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Condensed lamellar phase in ternary DNA–DLPC-cationic gemini surfactant system: a small-angle synchrotron X-ray diffraction study

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Abstract

We report on a small-angle synchrotron X-ray diffraction study of dilauroylphosphatidylcholine (DLPC) liposomes aggregated with high molecular DNA in the presence of 1,4-butanediammonium-*N*,*N*′-dilauryl-*N*,*N*,*N*′,*N*′-tetramethyl gemini surfactant cations (C12GS). The aggregates prepared at the DLPC/C12GS/DNA phosphate group = 2:1:1.6 molar ratio in 0.0015 mol l^{-1} NaCl aqueous solution exhibit Bragg reflections due to lamellar lipid bilayer stacking and the Bragg reflection typical of one-dimensional DNA lattice with parallel strands intercalated between lipid bilayers. In this condensed fluid lamellar L_{α}^{c} phase, the interactions between DNA and charged bilayers damp the thermally induced bilayer undulations. The diffraction data obtained with the mixture of DLPC liposomes and DNA (at DNA phosphate group/DLPC = 0.8:1 molar ratio) indicate a DNA–lipid interaction in the absence of C12GS.

Keywords: Cationic liposome; DNA; Small-angle X-ray diffraction; 1,2-Dilauroyl-*sn*-glycero-3-phosphorylcholine; 1,4-Butanediammonium-*N*,*N*′-dilauryl-*N*,*N*,*N*′,*N*′-tetramethyl dibromide; Transfection

1. Introduction

Cationic bis(quaternary ammonium bromide) surfactants, also called gemini surfactants, can be formally considered as the dimers of quaternary ammonium surfactants [1–4]. The gemini surfactants display increased bactericidal activities in comparison to the respective monomeric surfactants [2,5,6] and are very potent as plasmid curing agents [7,8]. Gemini surfactants can also be used to increase the efficiency of the DNA transfer into bacterial cells [8,9].

It has been observed that gemini surfactants interact with the phospholipid bilayer in model and biological membranes [6,8,10–14] and with DNA [15,16]. After intercalation of cationic surfactants into bilayer between zwitterionic phospholipid molecules, the bilayer surface becomes positively charged [8,17–21] and the bilayer structure disturbed [6,22–28] depending on the surfactant concentration, alkyl chain length and counter-ion. Minimum bilayer structural perturbations occur when the surfactant and lipid hydrocarbon chains have approximately equal lengths of hydrocarbon chains [6,14,25,26]. The bilayers in phospholipid liposomes charged by cationic gemini surfactants interact electrostatically with DNA forming large light scattering aggregates which dissociate upon increasing ionic strength or in the presence of anionic surfactant [8,15]. Such self-assembled "cationic liposome–DNA complexes" are being widely studied as synthetic DNA vectors for cell transfection and non-viral gene therapy (see Refs. [29–35] for reviews). Because of the net negative charge of the cell plasmic membrane, their charge should be positive for efficient DNA transfer into target cells.

Recent X-ray diffraction and complementary experiments have unambiguously identified two different types of microscopic structure in these aggregates—lamellar condensed ("sandwich") phases with DNA intercalated between lipid bilayers, and condensed inverted hexagonal ("honeycomb") phase wherein DNA is located in its water tubes [36–45]. These experimental as well as theoretical studies (see Refs. [46–55]) have analyzed the physicochemical properties of these phases as well as mechanism of their formation in great detail. In the present paper, we study the microscopic structure of the aggregates prepared

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by mixing high molecular DNA with 1,2-dilauroyl-*sn*-glycero-3-phosphorylcholine (DLPC) liposomes positively charged by intercalation of gemini surfactant 1,4-butanediammonium-*N*,*N*′-dilauryl-*N*,*N*,*N*′,*N*′-tetramethyl cations (C12GS) between lipid molecules. The C12GS surfactant was selected from the homologous series of gemini surfactants [1–4,14] to minimize structural perturbations of the DLPC bilayer. The aggregates studied in the present paper were prepared at DLPC/C12GS/DNA phosphate group = 2:1:1.6 molar ratio, i.e. their net charge is positive. The primary focus of the present study is the temperature dependence of lattice parameters of the aggregates prepared.

