of a cubic phase in these lipids. It forms during the reverse H_{II}–L_{α} phase transition and coexists with the L_{α} phase in the whole temperature range between the H_{II} and L_{β} phases. The cubic phase has only been observed at sucrose concentrations of 1 M and above. In accordance with previous data, sucrose suppresses the L_{α} phase in both lipids and brings about a direct

Abbreviations: DHPE, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DDPE 1,2-didodecyl-*rac*-glycero-3-3-phosphoethanolamine; DAPE, 1,2-diarachinoyl-*rac*-glycero-3-3-phospho-ethanolamine; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DSD, differential scanning densitometry; L_{α}, lamellar liquid crystalline phase; L_{β}, lamellar gel phase; L_{β}', lamellar gel phase with expanded lamellar period; L_c, lamellar subgel (crystalline) phase; H_{II}, inverted hexagonal phase; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering

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PII S0005-2736(96)00156-3

L_β–H_{II} phase transition in DHPE. A rapid, reversible gel–subgel transformation takes place at about 17°C in both DPPE and DHPE. Its properties do not depend on the sucrose concentration. The observed new effects of disaccharides on the properties of lipid dispersions might be relevant to their action as natural protectants.

Keywords: Phase transition; Phospholipid; Polymorphism; Membrane; Time-resolved X-ray diffraction; Differential scanning calorimetry; Scanning densitometry

1. Introduction

The phase behavior of saturated phosphatidylethanolamines has been extensively characterized in a large number of studies (see, for example, the recent review [1]). Dipalmitoylphosphatidylethanolamine (DPPE) is known to form crystalline (L_c) and gel (L_β) lamellar phases at low temperatures and a lamellar liquid crystalline phase (L_α) at intermediate temperatures [2]. Aqueous dispersions of DPPE exhibit an L_c–L_α phase transition at 66°C in heating scans, following low-temperature sample preparation or prolonged equilibration at low temperature. It is replaced by an L_β–L_α transition at 63°C in subsequent heatings. Dihexadecylphosphatidylethanolamine (DHPE) displays an L_c–L_β transition at about 50°C in first heatings or after prolonged equilibration at low temperature, according to our calorimetric data. The L_β–L_α transition takes place at 68–69°C in both first and subsequent heating scans. Both lipids form inverted hexagonal phase H_{II} at elevated temperatures. In DHPE, the L_α–H_{II} transition takes place at 87°C, while in DPPE an H_{II} phase has been detected at 125–130°C [3]. The dispersions of DHPE and DPPE in excess of water do not form cubic phases. Such phases have been observed in shorter-chain phosphatidylethanolamines such as didodecylphosphatidylethanolamine (DDPE) [4,5].

It has been found that disaccharides such as trehalose and sucrose strongly modulate the phase behavior of phosphatidylethanolamines as well as that of other phospholipids and glycolipids. These sugars are natural protectants [6]. The interest in their influence on the lipid phase behavior stems out from their ability to protect cellular membranes from injuries resulting from stress conditions such as low temperature and dehydration. When present in the aqueous medium, the disaccharides favor formation of the H_{II} and L_β phases at the expense of the intermediate L_α phase [7–9]. Further studies have shown that a number of other low-molecular, water-soluble natural protectants (polyols, proline, etc.) exhibit similar influence on the lipid phase transitions (see Ref. [10] for a review). This general effect was ascribed to the kosmotropic (water-structure making) properties of the natural protectants and to their indirect (Hofmeister) interactions with the lipid–water interface [8,9,11]. As a consequence of their preferential exclusion from interfacial water, kosmotropic solutes tend to decrease interfacial area [12]. Thus they suppress the L_α phase, which has the largest surface area per lipid molecule, and favor formation of the H_{II} and L_β phases at its place ([9,25]). As these findings apparently contradict the spontaneous expectation that natural protectants must support the 'fluid bilayer' state of the membranes, in the present work we undertook a further, detailed examination of the phase behavior of DHPE and DPPE in sucrose solutions by means of time-resolved X-ray diffraction. This study revealed additional, more complicated effects of the disaccharides on the lipid phase behavior. Firstly, high enough sugar concentrations induce formation of a new, so far unobserved lamellar gel phase with expanded lamellar period. This phase replaces the common gel phase of the phosphatidylethanolamines at low temperatures. Secondly, the disaccharides induce also formation of a cubic phase in these lipids. This phase appears during the reverse H_{II}–L_α transformation and coexists with the L_α phase in the whole temperature range between the L_β and H_{II} phases.

Part of this work has been published as an abstract [13].

2. Materials and methods

2.1. Sample preparation

1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE) (Fluka AG, Basel) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) (Avanti

Polar Lipids, Inc., Birmingham, AL) were used without further purification. Aqueous dispersions of these lipids display narrow, cooperative melting transitions, within the limits of published values, thus providing a guarantee that the lipid purity corresponds to the claimed one of 99%. Sucrose from Sigma Chemie (SigmaUltra, > 99.5% purity, and Molecular Biology Reagent, 99 + % purity) was used for the X-ray experiments, and from Riedel de Haën AG, Seelze-Hannover, for the calorimetric and densitometric experiments. Sucrose solutions were prepared in water for HPLC (Fluka AG, Basel) with specific resistance $1.8 \cdot 10^7$ ohm · cm. Solutions of trehalose (Fluka AG, Basel) and maltose (Sigma), were also used for preparation of lipid dispersions.