2. Materials and methods

2.1. Chemicals

Highly polymerized calf thymus DNA (sodium salt) was purchased from Sigma (St. Louis, USA). DNA concentrations given are based on an average nucleotide molecular mass calculated from the known composition of this DNA [56]. 1,2-Dilauroyl-*sn*-glycero-3-phosphorylcholine (DLPC) was purchased from Avanti Polar Lipids (Alabaster, USA). Analytical grade sodium chloride was obtained from Lachema (Brno, Czech Republic). 1,4-Butanediammonium-*N*,*N*′-dilauryl-*N*,*N*,*N*′,*N*′-tetramethyl dibromide ($C12GSBr_2$) prepared by reaction of tertiary *N*,*N*,*N*′,*N*′-tetramethyl-1,4-butanediamine with 1-bromododecane as described in Ref. [1] and purified by manifold crystallization from a mixture of acetone and methanol was kindly provided by Professors F. Devínsky and I. Lacko. Aqueous solution of 0.0015 mol l^{-1} NaCl and pH ~ 5.6 was prepared with redistilled water. This solution was used as a solvent.

2.2. Preparation of liposomes

Multilamellar DLPC liposomes were prepared by dispersing the 74.4 mg of lipid in 3.4 ml of solvent. This dispersion was briefly sonicated in a bath sonicator and incubated thereafter at room temperature. A part of hydrated lipid dispersion was centrifuged on a benchtop centrifuge and the sediment with a small amount of aqueous phase was transferred into a 1-mm glass capillary (Hellma, Müllheim, Germany). To the multilamellar DLPC liposomes, the DNA solution in the solvent was added to reach the DNA phosphate group/DLPC molar ratio of 0.8:1. A part of the resulting mixture was centrifuged as described above and the sediment was transferred into a capillary as above. To prevent the evaporation of water, the capillaries were sealed. To the remaining part of DLPC + DNA mixture, the solution of $C12GSBr_2$ was added stepwise. After each addition, the sample was mixed by vortexing. During these additions, a formation of large light scattering aggregates was detectable by continuous increase of opalescence of the mixture. After reaching the molar ratio of DLPC/$C12GSBr_2$ = 2:1, the aggregates were sedimented by a centrifugation on a benchtop centrifuge. The sediment was substantially more viscous than in the previous two cases. It was placed between 25-μm thick mica windows in the 5-mm hole in the center of 2 × 2 cm × 0.8-mm steel plate (sandwich sample). The mica windows were glued to the steel plate by high vacuum silicon grease (Wacker, Munich, Germany) to prevent the evaporation of water. Before measurements, the samples were stored at 5–6 °C.

2.3. Small-angle X-ray diffraction

The small-angle diffraction (SAXD) data were obtained using the X13 double focusing monochromator-mirror camera of the EMBL Outstation at the Deutsches Elektronen Synchrotron (DESY) in Hamburg on the storage ring DORIS. At this beam line, the wavelength selected by a Ge(111) crystal is $\lambda = 0.15$ nm. The distance between the sample and the linear small-angle detector [57] was 270 cm. The experimental setup and data acquisition system were described in more detail earlier (see Refs. [41,45] and references therein). During measurements, the capillary or the sandwich was held in a thermostatically controlled sample holder and heated at a scan rate 1 K/min. Cooling scans were performed at the same scan rate. During the temperature scan, the diffractograms were recorded for 10 s every minute. At selected temperatures, the diffractograms were measured for 300 s. The reciprocal spacing

$$s = (2/\lambda)\sin\theta \qquad (1)$$

where 2θ is the scattering angle, was calibrated using silver behenate as standard [58]. The data were analyzed using the interactive data evaluation program OTOKO [59].

3. Results and discussion

Fully hydrated DLPC in excess water has been measured as a control sample. The SAXD patterns observed at different temperatures in the range of 6.5–91 °C consisted of two rather broad peaks (Fig. 1). It is known that fully hydrated DLPC can exist in different lamellar phases. Depending on temperature and incubation conditions, crystal-like L_c phase, gel ripple $P_{\beta'}$ phase with tilted acyl chains, ordered fluid (liquid crystal) L_x phase and disordered fluid (liquid crystal) L_α phase were found in fully hydrated DLPC [60–66]. In nonannealed samples, the $P_{\beta'} \rightarrow L_x$ and $L_x \rightarrow L_\alpha$ phase transitions occur at ~ − 1.8 and ~ 5 °C, respectively [60,64,65]. Annealing (a prolonged sample incubation at ~ 0 °C) is accompanied by complete suppression of $P_{\beta'} \rightarrow L_x$ transition and by formation of crystal-like L_c phase [63]. In short, chain phosphatidylcholines, including DLPC, the crystal-like L_c phase can be converted into disordered fluid L_α phase directly on heating. At a slow heating rate (0.2 °C/min), the endothermic $L_c \rightarrow L_\alpha$ phase transition has

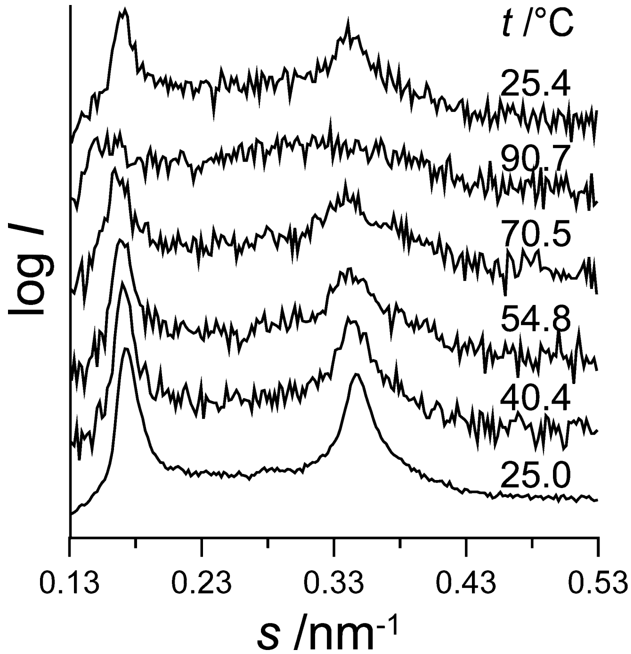

Fig. 1. Typical diffractograms of the DLPC L_α phase measured during the heating scan at indicated temperatures for 10 s. The diffractogram at 25.4 °C was recorded during the cooling scan and the diffractogram at 25.0 °C was recorded for 300 s before the temperature scans.

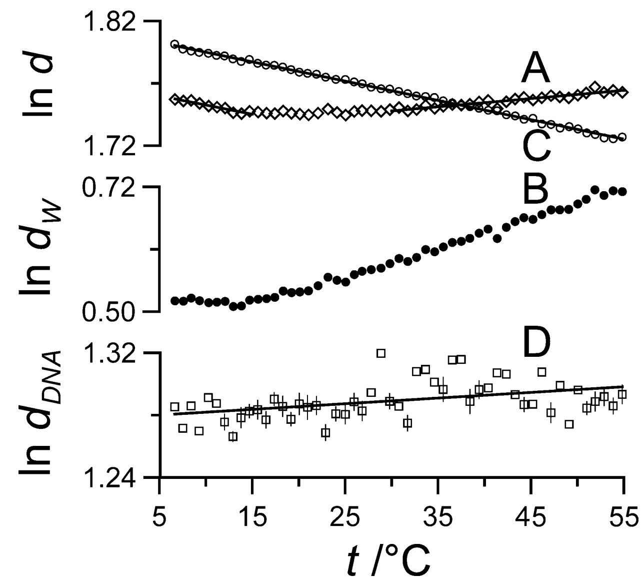

Fig. 2. Temperature dependence of the repeat period d (in nm), aqueous layer thickness d_W (in nm) and DNA interhelical spacing d_{DNA} (in nm). DLPC L_α phase—(A, B); DLPC + DNA + C12GS L_α^c phase—(C, D).

been observed to be broad (~ 5 °C) with excess heat capacity maximum at ~ 7 °C [63]. Our experimental protocol involved sample incubation at ~ 5–6 °C for about 24 h. However, in the sample holder, each sample was heated to 25 °C, equilibrated at this temperature minimally for 10 min, measured for 300 s, and cooled to about 6 °C thereafter. The SAXD patterns were measured then immediately during the heating scan between 6.5 and 91 °C in regular intervals. Our samples were thus nonannealed and we ascribe the observed peaks to the first and second order Bragg diffraction on the one-dimensional lamellar fluid L_α phase. It is further seen in Fig. 1 that the diffraction peaks become broader and superposed on the diffuse scattering with the increase of temperature. At the highest temperatures, the second order diffraction peak fully disappeared and only broad diffuse scattering was observed. This effect was reversible on cooling, though some hysteresis was observed at our relatively high scan rate (compare diffractograms taken at 25.0 and 25.4 °C in Fig. 1).