For the X-ray diffraction measurements, weighed amounts of lipid were dispersed in appropriate sucrose solutions to a lipid concentration of 3 wt%. Samples were vortexed, then heated up for 5 min to 85°C and vortexed again during cooling to room temperature. This procedure was repeated 3 times and followed by centrifugation at 14 000 rpm for 60 min using Eppendorf 5415C centrifuge. The free solution was removed from the vial with a syringe and the concentrated lipid pellet was used for the measurements. The final lipid concentration, determined by weight, was between 20 and 30 wt % in the different preparations. 'Cold' samples displaying L_c phase prior to first heatings were also used. These samples were homogenized by several successive cycles of freezing in liquid nitrogen, followed by thawing at room temperature and vortexing during the thawing step.

For the densitometric and high-sensitivity DSC measurements, sucrose solutions were added to weighed amount of lipid to a lipid concentration of 10–15 mg/ml. These samples were prepared by the procedure described above for X-ray samples, omitting the centrifugation step. Calorimetry of concentrated lipid dispersions was performed on samples containing 25 w % of lipid.

2.2. X-ray diffraction

For time-resolved X-ray diffraction experiments a sample holder was used as described in detail in Ref. [14]. The temperature gradient in the sample was less than 0.01°C axially (i.e., along the X-ray beam) and vertically, and less than 0.02°C horizontally. A stainless steel plate 0.7 mm thick was mounted in a brass holder connected to a PC-interfaced water bath (Huber Ministat, Offenburg, Germany). This setup allows to perform linear heating and cooling temperature scans in the range 0.1–2 K/min. Diffraction patterns were recorded on beam line X13 of the EMBL outstation at DESY in Hamburg. The camera comprises a double-focusing monochromator-mirror arrangement [15]. X-ray reflections in the small- and wide-angle regimes were recorded simultaneously using a data-acquisition system as described in Ref. [16]. With this system millisecond time-resolved experiments were feasible at high spatial resolution [17]. It consists of two linear detectors with delay line readout [18] connected electronically in series. In this configuration, both detectors appear as one single detector to the data-acquisition system [19]. One detector covers the small-angle region from $1/(250\ \text{Å})$ to $1/(20\ \text{Å})$, the second detector covers the wide-angle region from $1/(6\ \text{Å})$ to $1/(3.5\ \text{Å})$. To minimize the X-ray dose on the sample, a fast solenoid-driven shutter controlled by the data acquisition system was used to prevent irradiation of the sample in those periods when no diffraction data are taken. The signals of an ionization chamber to measure the incoming X-ray flux and the readings of a thermocouple placed in the sample holder next to the sample were stored together with the detector data. Raw data were normalized for the incident beam intensity. No further corrections were applied. No radiation damage of the lipids was evident from their X-ray patterns. Particular samples with longer exposure in the beam were checked by thin layer chromatography after the experiments. However, no products of lipid degradation were detected in these samples. The reciprocal spacing $s = 1/d = 2\sin(\theta)/\lambda$ with wavelength $\lambda = 1.5$ Å and scattering angle 2θ obtained from dry rat tail collagen with a long spacing of 650 Å in the SAXS region and from the spacings of tripalmitin in the WAXS region as calibration standards. The data were analyzed using the interactive data evaluating program OTOKO [20].

2.3. Differential scanning calorimetry

Calorimetric measurements were performed with a high-sensitivity differential adiabatic DASM-1M

calorimeter (Biopribor, Pushchino, Russia) with sensitivity better than $4 \cdot 10^{-6}$ cal $\cdot$ K^{-1} and a noise level less than $5 \cdot 10^{-7}$ W [21]. Heating runs, performed at a scan rate of 0.5 K/min, were followed by passive cooling in the calorimetric cell. Usually, a given sample was scanned 3–4 times in succession. The lipid concentration in the samples was 15 mg/ml. The thermograms were corrected for the instrumental baseline. The temperature at the maximum of the excess heat capacity curve was taken as the transition temperature. The calorimetric enthalpy ΔH of the transition was determined as the area under the excess heat capacity curve.

The calorimetric transitions in concentrated lipid dispersions, containing 25 w % of lipid, were recorded with a DuPont 1090 thermal analyzer. The samples were sealed in 20 μl calorimetric pans and scanned 2 times in succession at a scan rate of 1 K/min.