We have fitted each Bragg diffraction peak with a Lorentzian, and from the positions of fitted peak maxima, we have estimated the lamellar repeat period d using the Bragg equation. At temperatures above 55 °C, this procedure gives false results because of severe distortion of diffraction peaks. These data are not included thus in further discussion. The temperature dependence of d is shown in the semi-logarithmic plot in Fig. 2 (curve A). It is seen, that ln$d$ decreases up to about 15 °C and, after reaching a flat minimum around 20 °C, increases from about 30 °C on heating. The transversal thermal expansivity of DLPC L_α phase,

$$\alpha = \frac{1}{d}\left(\frac{\partial d}{\partial T}\right)_\pi \tag{2}$$

where T is the absolute temperature, is thus negative in the region of temperatures $t < 15$ °C and positive at $t > 30$ °C. The values of α obtained by least squares fittings are shown in Table 1.

The unit cell of lamellar fluid L_α phase consists of single lipid bilayer plus the aqueous phase layer between neighboring bilayers. The repeat period d is thus

$$d = d_L + d_W \tag{3}$$

where d_L is the steric thickness of bilayer and d_W the thickness of aqueous layer. Now we will show that the increase in the repeat period for $t > 20$ °C is accompanied by the increase in the aqueous layer thickness and in the number of water molecules located in it. We have found in the present paper that the repeat period is $d = 5.73 \pm 0.01$ nm at 20 °C. Harroun et al. [67] have measured the trans-bilayer distance of phosphate groups $d_{HH} = 3.08$ nm in the fluid lamellar DLPC phase at 20 °C. The distance between the phosphate groups and the boundary between the polar

Table 1
Transversal thermal expansivity of DLPC

	t/°C	$10^3\alpha$/K^{-1}
DLPC	6.5–15	− 1.48 ± 0.15
	30–55	+ 0.66 ± 0.05
DLPC + DNA	6.5–15	− 1.58 ± 0.21
	30–55	+ 0.92 ± 0.09
DLPC + DNA + C12GS	6.5–55	− 1.55 ± 0.01

and hydrocarbon region and the volume of hydrocarbon region are $d_{H1} = 0.43 \pm 0.02$ nm and $V_C = 0.674$ nm^3, respectively, in DLPC at 20 °C (see Ref. [68] and references therein). A combination of these data gives the DLPC bilayer hydrocarbon region thickness $2d_C = d_{HH} - 2d_{H1} = 2.22 \pm 0.04$ nm and surface area $A_L = V_C/d_C = 0.607 \pm 0.011$ nm^2. The thickness of the phosphatidylcholine bilayer polar region is $d_H = 0.9 \pm 0.1$ nm [69–71]. At 20 °C, the steric DLPC bilayer thickness is then $d_L = 2d_C + 2d_H = 4.02 \pm 0.14$ nm and the aqueous layer thickness $d_W = d - d_L = 1.71 \pm 0.15$ nm. The number of water molecules located in the aqueous layer (per one DLPC molecule) is thus $N_W = A_L d_W / 2V_W = 17.3 \pm 1.9$ where V_W is the molecular volume of water at 20 °C taken from Ref. [72]. In the fluid lamellar phase, an increase of temperature should induce an increased population of *gauche* rotamers in lipid acyl chains accompanied by lateral expansion of the bilayer. The parameter describing this expansion is the thermal area expansivity

$$\beta = \frac{1}{A_L}\left(\frac{\partial A_L}{\partial T}\right)_\pi \tag{4}$$

Evans and Needham [73] have estimated $\beta = 0.00417 \pm 0.00020$ K^{-1} in fluid dimyristoylphosphatidylcholine (DMPC) bilayers. Because β for DLPC is not available, we will use the known value for DMPC. Using this value, one can obtain the DLPC surface area at any temperature by extrapolation from the value obtained above at 20 °C. Similarly, the thermal expansion of DLPC molecular volume is characterized by the thermal volume expansivity

$$\gamma = \frac{1}{V_L}\left(\frac{\partial V_L}{\partial T}\right)_\pi \tag{5}$$

Since the value of γ is not available for DLPC, we will use $\gamma = 0.00071 \pm 0.00002$ K^{-1} calculated as the mean value for the fluid lamellar phase of DMPC from the volumetric data in Ref. [74]. From the molecular volume of DLPC $V_L = 0.993$ nm^3 at 20 °C [68], one can obtain then the molecular volume of DLPC in the fluid lamellar L_α phase at any temperature by extrapolation. The volume of the polar head group of phosphatidylcholines is $V_H = 0.319$ nm^3 and we will suppose in accord with Ref. [75] that it remains constant irrespective of temperature. At 54.8 °C, the repeat period found in the present paper is $d = 5.83 \pm 0.01$ nm. Using this repeat period and physical parameters summarized above, we have found a significant increase of both d_W and N_W in comparison to 20 °C (Table 2). The temperature dependence of d_W is shown in a semi-logarithmic plot in Fig. 2 (curve B). It is seen that d_W remains constant up to about 15 °C and then increases. Similar dependence has been observed for N_W—it remains constant up to 15 °C and then increases (not shown). The increase of d_W is thus accompanied by diffusion of water molecules from outside of lamellar phase in between lipid bilayers.

Table 2
Physical parameters of the lamellar fluid DLPC L_α phase

	20 °C	54.8 °C
d/nm	5.73 ± 0.01	5.83 ± 0.01
V_L/nm^3	0.993	1.018 ± 0.001
V_H/nm^3	0.319	0.319
V_C/nm^3	0.674	0.699 ± 0.001
d_{H1}/nm	0.43 ± 0.02	–
d_H/nm	0.9 ± 0.1	–
d_{HH}/nm	3.08	–
d_C/nm	1.11 ± 0.02	0.996 ± 0.024
A_L/nm^2	0.607 ± 0.011	0.702 ± 0.018
d_L/nm	4.02 ± 0.14	3.79 ± 0.05
d_W/nm	1.71 ± 0.15	2.04 ± 0.05
N_W	17.3 ± 1.9	23.9 ± 1.2

Simon et al. [76] observed the bilayer thickness decrease (~ 0.4 nm) and the aqueous layer thickness increase (~ 0.7 nm) in the fluid lamellar L_α phase of egg phosphatidylcholine (EPC) as the temperature was increased from 5 to 50 °C. They have predicted that the increase in aqueous layer thickness is caused by an increase in the undulatory fluctuations as a consequence of a temperature-dependent decrease in bilayer bending modulus. Petrache et al. [70] confirmed their experimental findings as well as their predictions. The undulatory fluctuations cause disorder in the relative positions of unit cells (long-range disorder) resulting in broadening of diffraction peaks and apparent disappearance of higher order Bragg diffraction peaks—the long-range disorder removes the intensity from the central part of the diffraction peak and spreads it into a diffuse scattering, and the magnitude of this effect increases with increasing diffraction order [70,77]. In the present paper, we have observed the broadening of first and second order diffraction peaks and disappearance of the second order peak of DLPC as the temperature was increased (Fig. 1). The width of the fitted second order Bragg peak Δs_2 and the ratio of the widths of second and first Bragg diffraction peaks $\Delta s_2/\Delta s_1$ increase nonlinearly with the temperature (see curves A in Figs. 3 and 4, respectively). This is evidence that the disorder induced by undulations increases with the increase of temperature. This effect is reversible—in the cooling scan, a decreasing tendency of Δs_2 and $\Delta s_2/\Delta s_1$ parameters has been observed.