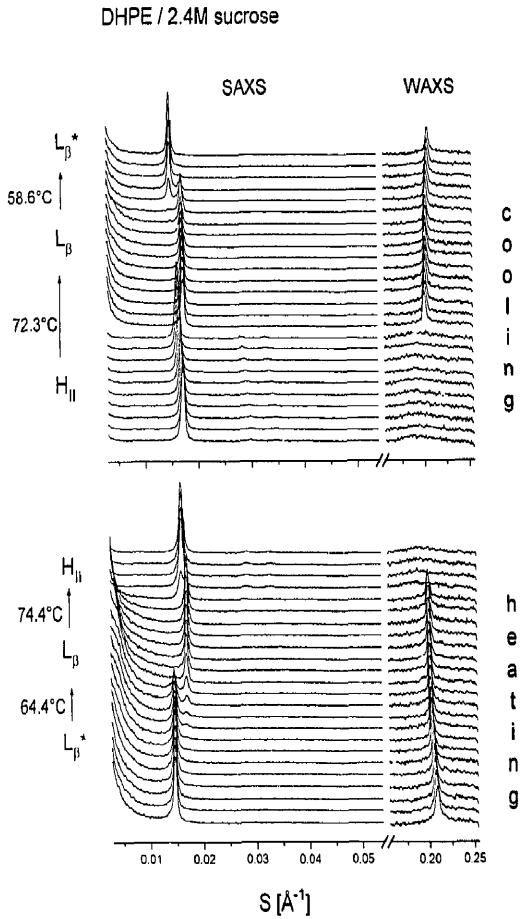

Fig. 1. The phase sequence $L_\beta' \leftrightarrow L_\beta \leftrightarrow H_{II}$ during heating and cooling of DHPE in 2.4 M sucrose solution. Scan rate 1 K/min. Diffraction data were recorded for 2 s at every 30 s. Every 5th frame is shown in the figure. SAXS and WAXS regions are designated.

2.4. Differential scanning densitometry

The specific volume of the lipid molecules as a function of temperature was calculated from the density difference between sucrose solutions and the lipid dispersions. This difference was recorded with two DMA-602H cells (Anton Paar KG, Graz) connected to a home-made unit for data acquisition and temperature control. Linear heating and cooling scans of the samples were performed at 0.5 K/min with a PC-interfaced water bath (Heto Lab Equipment, Allerod, Denmark). The lipid concentration in the samples was 10–15 mg/ml. The instrument constants were determined according to the specifications of the producer, using distilled water and air as standards. The densities of air and water as a function of temperature were taken from the CRC Handbook of Chemistry and Physics (66th Edn., 1985–1986)

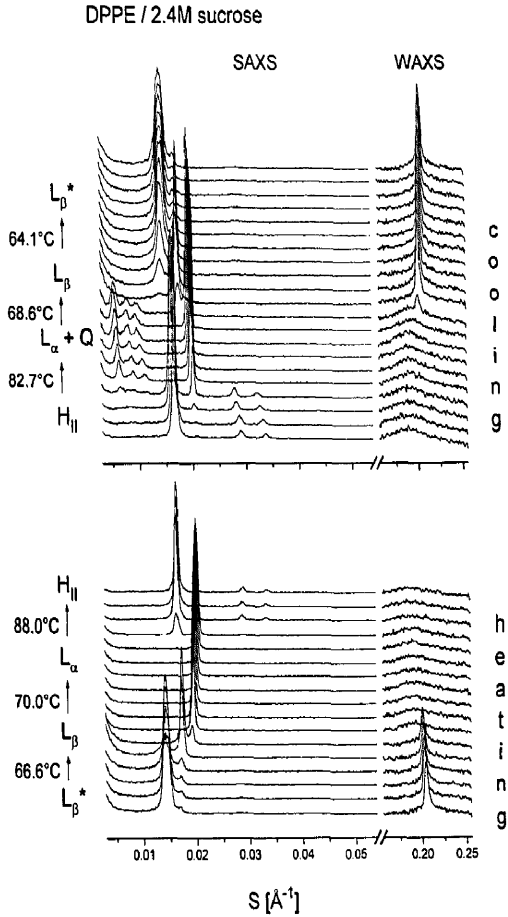

Fig. 2. Phase sequences during heating ($L_{\beta'} \rightarrow L_{\beta} \rightarrow L_{\alpha} \rightarrow H_{II}$) and cooling ($H_{II} \rightarrow L_{\alpha} + Q \rightarrow L_{\beta} \rightarrow L_{\beta'}$) of DPPE in 2.4 M sucrose solution. Scan rate 1 K/min. Diffraction data were recorded for 2 s at every 30 s. Every 5th frame is shown in the figure.

[22]. The partial specific volume $\bar{v}$ of lipids was calculated according to the equation

$$\bar{v} = \frac{1}{\rho_2}\left(1 - \frac{\rho_1 - \rho_2}{c}\right)$$

where ρ_1 and ρ_2 are the densities of the solution and solvent, respectively, and c is the lipid concentration.

3. Results and discussion

3.1. Formation of an expanded lamellar gel phase L_β^* in DHPE and DPPE in sucrose solutions at low temperatures

Cooling of DHPE and DPPE dispersions in sucrose solutions results in a relatively broad, but still cooperative transformation of the L_β gel phase into another gel phase, L_β^*, with expanded lamellar period. In Figs. 1 and 2, successive X-ray frames recorded during cooling and heating scans represent the process of formation of the new gel phase in these two lipids and its reverse transformation into the common gel phase at sucrose concentration of 2.4 M. In both heating and cooling direction the L_β^*–L_β transformation proceeds with well expressed coexistence of the two gel phases. It is reversible, with a hysteresis of 6–10°C at a scan rate of 1 K/min. The d-spacing of L_β^* exceeds by 8–10 Å the repeat period of L_β (Table 1). At the same time, no perceptible changes in the shape and position of the wide-angle reflection of the gel phase accompany this transition (Figs. 1 and 2). The transition temperature, width and hysteresis, as determined from the amplitudes of the first-order low-angle diffraction peaks of the two phases, are illustrated in Fig. 3. With decrease of sucrose concentration from 2.4 M to 1 M the temperature of the L_β^*–L_β transition in DHPE dispersions drops by about 25°C (Fig. 4, Table 2). The transition temperatures summarized in Table 2