Typical diffractograms of DLPC + DNA + C12GS aggregates are shown in Fig. 5. Besides the Bragg peaks due to lamellar bilayer stacking, a single Bragg peak (marked by arrow) is seen between the first and second order lamellar reflections. Similar peak has been observed in other DNA aggregates with cationic liposomes and has been ascribed to diffraction on parallel DNA strands intercalated between lipid bilayers in the condensed fluid lamellar phase L_α^c [36–38,44,45]. We have fitted each Bragg diffraction peak with a Lorentzian, and from the positions of fitted peak maxima, we have calculated the lamellar repeat period d and the DNA interhelical spacing d_{DNA} using the Bragg equation. The DNA interhelical spacing varied between 3.55 and 3.72

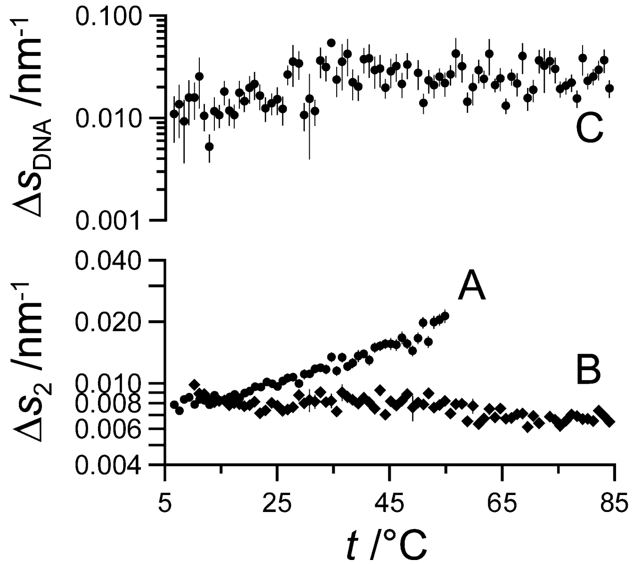

Fig. 3. Temperature dependence of the second order lamellar Bragg diffraction peak width Δs_2 and of the first order Bragg diffraction peak width of the one-dimensional DNA lattice Δs_{DNA}. DLPC L_α phase—(A); DLPC + DNA + C12GS L_α^c phase—(B, C).

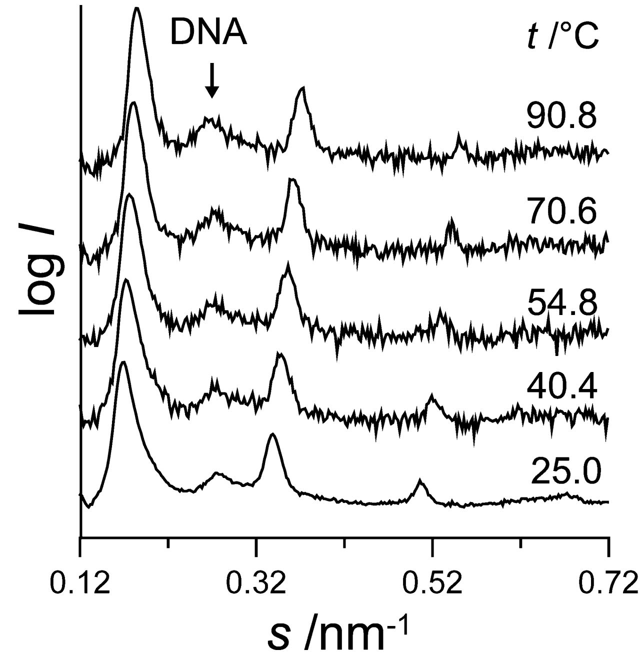

Fig. 5. Typical diffractograms of the DLPC + DNA + C12GS L_α^c phase measured during the heating scan at indicated temperatures for 10 s. The diffractogram at 25.0 °C was recorded for 300 s before the temperature scan.

nm with the mean $d_{DNA} = 3.64 \pm 0.02$ nm. This is close to $d_{DNA} = 3.53$ nm found at 45 °C in isoelectric DNA complexes with cationic DMPC + dimyristoyltrimethylammoniumpropane (DMTAP) liposomes at DMPC/DMTAP = 1:1 molar ratio [45]. Koltover at al. [44] have observed $d_{DNA} = 3.54$ nm in the isoelectric condensed fluid lamellar phase of DNA + dioleoylphosphatidylcholine + dioleoyltrimethylammoniumpropane at phospholipid/surfactant = 1:1 molar ratio. The surface area of C12GS in EPC bilayers estimated by X-ray diffraction is 0.69 ± 0.02 nm^2 with two cationic head groups [14]. This matches exactly the projected charge density of DNA—two anionic phosphate groups per 0.68 nm^2 [44]. The temperature dependence of the interhelical spacing is shown as a semi-logarithmic plot in Fig. 2 (points D). The scatter of the d_{DNA} values was relatively large to see clear temperature dependence of d_{DNA}, though it seems that it slightly increases on heating with the thermal expansivity 0.00036 ± 0.00011 K^{-1}. This is less than 0.0016 K^{-1} found in the isoelectric DNA + DMPC + DMTAP complexes [45].