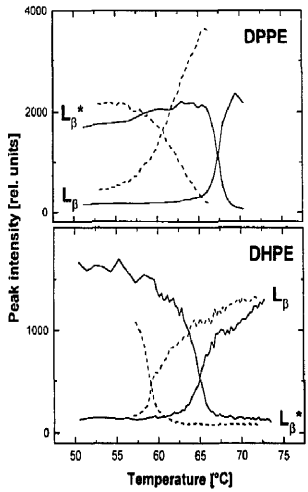

Fig. 3. Intensity of the first-order low-angle reflections from the L_β^* and L_β phases of DHPE and DPPE dispersed in 2.4 M sucrose solution during heating (———) and cooling (- - -) through the L_β^*–L_β phase transition at 0.5 K/min.

Table 1
Low-angle spacings d [Å] of the different phases in DHPE and DPPE for different sucrose concentration at constant temperatures

Sucrose [M]	L_β^*		L_β	L_α	H_{II}
	20°C	25°C	65°C	78°C	94°C
DHPE					
0.0	absent	absent	62.00	52.68	59.65 (68.88)
0.5	absent	absent	61.40	52.25	59.10 (68.24)
1.0	69.12	69.12	60.80	52.25	59.10 (68.24)
1.25	69.50	69.50	60.22	51.80	58.00 (66.97)
1.5	69.50	69.50	59.65	51.40	57.50 (66.39)
2.4	69.20	69.20	58.15	absent	54.10 (62.47) *
DPPE					
2.4		70.5 (60°C)	58.10	50.84	60.10 (69.40)

For the hexagonal phase, the lattice parameter $a = 2d/\sqrt{3}$ is given in brackets.
* Extrapolated value.

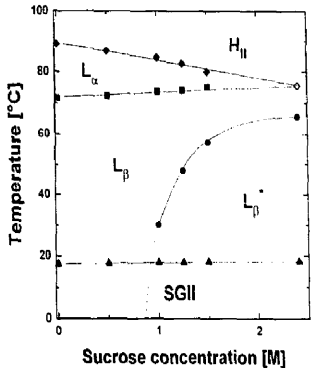

Fig. 4. Heating transition temperatures vs. sucrose concentration in DHPE dispersions. These temperatures were determined from the X-ray data as the temperatures of equal intensities of the low-angle spacings of the coexisting phases. The dashed line is an extrapolation of the L_β^*–L_β transition temperature line until crossing the X-axis at 0°C.

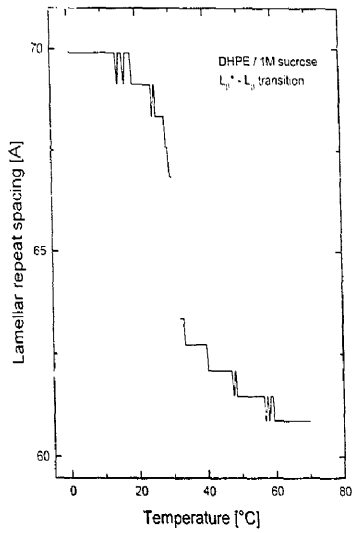

Fig. 5. Lamellar periods of the L_β^* and L_β phases of DHPE in 1 M sucrose during heating at 1 K/min. Identical shift of the L_β^* lamellar period was observed also in the region of the cooling phase transition (not shown).

were determined from the X-ray data as the temperature of equal intensities of the low-angle reflections of L_β^* and L_β. The L_β^* phase is absent at 0.5 M sucrose in the whole temperature range down to 0°C. From an extrapolation of the transition temperatures, we expect that the L_β^*–L_β transition line in Fig. 4 will cross the X-axis set at 0°C at about 0.8–0.9 M sucrose.

At sucrose concentrations of 1.25, 1.5 and 2.4 M the lamellar spacings of the coexisting gel phases do not change appreciably in the transition region. At sucrose concentration of 1 M, the disruption and formation of the L_β^* phase proceed with a strong shift of its expanded lamellar period towards the spacing of the L_β phase (Fig. 5). We note also that in many of the studied samples the conversion of the L_β gel

Table 2
Transition temperatures [°C] of DHPE and DPPE in sucrose solutions as determined by X-ray diffraction at scan rate 1 K/min

Sucrose [M]	$L_\beta^* \leftrightarrow L_\beta$		$L_\beta \leftrightarrow L_\alpha$		$L_\alpha \leftrightarrow H_{II}$	
	heating	cooling	heating	cooling	heating	cooling
DHPE						
0.0	absent	71.7	69.0	89.2		86.0
0.5	absent	72.3	69.9	86.8		81.9
1.0	30.1	20.0	73.6	70.2	84.6	79.0
1.25	48.0	39.5	74.0	71.1	82.7	77.7
1.5	57.2	51.0	75.0	72.2	80.0	74.3
2.4	64.4	58.6	74.8	(direct $L_\beta \leftrightarrow H_{II}$)		72.8
DPPE						
2.4	66.6		70.0	68.6	88.0	82.7

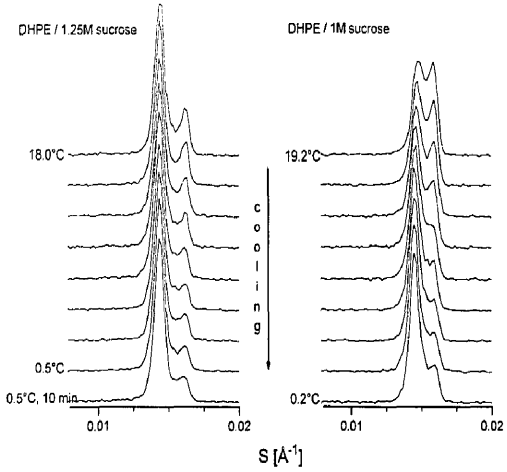

Fig. 6. Coexistence of the $L_{\beta'}$ and L_{β} phases in DHPE samples at about 0°C after cooling at 1 K/min. Sucrose concentrations 1.25 M (left) and 1 M (right). Diffraction data were recorded for 2 s at every 30 s. Every 5th frame recorded during the cooling is shown in the figure.

phase into $L_{\beta'}$ phase does not result in complete disappearance of the former phase. Stable traces of L_{β} phase, coexisting with the $L_{\beta'}$ phase, may be observed even upon incubation at 0°C (Fig. 6). The incomplete L_{β} phase conversion might be a consequence of heterogeneous sucrose distribution in the

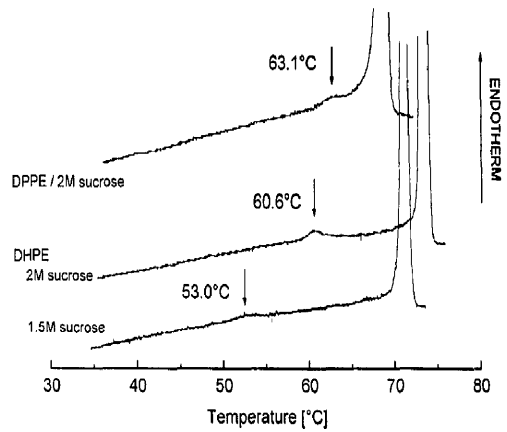

Fig. 7. Heating thermograms of the $L_{\beta'}$–L_{β} transition in DHPE and DPPE dispersed in sucrose solutions recorded at 0.5 K/min. The area under the calorimetric peaks indicated by arrows corresponds to an enthalpy of about 65 cal/mol and 35 cal/mol for DHPE in 2 M and 1.5 M sucrose, respectively, and about 40 cal/mol for DPPE in 2 M sucrose. Lipid concentration 15 mg/ml.

interlamellar aqueous spaces of the lipid phases. We did not attempt to investigate systematically the influence of different procedures for sample preparation on the degree of the L_{β}-$L_{\beta}^{`}$ gel phase conversion. The data presented here were obtained with samples homogenized at high temperature. 'Cold samples', prepared by the freeze-thaw procedure described in Section 2, also form an expanded lamellar gel phase. These samples were not analyzed in detail as they display also a strong background scattering and other poorly resolved structures at very low angles.

Corresponding in temperature phase transitions were detected by high-sensitivity DSC at a lipid concentration of 15 mg/ml (Fig. 7) which is rather high for this method (DASM-1M apparatus). The area under the calorimetric peaks indicated by arrows in Fig. 7 corresponds to an enthalpy of about 65 cal/mol and 35 cal/mol for DHPE in 2 M and 1.5 M sucrose, respectively, and about 40 cal/mol for DPPE in 2 M sucrose. These transitions were not observable at lipid concentrations of less than 1 mg/ml typically used in high sensitivity DSC. Even at the concentration employed, the detection of these low-enthalpy peaks on the thermograms was not perfectly reproducible, following the same temperature protocol. To check for possible effects caused by the high lipid concentration in the X-ray samples, we made calorimetric measurements on identically prepared samples using a DuPont 1090 heat-flow calorimeter. However, the latter measurements did not reveal any thermal events associated with the $L_{\beta}^{`}$-$L_{\beta}$ transformation. On the other side, the calorimetric measurements of concentrated and diluted lipid dispersions produced coinciding values for the temperature and enthalpy of the lipid melting transitions. Thus we conclude that the $L_{\beta}^{`}$-L_{β} transitions in DPPE and DHPE are indeed associated with very low heat absorption. This conclusion is in accordance with the absence of specific volume changes at the temperatures of these transitions (Fig. 8).

In summary, the very low heat absorption associated with the $L_{\beta}^{`}$-L_{β} transformation (Fig. 7), the absence of changes in the wide-angle peak of the gel phase (Figs. 1 and 2) and the lack of a specific volume change at the transition temperature (Fig. 8) clearly show that this transition is not associated with significant reorganization of the lipid bilayers. Thus, we assume that the expanded lamellar period of the

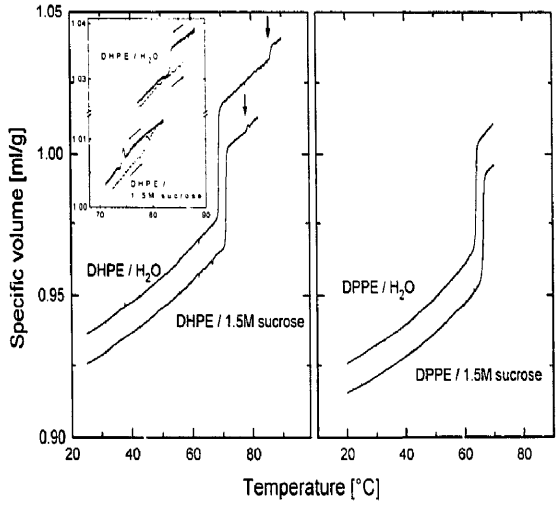

Fig. 8. Specific volume vs. temperature for DPPE and DHPE dispersions in water and in 1.5 M sucrose solutions recorded during heating at 0.5 K/min. The arrows in the left panel indicate the L_{α}-H_{II} transition; the same transition is shown in the inset in enlarged scale during heating and cooling at 0.5 K/min. The lipid concentration is 15 mg/ml.