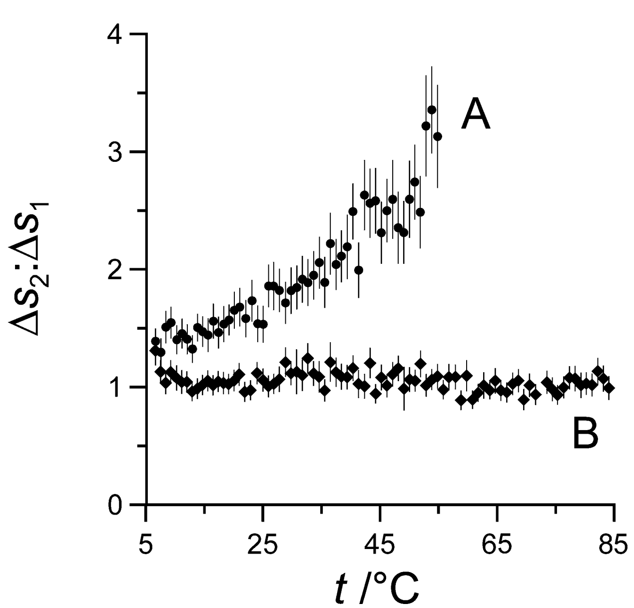

Fig. 4. Temperature dependence of the ratio of second and first order lamellar Bragg diffraction peak widths $\Delta s_2/\Delta s_2$. DLPC L_α phase—(A); DLPC + DNA + C12GS L_α^c phase—(B).

The diameter of hydrated DNA is 2.4 nm [37]. Subtracting this diameter from the DNA interhelical spacing, one calculates the distance between DNA molecules ~ 1.2 nm. This relatively large distance indicates that the one-dimensional DNA sublattice in the condensed fluid L_α^c phase can be disordered. The lateral topological disorder as well as thermally induced fluctuations contribute to the diffraction peak broadening. In Fig. 5, one can see that the DNA peak is broader than the other peaks. The fitted DNA peak width Δs_{DNA} increases up to about 35 °C and then decreases or remains constant (see Fig. 3, points C). Simultaneously, the intensity of the DNA peak increases above 35 °C (not shown). Artzner et al. [41] observed that the temperature cycling increased the DNA peak intensity and decreased its width in aggregates of DNA with DMPC and cationic surfactant DMTAP. They concluded that the topological DNA disorder is quenched after sample preparation before the temperature cycling. The aggregates studied in the present paper were not cycled before the measurements.

The observed dependence of the DNA peak width and its intensity is thus most probably caused by two effects acting simultaneously—by the thermally induced fluctuations causing an increase in peak width and decrease in its intensity, and by removal of quenched topological disorder which acts in the opposite direction.

The temperature dependence of the lamellar repeat period d in the condensed fluid lamellar phase L_{α}^{c} is shown as a semi-logarithmic plot in Fig. 2 (points C). It is seen that the repeat period decreases in the whole temperature range. The transversal thermal expansivity obtained from this plot is the same (within experimental error) as observed in pure DLPC in the region of 6.5–15 °C (see Table 1). The alkyl chain length of C12GS is the same as the acyl chain length of DLPC, so we can supposed that the steric bilayer thickness in DLPC + DNA + C12GS aggregates is close to that in pure DLPC, or slightly smaller because of larger surface area of C12GS (see above). Subtracting this diameter and the steric bilayer thickness of DLPC from the repeat period, one obtains that the thickness of aqueous layer between DNA and bilayer surface increases from 0.23 nm at 6.5 °C to 0.29 nm at 55 °C. The thermally induced decrease of the repeat period is thus caused by the change in the bilayer thickness in DLPC + DNA + C12GS aggregates.