$L_{\beta'}$ phase results from a cooperative increase of the interlamellar space of the L_{β} gel phase which is reversible with temperature. Similar expanded low-temperature gel phases were observed also in solutions of trehalose and maltose (data not shown). On the basis of these observations we consider the formation of a low-temperature gel phase with expanded lamellar period in phosphatidylethanolamines as a common effect of disaccharides. Noteworthy, our experiments currently in progress demonstrate that the monosaccharides fructose and glucose are also able to induce the appearance of an expanded gel phase. A detailed comparative description of the effects of different sugars on the properties of the $L_{\beta'}$ phase will be given subsequently.

3.2. Suppression of the L_{α} phase in DHPE and DPPE by sucrose

In accordance with previous data, the present X-ray study clearly demonstrates that sucrose favors formation of L_{β} and H_{II} phases at the expense of the L_{α} phase (Table 2, Fig. 4). The L_{β}–L_{α} and L_{α}–H_{II} transition temperatures increase, respectively decrease, linearly with sucrose concentration. The slopes of the transition lines determined from the diffraction data are equal to those determined by DSC in both diluted and concentrated DHPE dispersions. The L_{β}–L_{α} and L_{α}–H_{II} transition lines for DHPE intersect at sucrose concentration of about 2.4 M. At this point, the sequence L_{β}–L_{α}–H_{II} reduces to a direct L_{β}–H_{II} transformation in both heating and cooling scans (Fig. 1). Sucrose lowers the L_{α}–H_{II} transition in DPPE to temperatures below 100°C (Fig. 2). In 2.4 M sucrose solution, this transition takes place at 88°C and 82.7°C in heating and cooling, respectively (Table 2). These effects of sucrose as well as the identical effects induced by other disaccharides have been earlier attributed to indirect interactions of the disaccharides with the lipid–water interface (the Hofmeister effect) [8,9]. Sugars tend to be excluded from interfacial regions and to reduce in this way the amount of interfacial water. In turn, this brings about a tendency to reduction of the interfacial area. Since this area is smaller in the adjacent L_{β} and H_{II} phases, addition of sugars leads to the observed suppression of the L_{α} phase.

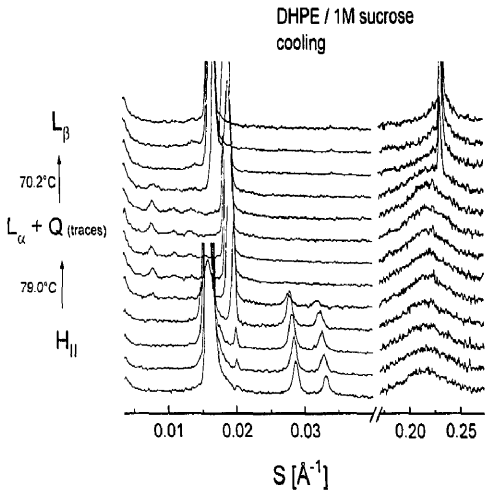

Fig. 9. X-ray diffraction patterns illustrating the formation of a cubic phase in DHPE dispersions in 1 M sucrose during cooling through the H_{II}–L_{α} transition. These patterns have been obtained by averaging by three the successive X-ray patterns recorded during a cooling scan at 1 K/min. Diffraction data were recorded for 2 s at every 30 s.

3.3. Formation of a cubic phase during H_{II}–L_α transitions in sucrose solutions

A remarkable sucrose effect is the induction of a cubic phase upon cooling of both DPPE and DHPE through the H_{II}–L_α transition. This phase coexists with the L_α phase and can be destroyed only by cooling to the L_β phase. It is particularly well developed in DPPE dispersed in 2.4 M sucrose (Fig. 2), but traces of this phase are clearly seen at higher magnification also in DHPE dispersed in 1, 1.25 and 1.5 M sucrose (Fig. 9). Traces of a cubic phase do not appear in DHPE dispersed in water and in 0.5 M sucrose. The three well-resolved peaks at low angles shown in Fig. 2 appear to index on a cubic lattice in the ratio $\sqrt{2}$: $\sqrt{4}$: $\sqrt{6}$ with a lattice constant of about

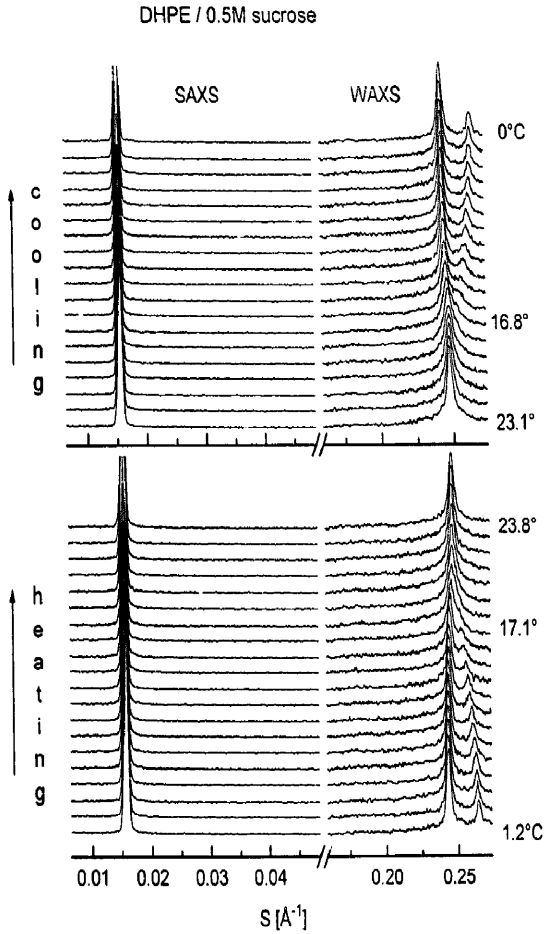

Fig. 10. Reversible splitting of the wide-angle peak of the gel phase of DHPE in 0.5 M sucrose solution at low temperature upon cooling (upper panel) and heating (lower panel) at scan rate of 1 K/min (we denote it as the 'Y-transition' because of the Y-shaped wide-angle pattern). Diffraction data were recorded for 2 s at every 30 s. Every 2nd frame is shown in the figure. No changes of the lamellar period accompany this transition.

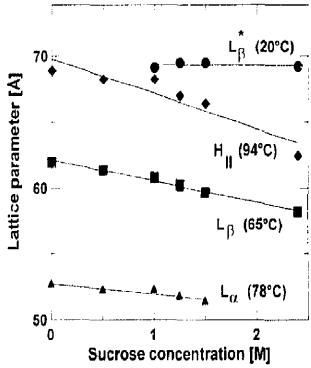

Fig. 11. Structural parameters of the lipid phases as a function of the sucrose concentration at constant temperatures.

220 Å at 82.5°C and 260 Å at 71.7°C. For the lack of a $\sqrt{3}$ reflection, we tentatively assign this phase to the body-centered space group Im3m.

3.4. A reversible gel–subgel transformation in DHPE and DPPE (Y-transition)

Cooling of DHPE and DPPE samples to temperatures below 20°C revealed another phase transformation in the studied lipids displayed as a splitting of the wide-angle peak of the gel phase (Fig. 10). Lowering of the temperature to 0°C resulted in a gradual separation of the two wide-angle peaks. This splitting, which we denote as the Y-transition, is accompanied by no changes in the low-angle X-ray patterns of both L_β and L_β^* phases. The Y-transition is fully reversible with a very small or no hysteresis and its temperature of 17°C upon heating is independent of the sucrose concentration (Fig. 4). It takes place in DHPE and DPPE dispersed in pure water as well. The properties of the Y-transition appear to be very similar to the properties of the reversible subgel–gel (SGII ↔ $L_{\beta'}$) transformation taking place at about 7°C in dipalmitoylphosphatidylcholine (DPPC) dispersions [23,24]. Our preliminary data show that, similarly to DPPC, the subgel phase below the Y-transition in the phosphatidylethanolamines is also a metastable precursor of the equilibrium crystalline low-temperature phase. A more detailed study of this transition is currently in progress.

3.5. Structural parameters of the lipid phases as a function of sucrose concentration

At constant temperature, the lattice parameters of the L_β, L_α and H_{II} phases of DHPE linearly de-

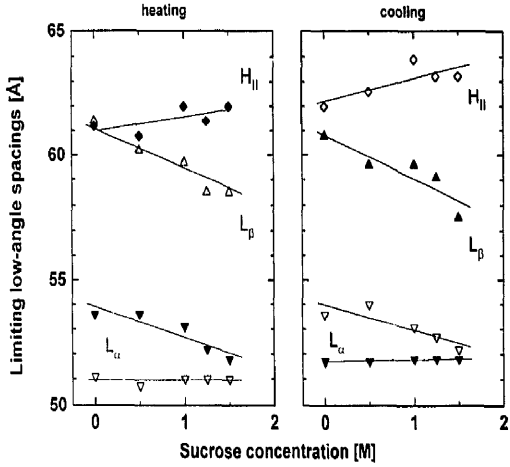

Fig. 12. Lattice parameters of L_β, L_α and H_{II} phases of DHPE at the L_β–L_α and L_α–H_{II} transition temperatures as a function of the sucrose concentration. Open symbols denote the disappearing phase, full symbols denote the nascent phase.