The temperature dependence of the width of the fitted second order lamellar Bragg peak Δs_2 and the ratio of the widths of second and first lamellar Bragg diffraction peaks $\Delta s_2/\Delta s_1$ are shown in Fig. 3 (points B) and 4 (points B), respectively. A small decrease in Δs_2 above 45 °C at a constant ratio $\Delta s_2/\Delta s_1$ is seen. Comparing points A and B in these figures, it is evident that the thermally induced long-range fluctuations observed in pure DLPC (see above) are damped by the DNA intercalated between bilayers. A small decrease in Δs_2 (and Δs_1) above 45 °C correlates with the small decrease in Δs_{DNA}. This indicates that not only the DNA topological disorder, but also short-range bilayer disorder in the direction perpendicular to the bilayer surface is removed on heating. It has been suggested that the presence of negatively charged DNA between bilayers can induce a partial lateral segregation of cationic surfactants in bilayers to minimize the electrostatic energy of the whole system, i.e. lateral "demixing" in plane of the bilayers can occur [51–53]. Such a demixing has been observed experimentally in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine liposomes containing various cationic surfactants and interacting with polyadenylic acid [78]. Because of a small difference in the lengths and surface areas of DLPC and C12GS molecules, this demixing can induce thickness modulation in the bilayer. Another source of short-range disorder can be a DNA-imposed elastic deformation of bilayer [41,46]. The increase of temperature increases lateral mobility of DLPC and C12GS molecules and *trans-gauche* isomerization of their hydrocarbon chains. This could be a mechanism responsible for the removal of short-range disorder on heating.

Boukhnikachvili et al. [40] have studied DNA complexes with polycationic lipid dioctadecylamidoglycylspermine (DOGS) prepared in a 0.15 mol l^{-1} NaCl solution. At pH = 8, DOGS forms a lamellar phase having the lamellar repeat period $d = 7.01$ nm. In the DOGS + DNA complexes, the lamellar repeat period was decreased to $d \approx 6.45$ nm and, simultaneously, the intensity of diffraction peaks due to DOGS lamellae was increased. The authors concluded that "DNA obviously improves the organisation of DOGS bilayer stacking, even accelerating it". Their observations and conclusions are thus in accord with our results concerning the effect of DNA on the disorder in lamellar phase. Noteworthy, no diffraction peak corresponding to DNA sublattice in the lamellar DOGS + DNA complexes has been observed in Ref. [40]. Though the authors have not noted or commented on the absence of the DNA peak, one can conclude that the DNA topological disorder was most probably too high in their preparation.

We have measured also the DLPC + DNA sample prepared without C12GS. Only lamellar phase was observed in the studied temperature range and diffraction patterns were similar to those observed in fully hydrated pure DLPC. We have not observed any diffraction peak indicating a correlation between DNA molecules. The calculated lamellar repeat period d displayed similar temperature dependence as that in pure DLPC. The thermal expansivity $\alpha = -0.00158 \pm 0.00021$ K^{-1} calculated from the d dependence in the range of 6.5–15 °C was (within the experimental error) the same as that in the pure DLPC (see Table 1). However, it was significantly larger when calculated in the range of 30–55 °C (see Table 1). This indicates interaction between DNA and bilayer even in the absence of cationic surfactant. It has been found in calorimetric experiments [79–81] that DNA influences the gel-fluid phase transition in dipalmitoylphosphatidylcholine (DPPC) liposomes. The calorimetric enthalpy of this transition was significantly decreased in the presence of high molecular DNA. Similar effect on the gel-fluid transition calorimetric enthalpy has been observed later by Zhdanov and Kuvichkin [82] who studied the interaction of polyA/polyU duplex with DMPC liposomes. They have also observed a small but significant shift (0.7 ± 0.1 °C) in the duplex melting temperature in the presence of DMPC. Changes in the melting of high molecular DNA have been observed in the presence of EPC liposomes [81]. Finally, we have found recently by using the sychrotron SAXD that the high molecular DNA decreases the rippling period d_r of P_{β} phase and affects the lamellar repeat period d in $P_{\beta'}$ and L_{α} phases of fully hydrated DPPC [83]. The molecular mechanism of the direct DNA–phosphatidylcholine interaction is not clear. It might be polyanion DNA interaction with the lipid $N^{+}-P^{-}$ dipole. It is known that interaction of this dipole with small inorganic and organic ions and charged peptides changes the conformation of the lipid head group [84–86]. The second possibility could be a local unwinding of the DNA and interaction of DNA bases with the bilayer [87].

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