crease with increase of sucrose concentration (Table 1, Fig. 11). As is typical for kosmotropic solutes, the sucrose concentration in bulk water must be higher than that in interfacial (interlamellar) water. We consider therefore the observed reduction of the lattice parameters as an osmotic effect caused by a partial exclusion of sucrose from the interface and associated with it reduction in the amount of interlamellar water. It is interesting to note that the *d*-spacing of the L_α phase is about 2 times less sensitive to sucrose compared to the gel and inverted hexagonal phases. It decreases by 0.85 Å per mol of sucrose, while the lattice spacings of L_β and H_{II} decrease by 1.6 Å and about 2 Å per mol of sucrose, respectively. On the other side, the surface area per lipid molecule in the L_α phase must be higher in comparison to the latter two phases. According to the data of Seddon et al. [4] for other phosphatidylethanolamines, DDPE and DAPE, the surface area of the L_α phase exceeds by 15 to 30% the surface areas of the H_{II} and L_β phases. This may explain, at least partially, the lower sensitivity of the L_α phase lattice spacing to sucrose concentration. However, the absence of data about the surface areas of the different DHPE phases precludes at this stage quantitative estimates of the reduction of interfacial water, expressed as water volume (equivalently, number of interfacial water molecules) per mol of sucrose.

By contrast with the above three phases, the lamellar period of the L_β^* phase of DHPE does not depend on sucrose concentration. Its value remains practically constant in the range from 1 to 2.4 M of sucrose (Table 1, Fig. 11). We consider the lack of osmotic sensitivity as an important characteristic of the L_β^* phase. It clearly indicates a rigid structure of its expanded interlamellar space. However, on basis of the present data we cannot propose a molecular interpretation for this structure.

Since, on one side, the structural parameters of the lipid phases depend on both sucrose concentration and temperature and, on the other side, the phase transition temperatures also depend on the sucrose concentration, it is interesting to assess the sucrose effects on the DHPE structural parameters not only at constant temperature, but also at the phase transition temperatures (Fig. 12). We note that the lamellar period of the L_α phase at the L_α–H_{II} phase transition practically does not depend on the sucrose concentration, i.e., the L_α–H_{II} transformation occurs upon reaching certain lamellar repeat spacing, independently on the temperature and sucrose concentration. The low-angle spacing of the H_{II} phase increases with the sucrose concentration. By contrast, the spacings of the L_β and L_α phases in their coexistence region decrease with increase of sucrose concentration in both heating and cooling scans.

4. Conclusions

(1) A new lamellar gel phase (L_β^*) with expanded lamellar period was found at low temperatures in dihexadecylphosphatidylethanolamine (DHPE) and dipalmitoylphosphatidylethanolamine (DPPE) dispersions in concentrated sucrose solutions (1–2.4 M). It forms via a cooperative transition upon cooling of the L_β gel phase of these lipids. The transformation between L_β and L_β^* is reversible, with a temperature hysteresis of 6–10°C and a transition width of about 10°C. No specific volume changes and a very small heat absorption of about 0.05 kcal/mol are associated with this transition. The L_β^*–L_β transition temperature strongly depends on the disaccharide concentration. It drops by about 25°C in DHPE with decrease of sucrose concentration from 2.4 M to 1 M. The low-temperature gel phase L_β^* has a repeat spacing by 8–10 Å larger than that of the L_β gel phase and a single symmetric 4.2 Å wide-angle peak. It has been recorded in 1, 1.25, 1.5 and 2.4 M solutions of sucrose, but not in 0.5 M of sucrose. Other sugars (trehalose, maltose, fructose, glucose) induce similar expanded low-temperature gel phases in DHPE and DPPE. The very low heat absorption associated with the L_β^*–L_β transformation, the unchanged wide-angle reflection of the gel phase and the lack of a specific volume change at the transition temperature clearly show that this transition is not associated with significant reorganization of the lipid bilayers. We conclude that the expanded lamellar period of the L_β^* phase results from a cooperative increase of the interlamellar space of the L_β gel phase which is reversible with temperature. The lamellar period of L_β^* does not depend on the sucrose concentration, while the lattice spacings of the L_α, L_β and H_{II} phases decrease linearly with increase of sucrose concentration. The

lack of osmotic sensitivity of L_β^* implies a rigid structure of its interlamellar space.

(2) Sucrose induces the appearance of a cubic phase in DPPE and DHPE. This phase forms during the reverse H_{II}–L_α phase transition and coexists with the L_α phase in the whole temperature range between the H_{II} and L_β phases. In DPPE dispersions in 2.4 M sucrose, three well-resolved peaks at low angles appear to index on a cubic lattice in the ratio $\sqrt{2}$: $\sqrt{4}$: $\sqrt{6}$ with a lattice constant of about 220 Å at 82.5°C and 260 Å at 71.7°C. For the lack of a $\sqrt{3}$ reflection, we tentatively assign this phase to the body-centered space group Im3m. In DHPE samples, traces of a cubic phase have been observed at all sucrose concentrations above 0.5 M.

(3) A rapid gel--subgel transformation takes place at about 17°C upon cooling of both DPPE and DHPE dispersions. It is manifested as a splitting of the wide-angle reflection of the L_β phase into two peaks that monotonously separate with decrease of temperature, with no accompanying changes of the lamellar period of the gel phase. This transformation is readily reversible with no or very small temperature hysteresis and its properties do not depend on the sucrose concentration.

Acknowledgements

The authors acknowledge support from Deutsche Forschungsgemeinshaft and from grants K-402/94 and K-407/94 of the Bulgarian National Science Foundation. We thank the European Union for support of the work at EMBL Hamburg through the HCMP Access to Large Installations Project. Contract Number CHGE-CT93-0040.

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